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Ciclo di dottorato XXX

Epidemiological study in the Lomellina's area: associated neurological findings.

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INTRODUCTION

Dementia: general concepts

The world population is aging at a phenomenal rate. People over ultra-sixty-five currently living in Europe are around 151 million (Economic And Financial Affairs, 2012) and the number is expected to double by the year 2060. In the past few years the scientific community has shown a growing interest in the quality of life of the elderly population, mainly focussing on the diseases that specifically affect this age group such as dementia. Dementia is a syndrome resulting from a range of progressive or chronic diseases of the brain, leading to the disturbance of several higher cortical functions (WHO 2012). According to the International Classification of Diseases 10th Revision (ICD10), this decline in cognitive function is comprised of two major components: 1- a decline in memory and 2- a decline in other cognitive abilities (WHO 1992), that must have been present for at least 6 months.

There are several aetiological types of dementia, including primary neurodegenerative diseases (such as Alzheimer disease, Lewy body dementia and Fronto-Temporal dementia) and vascular dementia (WHO 1992, AIHW; 2012). However in many patients with multiple pathologies, features of primary neurodegenerative disorders and vascular disorders coexist, leading to a diagnosis of "mixed dementia" (WHO 2006; AIHW-2012).

An important concept introduced more recently is the Mild Cognitive Impairment (MCI), that refers to a population of elderly subjects which, not being compromised in their daily operation, present only minimal impairment, and are only potentially at risk of developing Alzheimer's disease (Petersen et al., 2001). A classification of three different MCI phenotypes (Winblad et al., 2004) has been proposed: a-MCI single domain or

amnesic form (an isolated cognitive deficit) / b-MCI multiple domain (Involving more cognitive functions) and c-multiple domain MCI.

The different forms of dementia are responsible for morbidity and disability in a consistent percentage of patients. A recent study (Abbott, 2011) estimated that about 10 million people in Europe have a diagnosis of dementia and this number is expected to increase to 19 millions in 2050. The socio-economic impact of such diseases is extremely significant (Brodaty, Seeher, & Gibson, 2012).

Dementia risk factors and prevention

AD is a slowly progressing disease, that starts certainly decades before clinical onset (Vellas, Gillette-Guyonnet, & Andrieu, 2008). Delaying onset by as little as 5 years could halve the number of people afflicted with the illness. It is therefore essential to promote the importance of the early diagnosis of neurodegenerative diseases to allow timely identification of reversible causes and to start targeted therapies that can slow disease progression (Musicco, Caltagirone, Sorbi, & Bonavita, 2004). This could also results in the ability to timely schedule the necessary measures for the management of the related problems to the disease and reduce the social and economic costs (Mittelman, Haley, Clay, & Roth, 2006).

For this reason, many potential risk and protector factors have been investigated during the last few years including lifestyle and dietary changes, physical and mental exercises, and metabolic and vascular risk factors.

The association between dementia and dismetabolic risk factors has been widely recognized in the literature, particularly the relationship between vascular comorbidity arising from conditions such as hypertension and hypercholesterolemia and cognitive performance in Alzheimer's disease. Blood pressure values, blood cholesterol concentration and neuropsychological tests were investigated with particular attention to attentive, executive, amnesic, language, visual/visual and visual/spatial capabilities. It

was shown that impairment in specific cognitive tasks were more associated with the number than to the severity of vascular comorbidity, particularly in verbal and visual referral tasks, visual-constructive and spatial tests, verbal reasoning, and ability to pass from one task to another. Vascular comorbidity caused by high blood pressure and high levels of cholesterol in the blood is then associated with the reduction of specific cognitive abilities in Alzheimer's. This is probably related to the association with cerebrovascular damage, in particular white matter disease (Dickstein et al., 2010).

Interestingly, in 2012 Tom Russ at al (Russ, Batty, Hearnshaw, Fenton, & Starr, 2012) identified through a meta-analysis a variation in rates of dementia in affluent countries according to geographical scales. Rural living was associated with an increased risk of Alzheimer disease, and there is a suggestion that early life rural living further increases this risk.

It became clear in the past years that to control all the risk factors and establish preventive approaches targeted strategies were required. For this reason, Bruno Vellas et al. introduced the concept of the "multi-domain preventive approach", that is based on the hypothesis that cumulated exposition to all of these factors-nutrition, physical and mental exercises could optimize the protective role of each of them against cognitive decline (Vellas et al., 2008). However, such approach requires an appropriate setting to be fulfilled.

The role of memory clinics in dementia management

The dedicated setting for the application of this approach were identified in the Memory clinics (MCs), specialised health care facilities in which multidisciplinary teams provide a thorough physical, neuropsychological, functional and psychiatric assessment, a diagnostic disclosure, post-test counselling and appropriate drug treatment. (Kelly, 2008).

MCs represent the starting point to develop preventive multi-domain approaches

in patients with higher risk of cognitive impairment (particularly patients with subjective memory complaint, family history of AD, or vascular and metabolic risk factors). Remarkers et al (Ramakers & Verhey, 2011) with their study, showed that MCs in the Netherlands had outgrown the primarily university-based setting and have focussed less on scientific research, and had taken a place in the regular care of people with cognitive problems and people in early phases of dementia. The impact was a reduction of 29% of cases per year of patients categorized as dementia has been demonstrated, from 1998 to 2009, as a result of an increase of 14% of patients with Mild Cognitive Impairment (MCI).

MCs are a relatively new phenomenon in the care for people with dementia; the first MCs were set up in North America during the mid-1970s, and in the UK shortly thereafter. The focus on MCs at that time can be related to the changing view of Alzheimer's disease (AD) from senile dementia as an inevitable result of ageing to a disease (Thal, Kawas, Galasko, Salmon, & Sundsmo, 2006). In 1986, the first MC was developed in the Netherlands in Maastricht (Lee, 2005). Since then, MCs have been the focus of strong development, and today are a widely accepted health care service involved with the early diagnosis and treatment of people with dementia or other memory problems (Jolley, Benbow, & Grizzell, 2006). MCs developed in various ways, leading to large variations in their organisation, working methods and services. In Israel, in the 2007, Werner et al (Werner, Heinik, & Aharon, 2001) described the characteristics and activities of 25 memory clinics in Israel in 1998 and after in the 2007 using a mail survey. The results showed a development in the process and organizational characteristics of memory clinics in Israel over the years, probably as a consequence of the development of knowledge in the area of cognitive deterioration.

Jansen et al (Jansen et al., 2017), promoted a study to evaluate the Diagnostic and Prognostic Value of Neuropsychological Assessment in Memory Clinic Patients, that found an increase in correctly classified cases and that the use neuropsychological tests in the diagnostic process increased diagnostic confidence.

Dementia management in Lomellina

The province of Pavia has five MCs which have been active for several years (data available on AIMA website). Three of these are located in the city of Pavia or nearby, the other two are located in the Oltrepò Pavese's area. The lack of adequate structures for specialist investigations on neurodegenerative diseases in the Vigevano and Lomellina area led to the establishment of the "Diagnosis and Disease Service" in 2012-2013. This project was defined "a Multifunctional Dementia Centre" was organized according to the guidelines of the National Prevention Plan 2010-2012 promoted by the Ministry of Health, which proposed to undertake a set of activities to improve the quality of Care and overcome territorial inequalities. The final aim was to collect socio-demographic and health's data form the elderly population of Vigevano.

Information were obtained about the patients exploiting the service during the first year. The mean age of about 750 subjects involved in the Centre was 81 years. Of these patients, 410 did not have a diagnostic indication, although there were among these 218 people who were subsequently diagnosed with dementia. Although in many cases the clinical diagnosis was dementia, for 410 patients this was the first neurological specialist visit since the beginning of the illness. Considering the entire sample, 336 patients never performed a neuro-radiological examination.

This preliminary data reflect the social and clinical situation of a population sample that had no access for a long time to a service dedicated to dementia diagnosis and treatment.

Aims and Methods

Aims

The present study is part of a previous project in development, and aims to evaluate the differences between the patients referred to a newly established MC located in the Lomellina area (the "Multifunctional Dementia Centre") and those referred to the larger MC in IRCCS Mondino in Pavia, a neurological with a very wide catchment area that has been operational for several years.

Our primary endpoint is the differences in the diagnostic categories of dementia between the two groups.

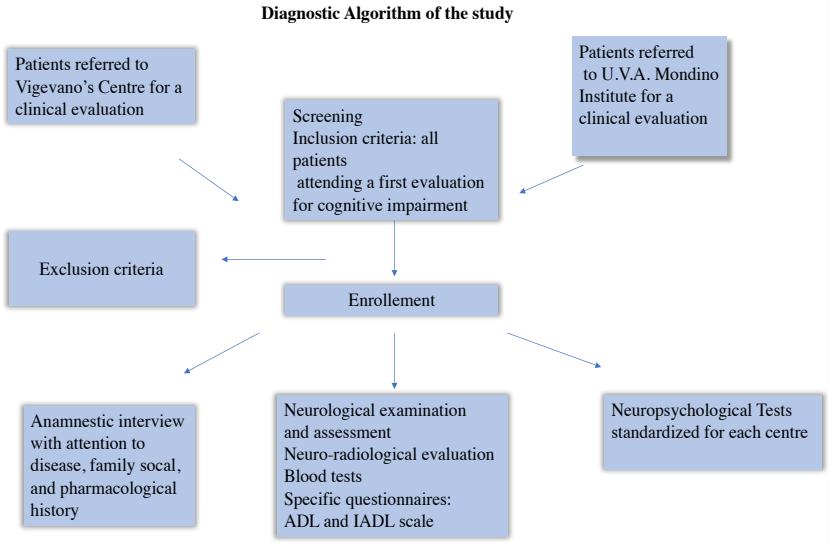
Secondary endpoints are the differences in neuropsychological profiles, dementia severity, comorbidities and neuro-radiological pattern between the two groups.

This data have the final goal to assess the current situation in the Lomellina area, providing evidence that could support a long term impact of the Vigevano AEU in the long term.

Methods

This was a two years multicentre observational prospective study of patients attending C. Mondino National Institute of Neurology Foundation IRCSS MC (Pavia) and and the Multifunctional Dementia Centre (Vigevano). This study was approved by Ethic committee (IRCCS San Raffele, Milan, and IRCCS San Matteo, Pavia) and IRCCS San Matteo.

Type of study: prospective observational multi-centric study.



Patients

We enrolled all patients referred for a first neurological evaluation at Pavia's MC (210 patients) and at Vigevano MC (441 patients) from 20th July 2015 to 30th July 2017.

Inclusion criteria and data collection procedure

All patients attending a first evaluation for cognitive impairment were screened as potential cases and underwent a selection visit. The patients willing to provide their informed consent were then enrolled in the study. If the patients were incapable of providing their informed consent a family member or the support administrator consent was required (in this case, the data collection was immediately anonymised).

Exclusion criteria were:

- presence of a disease potentially associated with cognitive impairment such as brain tumour, autoimmune disease, infectious disease and metabolic disorders
- impossibility to provide an informed consent and absence of a legal guardian.

The following procedure was used to collect the data in a dedicated case report form (CRF):

Anamnestic interview comprehensive of:

Disease history: the past clinical records of the patient were collected from discharge letters and outpatients evaluation. Relevant information included history of current, chronic and past illness, hospitalizations, previous surgeries, accidents or injuries (particularly head trauma), infectious diseases. Particular relevance had the investigation regarding dismetabolic diseases such as carotid stenosis (<50%) - moderate (50-70%) severe (> 70%) / BPCO / diabetes mellitus / Atrial fibrillation / Hyperthyroidism / Autoimmune diseases / Hypertensive ischemic heart disease and hypertension.

Family history: exploring the possibility of heredo-familial disorders focusing on the patient's lineage.

Social history: including patient's age, marital status, educational level, occupation and personal habits.

Pharmacological history: current and past medications, pharmacologic allergies, sedative load and anticholinergic load.

Sedative load definition: The sedative load (SL) was calculated for each subject according to a published model (Allegri et al., 2017). Medications were classified as follows: Group 1, primary sedatives; Group 2, medications with sedation as a prominent side effect or preparations with a sedating component; Group 3, medications with sedation as a potential adverse effect; Group 4, all the other medications with no known sedative properties.

Anticholinergic load definition: The AL was calculated according to the scoring system of the Anticholinergic Drug Scale. On the Anticholinergic Drug Scale, drugs are rated from 0 to 3, with level zero indicating no anticholinergic activity, and level 3 indicating an elevated anticholinergic activity. The anticholinergic scores relative to each drug taken by the patient were summed up to obtain the total score.

Neurological examination: the physician evaluation included mental status, cranial nerves, muscle tone and strength, sensory functions, deep tendon reflexes, coordination, gait and walking.

Neuropsychological assessment: this was performed by a trained psychologist at the Pavia-Vigevano Neuropsychological Center or at the Alzheimer Unit of the IRCCS Foundation Casimiro Mondino, consisted of an analysis of the cognitive and affective behavioral items and was divided in two moments:

- Anamnestic interview aimed to explore both cognitive difficulties reported by the patient and the main relevant details regarding the socio-family context and the life history.

- A psychometric evaluation during which all patients underwent a specific neuropsychological examination. Although most neuropsychological tests were similar, some differences in the clinical practice between the two centers could not be avoided.

The neuropsychological tests were:

Mini Mental State Examination (MMSE): is a brief 30 – item test, witch measures orientation to time and place, immediate recall, short-term memory, calculation, language and constructive ability. The maximum score is 30, scores below 24 suggest the presence of cognitive impairment.

Attentive atrices: tool for assessing selective attention skills. Score ranges from 0 to 60. Scores below 36 are specific for a selective attention loss.

Trial Making Test: tool for assessing sustained and divided attention skills. The score's difference is evaluated on the time taken to complete the two test forms A and B. A score greater than 93 in form A is indicative of difficulty in sustained attention. A score greater than 283 in form B is indicative of difficulty in the split attention.

Raven's progressive Matrices a tool for evaluating logic-deductive skills on a visual-spatial basis. Score ranges from 0 to 36. Scores lower than 18.6 are indicative of cognitive impairment.

Verbal fluency test (categorical and phonemic): tool for evaluating the ability to access semantic and phonemic basis. The score haven't a maximum limit. Scores lower than 17 and less than 24, respectively for phonemic and semantic fluidity are indicative of cognitive impairment.

Digit Span: tool for the evaluation of short-term auditory memory capacity. Score ranges from 0 to 9. Scores below 3.50 are indicative of cognitive impairment.

Corsi Blocking Tapping Task: tool for evaluating short-term and visuo-spatial memory abilities. The score varies from 0 to 9. Scores below 3.25 are indicative of cognitive impairment.

Story recall test: tool designed to evaluate verbal long term episodic memory. The score varies from 0 to 28. Scores lower than 7.50 are indicative of cognitive impairment.

Constructional Apraxia Test: tool designed to evaluate the capacity of constructive apraxia. Score ranges from 0 to 14. Scores below 7.75 are indicative of cognitive impairment.

Frontal Assessment Battery: tool for evaluating frontal and executive capabilities. The score varies from 0 to 18. Scores - lower than 13.4 are indicative of cognitive impairment.

Neuro-radiological evaluation (TC scan and/or MRI, and when indicated positron emission thomography-PET)

Blood tests including: blood cell count, full lipid profile, glycemia, glycosylated hemoglobin, omocysteine, vitamin B12, folate, thyroid function, liver and kidney function.

(Laboratory and neuro-radiological data performed according to the general practitioner prescription, were considered useful for our study and collected only if performed within three months prior to the first visit.)

Specific questionnaires:

ADL (Activities of daily living): investigates the tasks required to independently begin the day in the morning such as, bathing, dressing, toileting, brushing teeth, eating.

IADL (Instrumental activities of daily living): investigates the tasks required during the day and that are required to lead an independent life style, such as cooking, driving, using the telephone or computer, shopping, keeping track of finances, managing medication.

The data collected for each patient during the visits were included in a database built for the purposes of the study. Each patient was assigned, in addition to an identification code, a number that expressed the Centre's belonging.

Statistical Analysis

The collected data were included in a database built through Excel for Windows. Statistical analysis, performed using the SPSS 15.0 statistical package for Windows, were carried out in accordance with the objectives outlined above.

Categorical variables were compared using Fisher and Chi-Square test and continuous variable were analysed with T-test or ANOVA. If the normality assumption was not fulfilled the equivalent non-parametric tests were used. P-values <0.05 were considered significant.

Results

Demographic data

In the period between July 2015 and September 2017 we enrolled a total number of 651 patients, 210 from Pavia and 441 from Vigevano. Patients were predominantly females (63,9%), and the mean age was 78,54 (SD 9,07) (Table 1). No differences in gender were found between patients recruited in the two centers, but the two groups had a different social profile: patients form Pavia were more frequently married (54,8% versus 45,6%) and still involved in job activities and had a higher level of instruction (mean years of education: 7,18 versus 6,31 , p= 0.000). A possible explanation could be the older age of Vigevano's population (p 0,000).

Table 1: Demographic characteristics of the study population

Measures	***Vigevano***	***Pavia***	***Total***	
	(n = 441)	***(n = 210)***	***(n = 651)***	***P-value***
Sex, n (%)				
Male	159 (36,1)	76 (36,2)	235 (36,1)	0,973
Female	282 (63,9)	134 (63,8)	416 (63,9)	
Age mean (SD)	79,88 (8,64)	75,71 (9,31)	78,54 (9,07)	0,000

Length of education in years, mean (SD)	5,88 (3,17)	6,91 (2,95)	6,20 (3,14)	**0,000**
Length of Retirement in years, mean (SD)	21,61 (10,86)	18,69 (8,07)	20,45 (10,15)	**0,008**
Marital Status n (%)	201 (45,6)	115 (54,8)	316 (48,5)	
Married	201 (45,6)	115 (54,8)	316 (48,5)	0,028
Widowed	240 (54,4)	95 (45,2)	335 (51,1)	

Clinical data

We regrouped the different type of dementia by etiopathogenetic affinity and we created 8 different groups; then we analysed the diagnostic differences between the two populations. In particular we divided populations in: first group including AD patients; the second including vascular dementia's patients; the third including mixed form of different type of dementia; the fifth with Fronto-temporal disease; the one named "secondary dementia" including Parkinson disease and Parkinsonism, Hydrocephalus, Pseudo-depressive dementia and other type of dementia; the group including MCI single or multiple domain; one group including a non specified cognitive impairment and the last one including healthy patients (Fig. 1)

In Vigevano's group there was prevalence of patients affected by vascular dementia (8.6% versus 0,3%; p 0,000), non specified cognitive impairment (24% versus 16,2%; p 0,000) and MCI (15% versus 6%; p 0,000). In the Pavia's group we found a prevalence

of mixed type dementia (9,7% versus 6,6%; p 0,000) and secondary dementia (9% versus 4,1%; p 0,000). This difference could be explained in a long term follow-up absence for Vigevano's patients. We did not find a significant difference in patients with an AD diagnosis (15,4% versus 15,7%; p 0,000). Patients with mixed type and secondary dementia have, on the contrary, been numerically higher in Pavia. The data, not far from the one expected, could be attributed to the possibility of an effective primary prevention and early treatment (Fig. 2)

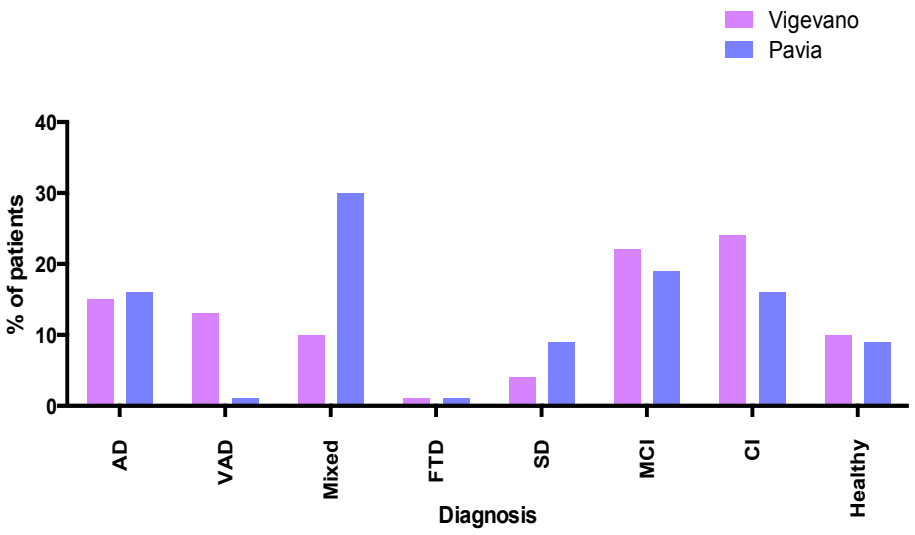


Fig.1 Comparison between different type of diagnosis among the two population

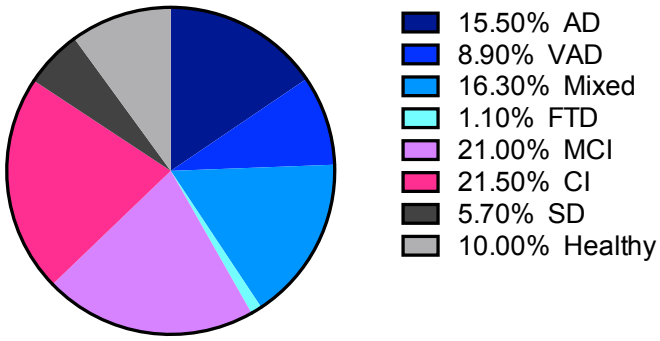


Fig 2. Distribution of different type of diagnosis among the two population

Comorbidities

During the observational study we investigated the major comorbidities affecting the patients (Table. 3) The most common was hypertension, which was present in 67,3% of the population, immediately followed by hypercholesterolemia (32.3%) and diabetes (21.4%). Other relevant comorbidities included cardiopathy (16.6%), atrial fibrillation (14.4%), carotid atheromasia (15.2%) and BPCO (10.4%). The remaining comorbidities such as autoimmune disease or hypertriglyceridemia were found in <5% of patients.

Comparing the two centres, patients form Vigevano showed higher prevalence of comorbidities, being more frequently affected by hypertension (p 0,006) atrial fibrillation (p 0,001), carotid atheromasia (p 0,001), chronic bronchitis (p 0,000), thyroid disease (p 0,000).

We also collected data regarding psychiatric manifestation, that could be interpreted both as a possible manifestation of the neurological disease or as comorbidities. Anxiety, depression and insomnia were commonly found, and were more frequent in patients from Vigevano (respectively p=0,003, p=0,000 and p=0,000). In addition, patients from

Vigevano presented more often with hallucinations (p=0.005) and eating disorder (p 0,000), whilst euphoria and disinhibition were more frequent in patient's form Pavia.

Table 2. Comparison of psychiatric manifestations measures among two populations

Measures ***N (%)***	***Vigevano*** ***(n = 441)***	***Pavia*** ***(n = 210)***	***Total*** ***(n = 651)***	***p value***
Delirium	41 (9,3)	10 (4,8)	51(7,8)	0,044
Hallucinations	42(9,5)	7 (3,3)	49 (7,5)	**0,005**
Psyco-motor agitation	83 (18,8)	28 (13,3)	111(17)	0,082
Depression	217 (49,2)	42 (20)	259 (39,8)	**0,000**
Anxiety	157 (35,6)	50 (23,8)	207 (31,8)	**0,003**
Euphoria	5 (1,1)	5 (2,4)	10 (1,5)	0,226
Apathy	90 (20,4)	31 (14,8)	121(18,6)	0,083
Disinhibition	25 (5,7)	20 (9,5)	45 (6,9)	0,070
Irritability	100 (22,7)	37 (17,6)	137 (21)	0,139
Motor Activity	25 (5,7)	4 (1,9)	29 (4,5)	0,030
Insomnia	142 (32,2)	28 (13,3)	170 (26,1)	**0,000**
Eating Disorder	66 (15)	3 (1,4)	69 (10,6)	**0,000**

Neuro-radiological data

Data on brain imaging were collected directly from the images or, when unavailable, from the report. Both MRI and CT scan data were considered in the analysis. The presence of cerebral atrophy was found in almost half of the patients (40.6%), and in most cases was diffuse (33,6%). The second most common finding was cerebral vasculopathy (37,9% of patients). Patients from Vigevano had a higher prevalence of neuro-radiological alterations compared to patients from Pavia (p=0.000). One possible explanation could depend on the neuro-radiological data's absence in the Vigevano's groups (Table 3).

Table 3. Comparison of comorbidities and neuro-radiological measures among two populations

Measures	***Vigevano***	***Pavia***	***Total***	***p value***
N (%)	***(n = 441)***	***(n = 210)***	***(n = 651)***	
Hypertension	312 (70,7)	126 (60)	438 (67,3)	**0,006**
Hypercolesterolemia	149 (33,8)	61 (29)	210 (32,3)	0,227
Atrial Fibrillation	78 (17,7)	16 (7,6)	94 (14,4)	**0,001**
Cardiopathy	78 (17,7)	30 (14,3)	108 (16,6)	0,275
Carotid Atheromasia	81 (18,4)	18 (8,6)	99 (15,2)	**0,001**

BPCO	59 (13,4)	9 (4,3)	68 (10,4)	**0,000**
Diabetes	105 (23,8)	34 (16,2)	139 (21,4)	0,027
Autoimmune Disease	4 (0,9)	5 (2,4)	9 (1,4)	0,132
Hypertrigliceridemia	21 (4,8)	5 (2,4)	26 (4)	0,147
Cerebral Vasculopathy	110 (24,9)	137 (65,2)	247 (37,9)	**0,000**
Infarctual lesion	89 (20,2)	38 (18,1)	127 (19,5)	**0,000**
Focal lesions	242 (54,9)	35 (12,6)	277 (42,5)	**0,000**
Cerebral Atrophy	122 (27,7%)	142 (67,6%)	264 (40,6%)	**0,000**
Selective	8 (1,4)	70 (12,7)	78 (14,1)	**0,000**
Diffuse	114 (30,2)	72 (40,2)	186 (33,6)	

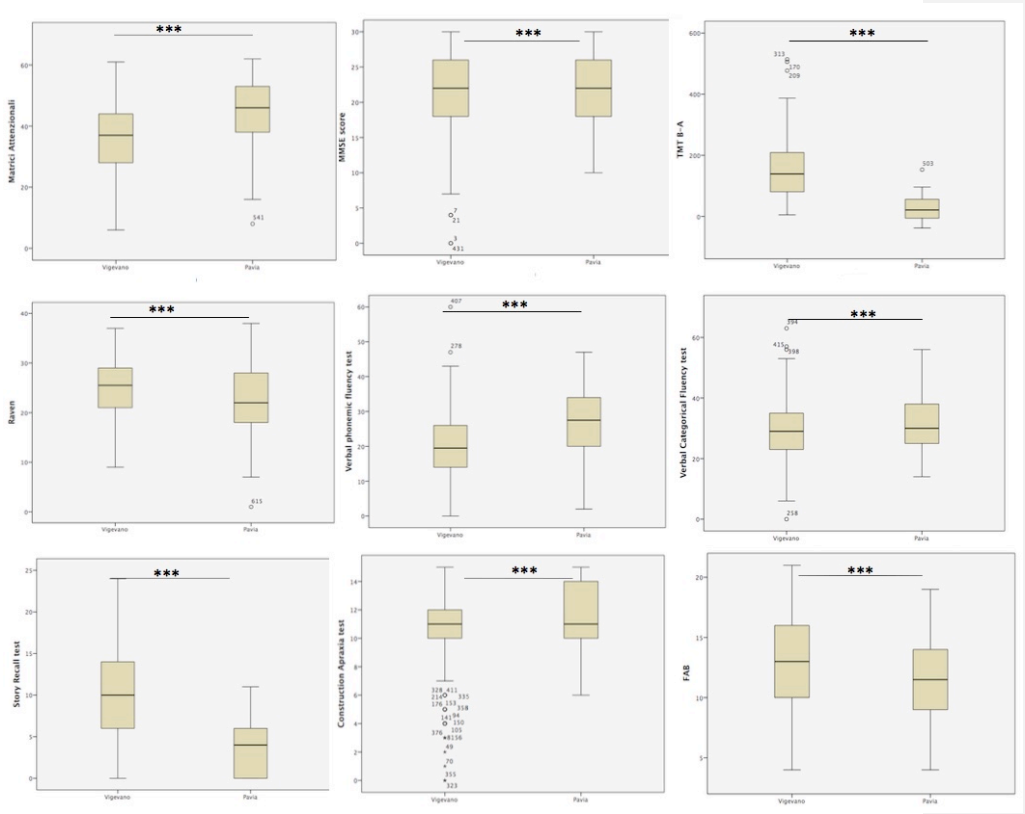
Neuropsychological profile and disability

We analysed also the neuropsychological difference between population's scores and we didn't find a significant difference in MMSE (mini mental state Examination). Vigevano's MMSE score was 22,06 and Pavia's one was 22,05. This difference may be caused by the older and more poli-pathological Vigevano' population but no longer compromised as regard the cognitive impairment. To better understand the population's difference in terms of cognitive domains, we performed a specific neuropsychological battery standardized for each centres. We found a significant differences between the two groups. Vigevano patient's showed a lower score in Attentive matrices (p 0,000), trail making Test B (p 0,000) and trail making Test A-B (p 0,000), Raven's progressive matrices (p 0,001), Token and Boston tests (p 0,000), verbal phonemic fluency test (p 0,000), digit span (p 0,000) and story recall test (0,000). The fact we did not find a MMSE score's difference was refuted by the group's neuropsychological domain's evaluation; the results showed how Vigevano's group had a more significant cognitive difficulties in the attentive-executive domain. The Pavia's sample resulted to have a short-term memory scores significantly lower than the other group (Table 4).

Table 4. Comparison of Neuropsychological scores among the two populations

Measures	*Vigevano (n =441)*	*Pavia (n = 210)*	*P value*
MMSE, Median (IQR)	22 (8)	22 (8)	0,689
Matrices Test, Median (IQR)	37 (16)	46 (15)	**0,000**
Trail Making Test B-A, Median (IQR)	139 (130)	21 (67)	**0,000**
Raven's progressive Matrix, Median (IQR)	25,05 (8)	22 (10)	**0,002**
Verbal categorical fluency test, Median (IQR)	19 (12)	27,50 (14)	0,187
Verbal phonemich fluency test, Median (IQR)	29 (12)	30 (13)	**0,000**
Corsi blocking tapping test, Median (IQR)	4,29 (1,08)	4,02 (0,84)	0,013
Story Recall Test, Median (IQR)	10 (8)	4 (6)	**0,000**
Constructional Apraxia Test, Median (IQR)	11 (3)	11 (4)	0,879
Frontal Assessment battery, Median (IQR)	13 (6)	11,5 (5)	0,834

Figure 3. Comparison of Neuropsychological significativity scores among the two populations



Daily and instrumental activities

As regard the functional activity in particular daily and instrumental activity (measured with specific ADL (Activity of daily Living) and IADL (Instrumental daily Activity) scores we found out that Vigevano's patients showed a lower scores than Pavia's one (p 0,000) (Table 5). This situation could be explained by the higher number of comorbidities

affected Vigevano's population that involved both personal and motor autonomy. The main reason could be the older age and the greater comorbidities number.

Disability was measured as well through questionnaires administered to the caregivers. We used the "Caregiver burden inventory" which was a self-administered test made of 24-item multi-dimensional questionnaire measuring caregiver burden with 5 subscales; all of the scores on the 24-item scale are summed and a total score > 36 indicates a risk of "burning out" whereas scores near or slightly above 24 indicate a need to seek some form of respite care. We found that caregivers from Vigevano presented a higher social burden(76,1%), emotional burden (75,8%) and physical burden (75%) compared to patients from Pavia (p 0,000). Vigevano's sample showed as well a significantly more compromised neurological examination than the Pavia's one; this difference could be explained both for the older patients than the older and more patients' comorbidities.

Table 5. Different GDS, ADL and IADL and Barthel scores

Measures	***Vigevano***	***Pavia***	
Mean (sd)	***(n= 441)***	***(n = 210)***	***P value***
GDS	6,06 (3,87)	5,43 (3,01)	0,128
ADL	3,57 (2,02)	5,58 (0,77)	**0,000**
IADL	2,94 (2,61)	5,55 (2,33)	**0,000**
Barthel scale	71 (25.96)	100 (-)	0,126

Drug load and caregiver's burden

We compared the population in term of correct drug prescription that is, in the elderly, a difficult task that requires careful survey of the current pharmacological therapies. In particular were evaluated the sedative and anticholinergic load (SL and AL, respectively), and the number of drugs and cholinesterase inhibitors taken by each patients (Table 6). We found out that Vigevano's patients (67%) were more medicated than Pavia's ones (32,3%); there was only 2 patients in Vigevano's group with a cholinesterase inhibitor's prescription (0,5%) and 14 in Pavia's group (6,7%). The sedative and anticholinergic load were higher (p 0,000) in Vigevano's group (67,6%) than in Pavia's one (32,4%).

Table 6. Drugs, Sedative and Anticholinergic load in the two study population (n = 651)

Measures ***N (%)***	***Vigevano*** ***(n= 441)***	***Pavia*** ***(n = 210)***	***P value***
Drugs	10 (67)	3 (2)	0,108
Sedative Load	1 (2)	1 (1)	**0,000**
Anticholinergic Load	0 (1)	0 (1)	**0,006**
Cholinesterase inhibitors	0 (0)	0 (1)	**0,000**

Table 7. Different diagnosis observed in the study population (n = 651)

Measures	*Vigevano (n = 441)*	*Pavia (n = 210*	*Total (n = 651)*	*Statistics p value*
Diagnosis n (%)				
AD	68 (15.4)	33 (15,7)	101 (15,5)	**0,000**
Vascular Dementia	56 (12,7)	2 (1)	58 (9)	**0,000**
Mixed Form	43 (9,8)	63 (30,9)	106 (16,3)	**0,000**
Secondary Dementia	18 (4,1)	19 (9)	37 (5,7)	**0,000**
Healthy patients	46 (10,4)	19 (9,3)	65 (10)	**0,000**
Cognitive impairment non specified	106 (24)	34 (16,2)	140 (21,5%)	**0,000**
Secondary Dementia	24 (5,4)	20 (9,8)	44 (1,9)	**0,000**
MCI	98 (22,2)	39 (18,6)	137 (21)	**0,000**
FTD	6 (1,4)	1 (0,2)	7 (1,1)	**0,000**

Conclusion

General objective of this research project is to assess on the Lomellina's territory the impact of a Clinical Center for early diagnosis for neurodegenerative disease evaluating the statistical and epidemiological differences among the demographic, socio-cultural and clinical features of the patient coming up to our centres for the first visit.

We paid as well attention to the characteristics of caregivers to assess the burden associated with mental and physical care of a family member, the socio-demographic characteristics and knowledge of the disease.

Given the limited size of the sample available to us after these two years of observation, we can say that the patients referred to Vigevano's Centre came to our attention in their old age (average 76) often with remarkable neurodegenerative disease, and more frequently of vascular aetiology, also considered the wide range of associated comorbidities. This result could be confirmed by some studies that explain how the prevalence of mixed dementia, defined as the coexistence of Alzheimer disease (AD) and vascular dementia (VaD), was likely to increase as the population ages (Lama et al, 2004). They found that cardiovascular risk factor control, especially for hypertension and hyperlipidemia, as well as other interventions to prevent recurrent stroke, could represent important strategies for preventing or slowing the progression of mixed dementia. Our population was older and presented a higher number of comorbidities. In the light of these founding could be essential the prevention and treatment of vascular risk factors, and particularly hypertension (Helemr et al 2004) which had an higher prevalence in Vigevano's population (67,3%). All this data, that is not different from what we observed and expected, data that confirmed how the absence of a suitable Memory clinic in the Lomellina's territory resulted in the lack of people's awareness of the neurodegenerative diseases but also with subsequent poor attention to early symptoms and therefore reduced chance of early detection. At the first visit, in fact, patients showed mild to moderate cognitive impairment.

By comparing data collected in the centres, through a specific questionnaire, we also detected the lack of knowledge of the disease by caregivers who are not adequately informed about the burden of care that such a disorder as dementia involves, resulting in high psychophysical stress. This situation, is slightly higher as to the caregiver related to the Vigevano's Centre confirming how low the awareness of the population in the area is, probably because of the absence of a specialized Centre there for so long. In practice, up to now our data reflected the social and clinic situation of a portion of population unable to take advantage of a service primarily dedicated to the diagnosis and treatment for dementia for a long time.

In the study of Andren et al (2008) is explained that the family caregivers experienced moderate burden especially in terms of isolation, disappointment and emotional involvement especially in rural areas as proved in this study as well.

As regard the patient's characteristics the obtained data showed a significant difference in autonomy in everyday and instrumental activities. Pavia's patients were more autonomous than those of Vigevano (p. 0,00). We also found a significant difference among the two groups for the numbers of taken drugs. Pavia's patients take less medication (T-test p 0,00). We find, as well, a significant difference in sedative and anticholinergic load (T-test 0,000 /p. 0,000). Vigevano's patients showed a higher sedative and anticholinergic load. This data could confirm the necessity of a selected medical and clinical interventions, aims to guarantee a better quality of prescriptions (Cherubini et al), not only for anti-dementia drugs but also for drugs prescription in general (Allegri et al).

Finally we compared the different diagnostic groups in the two centers and we found a significant difference in diagnosis between the two group, in particular for the diagnosis of vascular dementia, unspecific cognitive impairment and MCI. In literature it was explained that vascular risk factors and cerebrovascular disease could be the common causes of dementia. The authors underlined the importance of sharing risk factors for vascular dementia and Alzheimer's disease, as well as frequent coexistence of these pathologies in cognitively impaired older people and suggested convergence of the

aetiology, prevention and management of the commonest dementias affecting older people (Etherton-Beer CD's review 2014). Prevention remains the cornerstone of management the diagnosis of brain vascular disease and is crucial because of the potential to improve clinical outcomes through clear diagnosis, enhanced control of risk factors, lifestyle interventions and secondary prevention. May also specific pharmacological intervention be indicated for some patients with cognitive impairment and cerebrovascular disease. In our study we compare two different clinical centre, one settled in Pavia's area for a long time and one recently born in the Lomellina's area, that show different features in term of demographic, social and diagnosis data.

These differences are supported by a study comparing patient characteristics and family perceptions of patient's function at urban and rural memory disorder clinics (Wackerbarth et al 2014). The authors highlighted that the urban family members reported more memory problems, twice as many personality changes and more frequent behaviour problems or more adverse reactions to problems. A possible explanation was that clinic urban patient's were significantly more likely to be living in a facility and more educated than the typical rural ones.

I think that it was important to emphasize as well the geographical variation between the two areas. In particular we found a group's variation in geographical variance; the rural living could be associated with an increased risk of Alzheimer disease, there is a suggestion that early life rural living further increases this risk (Russ et al 2012). In our "rural" population we observed an increase number of vascular dementia, unspecified cognitive impairment and mild cognitive impairment's diagnosis than Alzheimer disease's diagnosis.

In the study, I pointed out the importance of a preventive diagnosis of neurodegenerative disease first of all by understanding genetic, biological, and symptomatic heterogeneity underlying Alzheimer's disease; this surely requires a deep understanding of mechanisms affecting complex brain systems and request a specific unit such as a Memory Clinic. I'd like to highlight as well, in term of early diagnosis, the potential Neuroimaging role. Functional MRI could be a powerful way to analyse and characterize intermediate

biological phenotypes of AD (Chiesa et al. 2017). Neuroimaging specific techniques could be useful to describe the differential effect of genetic risk factors for AD on brain functional connectivity in cognitively normal, preclinical, prodromal, and AD dementia individuals. Functional neuroimaging holds particular promise for the characterization of preclinical populations in the neurodegenerative diseases.

In previous studies, age, educational level cardiovascular comorbidity and familiar predisposition are risk factors to dementia. Our study supported these data: the majority of patients were over 76 years, they were diagnosed with Alzheimer's disease, vascular dementia, mild cognitive impairment or mixed form and they lived in urban-rural areas. In the literature female gender has been associated with increased risk of development of Alzheimer's disease (6,7). Of the total 650 patients with all dementia forms recorded, 416 patients were females and a statistical difference was observed among genders regarding dementia's forms. To assess the functional, cognitive, and behavioural status of the patients we used the MMSE, ADL and IADL scale, and a complete neuropsychological tests battery for each centre. Even if the MMSE reflects the main cognitive domains affected in dementia, additional scales are important for screening domains that are less assessed by the MMSE. We performed: Matrix test, Trial Making Test, Raven's progressive Matrix, Verbal fluency test (categorical and phonemic), Digit Span, Corsi Blocking Tapping Task, Story recall test, Constructional Apraxia Test, Frontal Assessment Battery. We found significant differences as regard the attentive-executive domain in witch Vigevano's group showed a worst performance. Neuropsychiatric symptoms are as well common in dementias. As our study displayed, a large number of patients presented hallucinations, psycho-motor agitation, depression, anxiety, apathy, irritability, insomnia and eat disorders.

A large proportion of the enrolled patients was newly diagnosed. According to the MMSE and Neuropsychological screening 15,7% of patients displayed Alzheimer disease, 9% vascular dementia, 16,4% mixed form, 0,8% Parkinson disease, 1,2% Lewy Body disease, 1,1% hydrocephalous, 20% Mild cognitive impairment single or multiple domain, 19% unspecified cognitive impairment and 11% of healthy subjects. Overall

prevalence of MCI among elderly is high; of those a notable percentage will convert to AD at an accelerated rate. Today there is a trend towards early diagnosis and treatment, towards altering progression of MCI. Patients and caregivers though abstain from confirming the existence of dementia and many physicians (i.e., general practitioners and specialists) overlook symptoms of MCI attributing them to normal aging manifestations . Comparing the diagnosis we noticed some differences between the groups: in the Lomellina's area dementia was diagnosed in a later stage of the disease in comparison to the Pavia's area, which may lead to negative effects in altering the progression of the disease itself as well as to the general well-being of patients. A higher number of comorbidities were evident in Lomellina's group patients, which was expected and probably due to certain inefficiencies of the health care system in this area. Some previous study showed that rural population is characterized as being less healthy overall in comparison to urban residents with a higher rate of hospitalization, for a range of causes, probably related to the often limited and delayed access to health care services. In Tountas et al study rural patients' contacts with health care professionals were less than urban residents' especially among the less educated. In our study, on the contrary, the larger part of patients had an 20 years schooling in both population.

The scope of this study was to assess the demographic and clinical characteristics of patients referring to a new centre compared to the old Pavia's one and to describe the differences in care management of dementia between the population's area. We found some differences (described before) that could support the importance of a growing need for interventions regarding the provision of services to patients with dementia and a growing need for the implementation of strategies to raise the awareness of the disease and to develop structures and capacities to manage the growing burden of dementia in Lomellina's area. We can suggest some future issues, such as a reinforcement of community support services and housing options accessible to dementia patients and caregivers or the creation of a even more efficient and personalized healthcare plan for patients with dementia and high risk population living in the Lomellina's area. In my opinion there are insufficiencies in specialists' care too to make an early diagnosis of

dementia in order to prevent or slow the transition in more severe forms of the disease. In the light of this findings, I believe in the importance of an improvement in health care delivery in Lomellina's areas and health care professionals' training as well as caregivers' education for a proper treatment of dementias; all these strategies aim to reach a substantial quality of life and general wellbeing among the elderly habitants of this area.

Role of the Neuroimaging in diagnosis of neurodegenerative diseases

As regard Neurodegenerative disease, neuroimaging with its various structural and functional modalities has provided evidence of neurobiological changes across the trajectory of normal ageing – MCI – dementia, and AD in particular (Chui, 2007). Structural MRI studies have identified the key areas of atrophy: the medial temporal lobe, reflecting entorhinal and hippocampal volume loss, and the posterior cingulate (Benisty et al., 2008). Furthermore, longitudinal studies have shown that acceleration of the annual rate of hippocampal atrophy as well as rates of cortical atrophy and ventricular expansion are good predictors of AD progression in MCI subjects (Small, 1985). However, a recent review of previous studies on the progression towards AD in MCI subjects revealed accuracy figures between 56% and 82% (Reichard, 2009). FDG PET studies have shown substantial impairment in temporal-parietal and posterior cingulate association cortices in MCI subjects who progress rapidly to dementia, and AD in particular (Neary & Snowden, 2002).Using amyloid-labelling ligands, such as the most popular C-11 Pittsburgh compound B, PET enables molecular imaging of regional cerebral patterns of amyloid pathology and has shown increased Aβ burden in progressive MCI, particularly in the lateral frontal cortex, posterior cingulate cortex, regions of the medial and lateral parietal lobe, and the lateral temporal lobe (McKhann et al., 2001). Both MRI and FDG PET are surrogate markers of neuronal degeneration in a model of temporal evolution of disease-specific pathology proposed by Jack *et al* (Arvanitakis, 2010). According to this hypothesis, amyloid biomarkers show abnormalities earlier than markers for neuronal degeneration, possibly 10–20 years before first symptom occurrence. In comparative studies in which different biomarkers were combined in a prediction model of MCI, FDG-PET together with episodic memory test was a strong predictor of the clinical transition to AD, whereas CSF biomarkers primarily reflected rate of longitudinal cognitive decline independent of disease severity (Coyle-gilchrist et al., 2016). In logistic

regression models combining clinical information with MRI imaging, CSF proteins and FDG PET, the latter added the most prognostic information (Graff-Radford & Woodruff, 2007).

The Theory Behind Magnetic Resonance Imaging

Introduction

When searching for the scientific discoveries that paved the way for functional magnetic resonance imaging to become the paramount neuroimaging technique today, scientists start with 1931. It is in this year that Isidor Isaac Rabi, a Polish American physics assistant professor at Columbia University, demonstrated that the nuclei of atoms displayed magnetic properties (Rabi, Zacharias, Millman, & Kusch, 1938). He succeeded in flipping the nuclei of the atoms immersed in a magnetic field with the use of a radio wave signal. He was awarded the Nobel Prize in physics in 1944 for his discovery of different elements' magnetic resonance properties. In 1946 Felix Bloch of Stanford University won the Nobel Prize for theorizing that any spinning charged particle, such as a proton, creates an electromagnetic field (Mackinnon et al., 2004). For 30 years magnetic resonance was used in chemistry to study magnetic properties of 'elements' nuclei. We have to fast for- ward to 1980 to admire the first commercial magnetic resonance imaging (MRI) scanner and jump ahead another 10 years, to the 1990s, to observe the first functional magnetic resonance image. The latter discovery was accredited to Seiji Ogawa, a physicist working in Bell Laboratories in New Jersey. He observed that oxy- gen-rich hemoglobin and oxygen-poor hemoglobin displayed different magnetic resonance properties and thus behaved differently from one another (Hattox & Nelson, 2007). He was able to utilize this intrinsic contrast in order to map which regions of the brain were more active than others, thus enabling functional magnetic resonance imaging (fMRI). This brief historic overview, far from exhaustive, shows that it took about fifty years to refine the theory behind nuclear magnetic resonance and build a

working scanner that was able to use the theoretical implications of magnetic dipole moment and nuclear spin momentum to obtain a reliable signal and convert it to a comprehensible image. While there exist a wide variety of different techniques to obtain an image of the anatomy of internal organs of the human body (e.g. X-ray, Computer Tomography scan, etc.), each one with its advantages and drawbacks, the MRI technique provides the operator with the possibility to tweak and adjust many parameters that in turn allow for an image flexibility that other imaging techniques lack. This flexibility coupled with its high spatial and temporal resolution and its non-invasive nature, in stark contrast to other methodologies that use radiation, with all the safety implications that this methodology requires, make the MRI the most used technique in both, the clinical and research, fields. It is with this introduction in mind that we will tackle the theory that lies at the heart of the nuclear magnetic resonance phenomenon.

Principles of Nuclear Magnetic Resonance (NMR)

Atoms are the building blocks of matter. Protons and neutrons together form the nucleus of an atom and elements differ from one another by the number of protons present in the nucleus. Protons are positively charged whereas neutrons carry no electrical charge. A spinning charged particle like a proton will generate an electromagnetic field and thus behave like a magnet in that it will have a North pole and a South pole. Additionally, each atomic nucleus will display different energy levels that can be derived by the spin quantum number S of that particular element. For example, Hydrogen has a spin quantum number S of 1⁄2.

Using the formula:

$$\#energy\ states = 2S+1 \quad (1)$$

we know that the Hydrogen proton has 2 energy states that we indicate with + 1⁄2 and – 1⁄2. The demonstration in a molecular beam laboratory that different elements had different spin quantum.

Precession and Larmor Frequency

When a human body is placed in a large magnetic field B_0, many of the free hydrogen nuclei align themselves with the direction of the magnetic field (Figure 1).

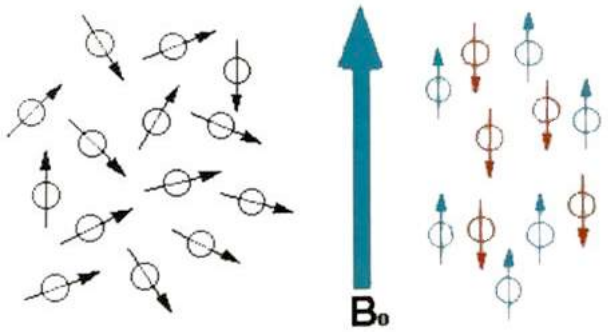


Figure 1. Visual representation of protons placed within an external magnetic field B0 that align either parallel or antiparallel to B0. Eventually, more and more protons, about 1 every million, will be in the low energy level state (i.e. MDM same direction of B0) resulting in a net magnetization vector M0, sum of all the μ, parallel to B0.

The nuclei precess about the magnetic field direction like gyroscopes. This behaviour is termed Larmor precession or ώ and is described by the following equation:

$$\dot{\omega}=\gamma B0 \quad (2)$$

where γ is the gyromagnetic ratio and is a nuclei specific constant. ώ is 42.6 MHz/Tesla for the hydrogen proton (Balling, L. C., & Pipkin, F. M., 1965). Thus, each proton μ will be slightly off and not be perfectly parallel to B0. This means that the magnetization vector M0, sum of all the magnetic dipole moments, will be the sum of two perpendicular components, Mz, also referred to as longitudinal magnetization, and Mxy, also referred to as transverse magnetization (Figure 2).

While all the protons precess with the same Larmor frequency, they tend to influence the each other relaxation. That's results in a mutual dephasing. As result, all the Mxy components of the M0 vector cancel each other out, leaving only the Mz component. This

means that if only the B0 field is applied, the value of the net magnetization vector M0 is equal to the value of its longitudinal component Mz.

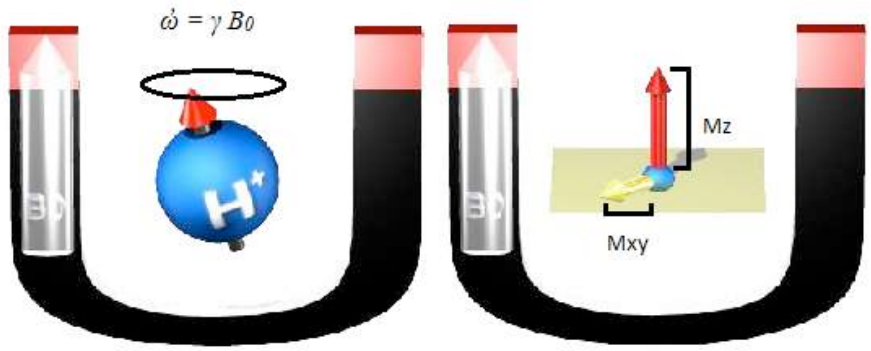


Figure 2. The longitudinal (M_z) and transverse component (M_{xy}) of the magnetization vector M. Image from https://www.imaios.com/en/e-Courses/e-MRI.

Radio Frequency Pulse

A radio frequency pulse (RF pulse) is an electromagnetic wave. If we transmit a radio frequency pulse, we are generating an oscillating magnetic field B_1. Setting the frequency of the RF pulse equal to the precession frequency of protons (i.e. the Larmor frequency) and transmitting the RF pulse perpendicular to B_0, will give rise to the nuclear magnetic resonance (NMR) phenomenon.

Two processes now begin simultaneously:

- First, phase coherence across all protons will increase, resulting in an increment of the transverse component, M_{xy}, of the magnetization vector M_0.
- Second, there will be an electromagnetic energy absorption by the atomic nuclei that will modify the spin equilibrium and tip protons from the low energy level state to

the high energy level state.

As more and more protons absorb electromagnetic energy and jump to the higher energy level state, the longitudinal component M_z of the magnetization vector M_0 disappears while the M_{xy} component reaches its maximum value (Fig 3). At this point, we observe a net magnetization vector M_0 that has spiralized down from the z-axis into the xy-plane and rotates at Larmor frequency. This is referred to as nutation.

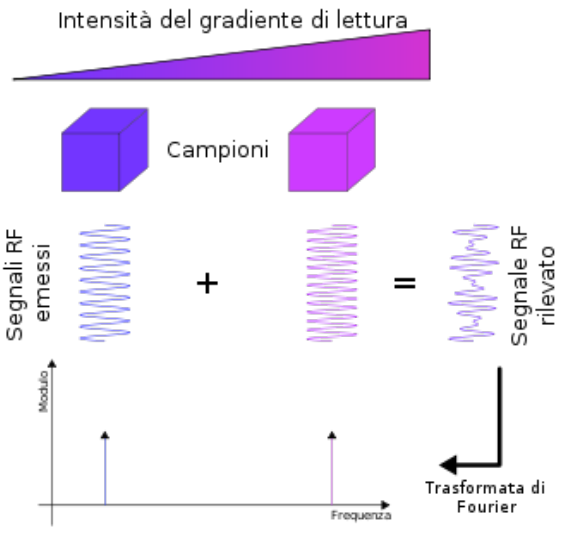


Figure 3.

As soon as the RF pulse is turned off, magnetic resonance ceases and the excitation period ends. The energy emission period follows the energy absorption period. Thus, the protons that had jumped to the higher energy level state now emit energy and relax back to the low energy level state. The energy emitted is the signal that a scanner picks up in a nuclear magnetic resonance (NMR) experiment.

Longitudinal and Transverse Relaxation: The FID signal

Two independent but simultaneous processes begin as soon as the RF pulse has been shut off: longitudinal relaxation (T1) and transverse relaxation (T2). Specifically, T1 represents the time constant after which the longitudinal component Mz of the magnetization vector M0 has recovered 63% (Figure 4) of its original value (i.e. before the RF pulse was transmitted). T1 is determined by spin-lattice interactions as protons give off energy to the surrounding lattice and return to their low energy level state. T1 values are tissue-specific and are directly proportional to external magnetic field strength. T2 represents instead the time constant after which the transverse component Mxy has lost 63% (Figure 5) of its original value (i.e. when RF pulse was on).

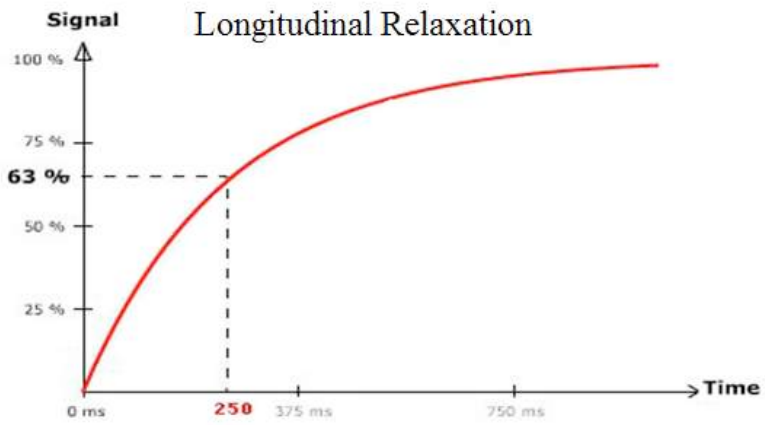


Figure 4. T1 relaxation time. Longitudinal signal strength as a function of time in ms

T2 is determined by spin-spin interactions, that is, protons' MDM interact with one another. This generates a mutual influence among protons that causes them to precess at different phases which consequently causes the M_{xy} component to return equal to zero.

T2 is also tissue specific. T2* is an adjusted value of T2 that takes into consideration field that also influence the decaying rate of the transverse component M_{xy}. T2* will always be shorter than T2 which will always be shorter than T1.

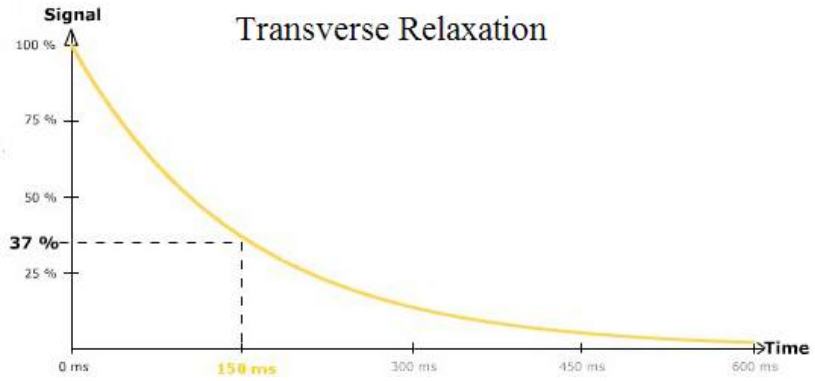


Figure 5. T2 relaxation time. Transverse signal strength as a function of time in ms.

Just like a spinning charged particle generates a magnetic field, a spinning magnetic field generates a current in a coil. The spinning magnetic field in this case is the net magnetization vector M_0 once the RF pulse has been applied to the B_0 field and the $M_z$component is equal to zero. This spinning magnetic field generates a current in an adjacent receiver coil. This current is referred to as the free induction decay (FID - Figure 6) signal and it is this signal that an MRI receiver coil measures.

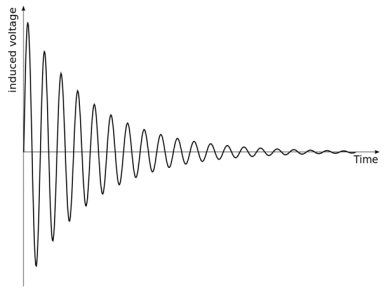


Figure 6. Free induction decay

Repetition Time and Echo Time

Different tissues have different T1. Thus, the rate of longitudinal recovery varies across tissues. Fat, grey matter, and white matter all have different T1. If we apply a second RF pulse while the longitudinal magnetization has not fully recovered, we will accentuate tissue differences according to the discrepancy between their T1. In a similar fashion, once we allow for the longitudinal component to fully recover, we can decide how much time after the RF pulse to sample our signal. Given that different tissues have different T2, our signal strength and thus our image will be influenced by our choice of the sampling interval. The time between each RF pulse is called the repetition time (TR). The delay between the emission of the RF pulse and the sampling time is the time echo (TE). By combining either long or short TR with either long or short TE, we will have different signals that will in turn yield different images featuring different contrasts (Figure 7).

Magnetic Field Gradient and Spatial Encoding

If the external field B0 is evenly applied, it will be impossible to determine signal location, because the MR signal would come from everywhere as all the protons resonate. In order to assign spatial information to the acquired signal, researchers turned to gradient coils. Because protons precess at Larmor frequency $\dot{\omega}$, which depends upon B0 strength, a variation of B0 results in a variation of protons precession rate. Field variation is

gradually applied using additional coils, called gradients, that produce a linear variation in magnetic field intensity in a direction in space. This variation in magnetic field intensity is added to the main magnetic field, which is far more powerful. The variation is produced by pairs of coils, placed in each spatial direction.

The direction of the magnetic field is not modified. By adding them to B0, a linear variation is produced in the total magnetic field amplitude, in the direction to which they are applied. Their action is considered as homogeneous on a plane perpendicular to the direction of application. This modifies resonance frequency, in proportion to the intensity of the magnetic field to which they are submitted (in accordance with Larmor's equation: the stronger the field, the faster they precess). This variation in Larmor frequency also causes a variation and dispersion of spin phases.

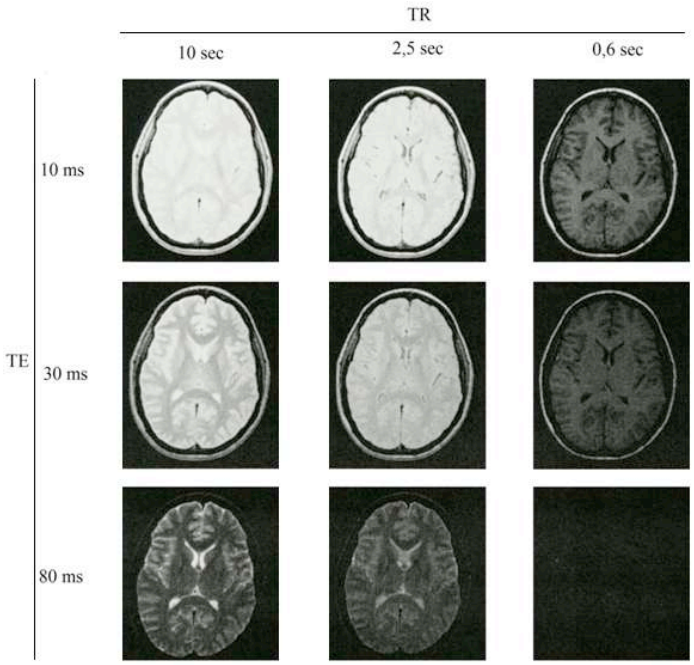


Figure 7. Effects of combining different time echo (TE) and time repetition (TR) on image output

Scan speed is dependent on performance of the gradient system. Stronger gradients allow for faster imaging, or for higher resolution; similarly, gradient systems capable of faster switching can also permit faster scanning. However, gradient performance is limited by safety concerns over nerve stimulation.

Some important characteristics of gradient amplifiers and gradient coils are slew rate and gradient strength. As mentioned earlier, a gradient coil will create an additional, linearly varying magnetic field that adds or subtracts from the main magnetic field. This additional magnetic field will have components in all 3 directions: x, y and z. However, only the component along the magnetic field (usually called the z-axis, hence denoted Gz) is useful for imaging. Along any given axis, the gradient will add to the magnetic field on one side of the zero position and subtract from it on the other side. Since the additional field is a gradient, it has units of gauss per centimeter or millitesla per meter (mT/m). High performance gradient coils used in MRI are typically capable of producing a gradient magnetic field of approximate 30 mT/m or higher for a 1.5 T MRI. The slew rate of a gradient system is a measure of how quickly the gradients can be ramped on or off. Typical higher performance gradients have a slew rate of up to 100–200 T·m−1·s−1. The slew rate depends both on the gradient coil (it takes more time to ramp up or down a large coil than a small coil) and on the performance of the gradient amplifier (it takes a lot of voltage to overcome the inductance of the coil) and has significant influence on image quality.

The K-Space

The image we acquire through the MRI scanner is just a collection of pixels whose intensity is coded on a grey scale and distributed along a coordinate system. Pixel intensity depends upon signal strength which in turn depends upon proton density (more specifically mobile protons), such that more protons equal more resonance which equals a greater FID signal. It is important to understand that the FID signal acquired during each TR is a superimposition of signals each with a different frequency and phase

originating from the entire slice. By a Fourier transformation (FT) we are able to create a frequency spectrum, translating the signal from a time domain to a frequency domain enabling us to extract all the signals that make up that complex FID signal. Because of the frequency encoding gradient, frequency information is tied to spatial information on our slice. Thus, we are able to assign spatial information to the signal. Subsequently, we Fourier transform the data again, this time in the vertical direction. This enables us to obtain spatial information based upon the phase encoding gradient.

The MRI data is stored in a matrix called data space whose x axis represents the time during which the frequency encoding gradient is applied while the y axis represents the time TR during each phase encoding gradient. Once this data space is digitized it shifts from a time domain to a spatial frequency domain. This is referred to as K-space. The change from data space to K-space entails a mathematical manipulation. It is out of the scope of this thesis to explain the entire process. We refer the reader to a physics textbook for further details about this topic. However, a fundamental aspect of K-space is the relationship between signal amplitude and distribution on K-space. High amplitude low spatial frequency information is stored at the centre of K-space whereas low amplitude high spatial frequency information is stored in the periphery of K-space.

Example of a pulse sequence

In the timing diagram in figure 8, the horizontal axis represents time. The vertical axis represents: (top row) amplitude of radio frequency pulses; (middle rows) amplitudes of the three orthogonal magnetic field gradient pulses; and (bottom row) receiver analog-to-digital converter (ADC).

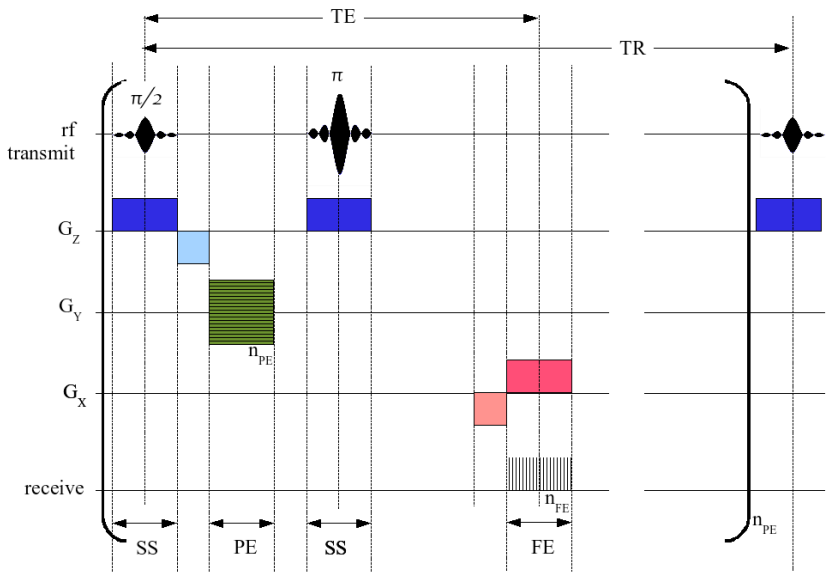


Figure 8. Simplified timing diagram for two-dimensional-Fourier-transform (2DFT) Spin Echo (SE) pulse sequence

Radio frequencies are transmitted at the Larmor frequency of the nuclide to be imaged. The three field gradients are labeled GX (typically corresponding to a patient's left-to-right direction and colored red in diagram), GY (typically corresponding to a patient's front-to-back direction and colored green in diagram), and GZ (typically corresponding to a patient's head-to-toe direction and colored blue in diagram). Where negative-going gradient pulses are shown, they represent reversal of the gradient direction, i.e., right-to-left, back-to-front or toe-to-head.

The first part of the pulse sequence, SS, achieves "slice selection". A shaped pulse (shown here with a sinc modulation) causes a 90° nutation of longitudinal nuclear magnetization within a slab, or slice, creating transverse magnetization. The second part of the pulse sequence, PE, imparts a phase shift upon the slice-selected nuclear magnetization, varying with its location in the Y direction. The third part of the pulse sequence, another slice selection (of the same slice) uses another shaped pulse to cause a

180° rotation of transverse nuclear magnetization within the slice. This transverse magnetisation refocuses to form a spin echo at a time TE. During the spin echo, a frequency-encoding (FE) or readout gradient is applied, making the resonant frequency of the nuclear magnetization vary with its location in the X direction. The signal is sampled nFE times by the ADC during this period, as represented by the vertical lines. Typically, nFE of between 128 and 512 samples are taken. The longitudinal magnetisation is then allowed to recover somewhat and after a time TR the whole sequence is repeated nPE times, but with the phase-encoding gradient incremented (indicated by the horizontal hatching in the green gradient block). The negative-going lobes in GX and GZ are imposed to ensure that, at time TE (the spin echo maximum), phase only encodes spatial location in the Y direction. Typically, TE is between 5 ms and 100 ms, while TR is between 100 ms and 2000 ms. After the two-dimensional matrix (typical dimension between 128 × 128 and 512 × 512) has been acquired, producing the so-called k-space data, a two-dimensional inverse Fourier transform is performed to provide the familiar MR image. Either the magnitude or phase of the Fourier transform can be taken, the former being far more common.

Fast Scanning Techniques and Eco Planar Imaging

Once the theoretical underpinnings of the MRI technique were understood, researchers focused on the development of new pulse sequences that decreased scanning time while increasing signal-to-noise ratio.

A classical spin-echo sequence, where a 180° refocusing pulse is used to create a train of echoes, gave way to more efficient scanning techniques referred to as fast spin echo (FSE) or turbo spin echo (TSE) technique. FSE techniques are characterized by a changing phase- encoding gradient for each echo, resulting in the filling of multiple lines of k-space during a single TR.

While TSE techniques significantly decreased scanning time, the true game changer was the development of the gradient echo (GE) sequence and its combination with innovative

K-space filling methods such as multi-shot echo planar imaging (EPI) (i.e. blipped, non-blipped, spiral). The two main features that set GE sequences apart from all the others is the absence of a 180° re-phasing pulse and a flip angle that is lower than 90°. After the RF pulse, a dephasing gradient is applied and then subsequently reversely applied in order to create the echo. This drastically decreases the echo time. On the other hand, a lower flip angle decreases the time between each TR as the longitudinal magnetization recovers faster from a 30° nutation than a 90° nutation. Thus, the combination of a short TE and a short TR echo means an acquisition time of 100 ms per slice. The compromise between a low spatial resolution image with a high temporal resolution one enabled researchers to look at the dynamic changes of the brain signal during a time series. Once the measured brain signal was attributed to a specific feature, be it water molecule movement or ratio of oxygenated to de-oxygenated hemoglobin, a new prolific field of research populated by functional magnetic resonance images, diffusion weighted images, and resting-state functional magnetic resonance images, was born.

Resting State Networks and the Role of the Cerebellum

Introduction

The invention of the magnetic resonance (MR) scanner opened a door on a world that was previously observable only by positron emission topography (PET) and electroencephalogram (EEG). Experimental psychologists long dreamed to have a non-invasive apparatus that measured *in vivo* brain activity with a high spatial resolution. In contrast with MR technique, PET requires the injection of a radio-isotope and the EEG is able to capture only the overall activity of the entire cerebral cortex and cannot localize brain activation.

In the first half of the XX century, advocates of behaviourism believed possible to explain human behaviour according to a mere stimulus-response paradigm, wherein operant and classical conditioning played a crucial role ((Holland, 1991) (Holland, 1991). As advocates of cognitive psychology grew larger in the 1960s, behaviourism, criticized

for his reductionist perspective, gave way to cognitive psychology and the human information processing model (Neisser, 1976). The *mind* was considered the processor of external environmental stimuli and capable of manipulating previously stored information. In between the input (external stimuli) and the output (behaviour) lies the mind with its ability to construct schemata about the external world. A person mental representation of the external stimulus became more important than the stimulus itself and new cognitive processes were needed in order to account for information storage and manipulation. Working memory, attention, pattern recognition, problem solving, language, and meta-cognition are all mental processes that were brought under the microscope of experimental psychologists and researchers during the 1970s (Baddeley, 2010). A systematic investigation of these mental processes began and response time and accuracy were two of the first measurements used to quantify the amount of processing each stimulus required, with longer response times indicating greater brain processing (Jordan, Heinze, Lutz, Kanowski, & Jäncke, 2001). It was a prolific time for psychology in general and for cognitive psychology in particular.

The aim of neuroscience is to investigate the micro and macro levels of the nervous system from all different aspects: developmental, molecular, cellular, evolutionary and medical. However, the subject of the psychological inquiry is intrinsically harder to investigate than that of the hard sciences. While perception and behaviour are still observable and quantifiable phenomena, emotions and thoughts are of an intrinsically subjective nature. Without delving into the philosophy of mind and its conundrums, it is worth noting that the identification of neural correlates of consciousness remains the holy grail of neuroscientific research.

In the last two decades, brain research has witnessed a tremendous growth and in the 1970s neuroscience, traditionally considered a specific branch of biology, started to claim a separate and independent role. Drawing on concepts from philosophy, linguistic, and psychology, and borrowing methodologies from chemistry, physiology and biology, a precise taxonomy of the sciences that cross path with neuroscience is not possible. To name a few, cognitive neuropsychology focuses on the relationship between the

aforementioned cognitive constructs and the brain structure and function. On the other hand, cognitive neuroscience focuses on the neural substrates of mental processes. While behavioural neuroscience tackles the neural correlates of behaviour. Lesion studies, case studies and neuropsychological testing populated much of the literature initial. The invention of imaging techniques and brain stimulation techniques (e.g. transcranial magnetic stimulation, dual current stimulation) were major contributors to the proliferation of neuroscientific publications seen in the past decade.

Functional magnetic resonance imaging (fMRI)

Functional magnetic resonance imaging (fMRI) is a leading candidate for assessing changes in functional connectivity (FC), suitable to study neurodegenerative diseases, such as AD, thanks to the short acquisition protocol without the need of performing a task. By measuring FC between spatially distinct brain regions, resting-state fMRI (rs-fMRI) allows the identification of several networks (RSNs) (Xu, Potenza, & Calhoun, 2013) (Smith et al., 2005) (Beckmann, Deluca, Devlin, & Smith, 2005) in which separate brain areas show MR signal correlations in the absence of any specific external stimulation (Biswal, FZ, VM, & JS, 1995). Because of the striking overlap between areas of amyloid deposition and areas involved in the default mode network (DMN) (Chen & Ogawa, 1999), most rs-fMRI studies in AD patients have focused the attention on alterations of the (Mohan et al., 2016). The DMN is active during episodic and autobiographical memory retrieval and shows decreased activity during cognitive tasks demanding attention to external stimuli (Fox & Greicius, 2010). In AD, the DMN has shown decreased FC in the precuneus and posterior cingulate cortex, revealing a good matching between reduced FC, cortical atrophy and memory impairment (Binnewijzend et al., 2012). Only a few studies suggested also the involvement of other RSNs, including visual cortex, basal ganglia and cerebellum (Rombouts, Barkhof, Goekoop, Stam, & Scheltens, 2005). Interestingly, the cerebellum is a common area that can be thought as a node of several RSNs including the DMN.

The Blood Oxygen Level Dependent (BOLD) Signal

Hemoglobin is a protein within red blood cells responsible for transporting oxygen. Each hemoglobin molecule can transport up to four oxygen molecules. When oxygen has not yet bonded with hemoglobin, it is referred to as deoxyhemoglobin. Leaving aside the quaternary and tertiary protein structure of hemoglobin, it is important to mention that each hemoglobin molecule has one iron ion. In deoxyhemoglobin this iron ion has four unpaired electrons. Because of these unpaired electrons, deoxyhemoglobin behaves as paramagnetic substance when placed within an external magnetic field. A substance is paramagnetic when it is susceptible to a magnetic field and it interacts with it creating field in homogeneities. On the other hand, oxyhemoglobin has four oxygen molecules bonded with those four previously unpaired iron electrons and behaves as a diamagnetic substance. Thus, if placed within a magnetic field, it does not interact with it. It is this different susceptibility to magnetic fields that lies at the heart of functional magnetic resonance imaging.

The T2 relaxation time, as seen in chapter one, is the time constant it takes for the transverse magnetic vector M_{xy} to lose 37% of its original value. Deoxyhemoglobin paramagnetic nature shortens T2 relaxation time resulting in a faster loss of protons'spin phase coherence, weakening the MRI signal. Thus, the areas of the brain where the blood will have a higher oxyhemoglobin concentration will also have a higher MRI signal intensity than those areas exhibiting a higher deoxyhemoglobin blood concentration. Brain tissues rely exclusively on oxygen and glucose to carry out cellular respiration and metabolism. The brain dependence on oxygen entails that an increase in neuronal activity must be corresponded with an increase in blood perfusion (Arthurs, O. J., & Boniface, S., 2002). The neurovascular coupling is the automatic mechanism whereby our body responds to an increase in metabolic activity with an increase in cerebral blood flow. The hemodynamic response function depicts cerebral blood flow measured in terms of % MRI signal change as a function of time (Figure 9). As we can see there is a two-second

delay from the stimulus onset and the rise in cerebral blood flow. A peak or plateau is reached within eight seconds and the system returns to baseline after roughly twenty seconds. It seems reasonable to say that activated areas will have a lower MRI signal as they let out more deoxyhemoglobin by consuming more oxygen. Counter intuitively, the opposite is actually truth.

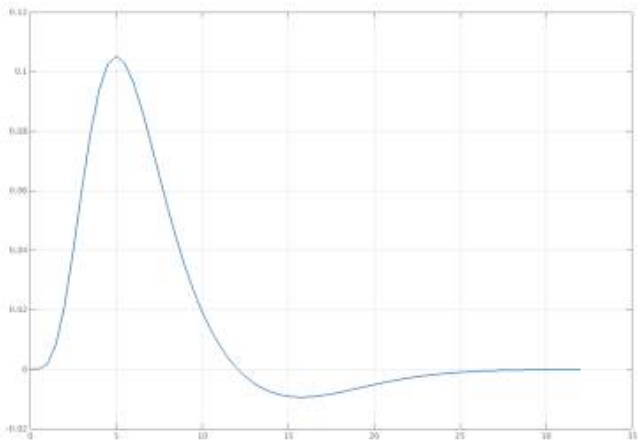


Figure 9. Canonical Hemodynamic Response Function (HRF)

Activated areas display higher MRI signal intensity. MRI signal is dependent on the ratio between oxyhemoglobin concentration and deoxyhemoglobin concentration. The body overcompensates the need for oxygen by dramatically increasing cerebral blood flow. Thus, the net result is an increase of oxyhemoglobin in those activated areas. The name given to the signal acquired exploiting the T2* contrast is called the blood oxygenation level dependent signal, or BOLD signal. A clear overview of the chain reaction triggered by from stimulus to BOLD is presented in Figure 10. However, it is important to note that the BOLD is only an indirect measurement of neuronal activity. The methodological debate on whether the BOLD represents neurons' action potential or simply local neuronal activity remains open (Heeger & Ress, 2002)Lastly, the BOLD increase in an activated area is only, at maximum, 5% greater than its original (i.e. inactivated) value.

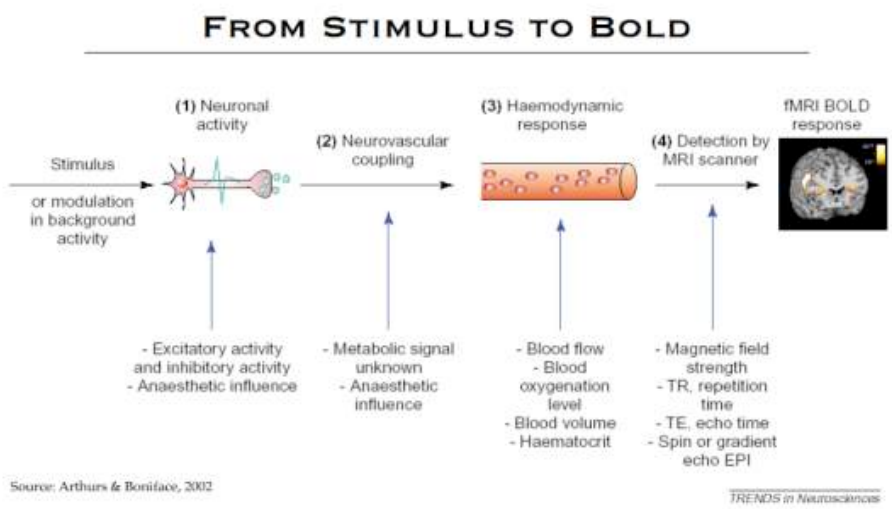


Figure 10. Protons placed within an external magnetic field B_0 will align either parallel. Image from Arthurs, O. J., & Boniface, S., 2002.

Resting State Networks

By the end of the 1990s, much of the fMRI research focused on acquiring data while the subject was performing a specific task (e.g. face recognition, pattern recognition, emotion identification, memory recall). Identifying those areas of the brain that were active during the experimental condition (i.e. task epoch) by comparing them to the activation level at baseline or rest condition remains a classic fMRI experiment. However, data interpretation is never straightforward and relies upon the assumptions that brain activity scales in a linear fashion and that cognitive processes are additive (Le Bihan, 2012). Furthermore, the noise present in fMRI data, the taxing and complicated statistical expertise required, especially in event-related designs, and the many confounds present (e.g. subject motion, physiological noise, autocorrelation in the data) have hindered the development of fMRI research.

The first one to look at the brain while at rest was Dr. Bharat Biswal, an electrical

engineer, in the laboratory of the Medical College of Wisconsin. In his first paper, he observed slow signal intensity oscillations in the sensorimotor cortex while at rest. From an electrical engineering point of view, it made perfect sense that spontaneous random activation within a circuit would spread over the entire circuit (Biswal et al., 1995). Thus, he concluded that the low-frequency fluctuations observed were not an artifact but represented, what he referred to, the brain resting state functional connectivity. Getting the manuscript published was no easy thing. Rejected from major journals, Biswal had to review it four times before it was finally published, three years after the data were collected. Although met with harsh criticism and general skepticism, Biswal further investigated the nature of these low-frequency fluctuations. Following closely Biswal's research was radiologist Dr. Marcus Raichle at the University of Washington. Raichle was the first one to hypothesize the existence of a default network that worked as a facilitator between internal and external environmental stimuli (Raichle, 1996).

The brain represents 2% of the total body weight and yet it accounts for 20% of the total energy consumption at rest (van den Heuvel, Stam, Kahn, & Hulshoff Pol, 2009). Task-induced activity causes, at the most, a 5% BOLD increase. Thus, researchers speculate that even when at rest, the brain is extremely active. However, this activity may be random and spontaneous and carry no relevant information. Resting state functional connectivity may simply represent the end-product of a long maturation process during which preferential neuronal pathways are created within the brain.

The area over which this random activity spreads indicates an interconnected network or area, hence the term intrinsic functional connectivity.

Once it was established that resting state BOLD fluctuations were not related to cardio and respiratory processes (Birn, Diamond, Smith, & Bandettini, 2006) and multiple researchers confirmed that the signal they previously considered noise was instead the DMN activity, research on resting state functional connectivity grew enormously. In particular a paper (Smith et al., 2004) from physicist Stephen Smith at Oxford University had a great impact on the scientific community Starting from the 30,000-subject BrainMap database.

Smith published the output from an analysis of resting state functional connectivity depicting the 10 most commonly recognized networks. Additional networks were then added to the list, reaching a total of about 20 networks (Figure 11).

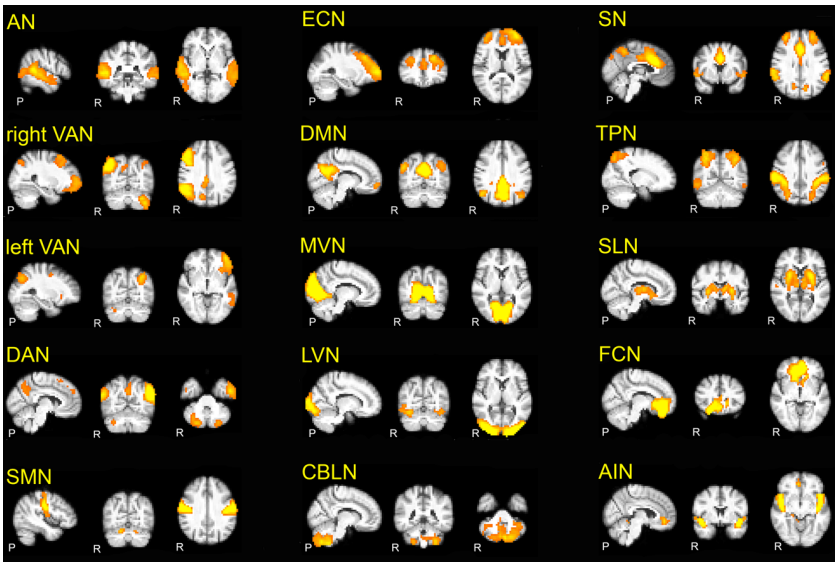


Figure 11. The most commonly recognized RSNs. Image from Castellazzi et al, 2014

The *AN* is responsible for auditory processing and is located in the bilateral superior temporal gyrus, involving the primary and secondary auditory cortices. The *SMN* is responsible for sensory-motor processing and includes the primary somatosensory, primary motor, premotor, and supplementary motor cortices as well as the cerebellum. The *LVN* and *MVN* are the networks responsible for visual processing, and are located in the lateral and medial parts of the occipital lobe, respectively, involving secondary and primary visual cortex, and also involve the cerebellum. The *TPN* is activated during task-oriented behaviours and includes bilateral dorsolateral and ventral prefrontal cortex, insula and primary sensory-motor areas. The *ECN* is responsible for executive processing, including perception, action selection, memory retrieval and emotion

evaluation: it includes bilaterally the superior, middle and ventro-lateral prefrontal cortices, the anterior cingulate, and the paracingulate gyri. The *VAN* is the networks involved in the attentional pro- cessing: *VAN (left and right)* is largely lateralized in the temporal-parietal junction, n the ventral frontal cortex, in the insula and in the contralateral cerebellum. The *SN* mediates the function of other networks and includes bilaterally the anterior cingulate cortex, premotor, supplementary motor areas, and anterior insula (Cauda et al., 2011).

The *DMN*, which is active during episodic and autobiographical memory retrieval and shows decreased activity during cognitive tasks demanding attention to external stimuli (Fox & Greicius, 2010) includes the bilateral inferior parietal cortex, the precuneus, anterior and posterior cingulate cortex, mesial-temporal structures including dorsolateral prefrontal cortex, thalamus and cerebellum. The *BGN* is involved in emotional processing and includes the amygdala, striatum and lateral globus pallidus, mammillary bodies, hypothalamus, and the ventral tegmental area of midbrain. The *FCN* is involved in core mental functions at the basis of human social behaviours (Spreng et al., 2009) and includes the bilateral anterior cingulate cortex and boundary areas between prefrontal cortex (middle and inferior frontal gyri) and orbitofrontal cortex (Janes et al., 2012). The CBLN network is intrinsic to cerebellum and includes bilaterally large areas centred in crus I and crus II, cerebellar tonsil, the IV, V, VI, VIII, and IX lobes, dentate, declive, vermis, culmen, uvula, tuber, pyramis, medulla, and nodule. Finally, the LN involves the bilateral post cerebellum and inferior parietal lobule, the left inferior temporal gyrus and precuneus, as well as the inferior, middle, and superior frontal gyri. *LVN*, *MVN*, *AN*, *TPN*, and *SMN* are networks directly related to sensory-motor processing, whereas *DMN*, *SN*, *FCN*, *ECN, BGN*, *VAN*, and *LN*, are prominently associated with higher cognitive functions.

The Cerebellum in Resting State

The cerebellum, literally "little brain", is a region of the brain situated behind the brain stem, more specifically behind the pons, and under both cerebral hemispheres. First inquired by Golgi and Cajal at the beginning of the XX century, the function of the cerebellum was constrained within the motor-movement domain. More specifically, the cerebellum acts as a movement-refiner and coordinator. In the recent years, researchers have extended the role of the cerebellum outside the motor-movement domain, attributing it a contribution to human cognition, language and emotion regulation (Schmahmann, J. D., & Pandya, D. N., 1997). The cerebellum presents a homogeneous cyto-architecture suggesting that a similar computational algorithm is used in all of its functions (Voogd, J., & Glickstein, M., 1998; Schmahmann, J. D., 2004). Despite making up only 10% of the total brain volume, the cerebellum counts half the neurons of the whole brain and while the neocortex operates on a scale of the tenth of a second, the cerebellum is able to produce an output on the scale of a thousandth of a second (D'Angelo et al., 2010). The cerebellum is not associated with consciousness and is not vital for survival, as nine cases have been reported in which the person was born without a cerebellum (Lemon, R. N., & Edgley, S. A., 2010). Given the functional relationship that is hypothesized across the regions that are part of a resting state network, the fact that the LN, DMN, ECN, and VANs all have a cerebellar component is *per se* supporting evidence of the contribution of the cerebellum to human cognition (Habas, C. et al. 2009; Krienen, F. M., & Buckner, R. L., 2009 O'Reilly et al., 2010). These networks all have a predominantly cognitive function to it. Additionally, trans-synaptic viral tract-tracing studies have shown that there exist anatomical connections between the cerebellum and the pre- frontal and parietal cortices (Schmahmann, J. D., 2004). Lastly, functional MRI studies that looked at cerebellum activation during executive functioning tasks such as the Wisconsin Card Sorting task, the Tower of London task and the go/no-go task, all indicate the activation of lobule VI, Crus I and VIIb (Marvel, C. L., & Desmon, J. E., 2010; Stoodley, C. J., Valera, E. M., & Schmahmann, J. D., 2010). Thus, researchers agree that the cerebellum

does play a role in human cognition and agree that the role of the cerebellum is exclusively to modulate a specific behaviour (be it movement or thought coordination) and not give rise to it. However, the exact computation performed by the cerebellum on the information-input is not clear yet.

Aims of the study

In this work, we characterized the changes occurring in multiple RSNs in three groups of patients: Alzheimer disease (AD), vascular dementia (VaD) and patients with combined cognitive impairment symptoms we labelled as mixed dementia or M subjects. A group of age-matched healthy controls (HC) has been considered for the statistical testing. We studied the RSN alterations occurring in each pathological group compared to HC with the aim to assess the group-specific profile of functional alterations.We then investigated the presence of hubs of alterations (nodes) at the intersection of multiple networks. We finally compared the functional impairment profiles of M, AD and VaD (compared to HC) to assess the similarities/dissimilarities of the M profile from those of AD and VaD subjects. This last step aimed to assess the potential of rs-fMRI to address the issue of the identification of the prevalent underlying disease in dementia subjects with combined cognitive impairment symptoms.

Materials and procedure

Subjects

A total of 90 subjects (mean age 70 ± 6) were recruited for this study among those suffering from subjective or objective memory complaint, attending regularly the Memory Clinic of the Neurological Institute C. Mondino, Pavia, Italy. Subjects were selected based on their neuropsychological examination using a standardized battery of tests which evaluated different cognitive domains (Spinnler and Dall'ora, 1987). Exclusion criteria were: age >80 years, significant medical, neurological (different from

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AD) psychiatric disease as well as significant cerebrovascular disease (Hachinski et al., 1975; Binnewijzend et al., 2012). In detail, patients with significant Central Nervous System (CNS) disorders other than AD (e.g., Parkinson's disease and other extra-pyramidal disorders, multiple sclerosis, epilepsy, significant focal or vascular intracranial pathology, clinical evidence of cerebrovascular accident, and/or previous head injury with loss of consciousness) were excluded. Written informed consent was provided by all the subjects or their lawful caregiver.

Fourteen patients were excluded because of data quality, mainly motion. Some images had such artifacts that were excluded on visual inspection. When in doubt, realignment parameters were also considered and data excluded if the subject had a maximum displacement in any of the cardinal directions (x, y, z) that was larger than 3 mm, or a maximum spin (x, y, z) larger than 3◦. After inspection, 115 subjects were included in this study. Based on the clinical evaluation, subjects were divided into three groups: 33 patients (14 females, mean age 74 ± 6) were classified as AD (NINCDS2-ARDAcriteria) (McKhann et al., 2011), 26 patients (21 females, mean age 70 ± 6) as VaD (Petersen et al., 2001, 2009; Petersen, 2009) and 19 patients (8 females, mean age 70 ± 6) as combined vascular and degenerative cognitive impairment (Petersen et al., 2001, 2009; Petersen, 2009). In order to obtain a reference metric for our findings, 34 HC (16 females, mean age 69 ± 5) were recruited on a volunteer base through a local recreational association ("Argento Vivo," Bereguardo, PV) and underwent the MRI examination.

Clinical And Neuropsychological Assessment

Subjects underwent clinical and neuropsychological testing, which evaluated different cognitive domains (Spinnler and Dall'ora, 1987; Spinnler and Tognoni, 1987) including: Mini- Mental State Examination (MMSE) (Folstein et al., 1975), trial making test part A and B (TMT-A and TMT-B) (Reitan, 1958), memory for prose (MP) (Novelli et al., 1986), category fluency (CF), and semantic fluency (SF) (Randolph et al., 1993), Rey complex figure copy test (ROCF-copy) and Rey complex figure recall (ROCF-rec) (Osterrieth, 1944; Caffarra et al., 2002). For each test age, gender, and education-

corrected scores were calculated from the raw scores. Corrected scores were then transformed in equivalent scores ranging from 0 (pathological) to 1 (lower limit of normal) and 2–4 (normal). Only corrected scores were used in the statistical analysis.

MRI Acquisitions

All data were acquired using a 3T Siemens Skyra MR scanner (Siemens, Erlangen, Germany) with a 32-channels head-coil. For each subject, an echo-planar imaging (EPI) 2D BOLD sequence was acquired for rs-fMRI with TR/TE = 3010/20 ms, voxel size=2.5mm isotropic, FOV=224mm, 60 slices for a total of 120 volumes. For anatomical reference, a volumetric 3D T1-weighted acquisition (3DT1) was also acquired using a MPRAGE sequence (TR/TE/TI=2300/2.95/900ms, flip angle 9°, 256 slices, slice, voxel size=1x1x1.2mm3, FOV=270mm).

MRI Analyses

All the MRI analyses were performed on a workstation with Linux Ubuntu 14.04 OS, running SPM12 (Wellcome Department of Cognitive Neurology, http://www.fil.ion.ucl.ac.uk/), Matlab R2016a (The MathWorks, Natick, Mass, USA http://www. mathworks.com/) and FSL (FMRIB Software Library, version 5.0.9, http://www.fmrib.ox.ac.uk/fsl/).

FMRI Analysis

For each recruited subject, rs-fMRI images were analysed using *Independent Component Analysis* (ICA) to characterize RSNs. ICA results were analysed using the Multivariate Exploratory Linear Optimized Decomposition into Independent Components (MELODIC) method as implemented in FSL. (Beckmann et al., 2005)

Data pre-processing

Individual subject's pre-processing consisted in motion correction, brain extraction, spatial smoothing using a Gaussian kernel of full-width-at-half-maximum (FWHM) of 4

mm, and high pass temporal filtering equivalent to 150s (0.007Hz). rs-fMRI volumes were then registered to the individual's structural 3DT1 scan using FMRIB's Linear Image Registration Tool (FLIRT) and subsequently to standard space (MNI152) using FMRIB's Nonlinear Image Registration Tool (FNIRT) with default options.

Independent component analysis (ICA)—identification of RSNs

Pre-processed functional data, containing 120 time points (volumes) for each subject, were temporally concatenated across subjects to create a single 4-dimensional data set. The dataset was decomposed into independent components (ICs), with an automatic estimation for the number of components, which resulted in spatial maps, each with an associated time course. Model order was estimated using the Laplace approximation to the Bayesian evidence for a probabilistic principal component model. Some of the ICs were identified as noise while others as RSNs, based on previous literature (Beckmann et al., 2005; Damoiseaux et al., 2006; Smith et al., 2009; Cole et al., 2010). This method is run on the entire dataset and decomposes data into spatial maps that are the ICs relative to the total pro- cessed dataset, or the multi-subject ICA components. This means that ICs are the same for each subject and represent the maps within which inference between groups (AD, VaD, M and HC) is then evaluated applying dual regression.

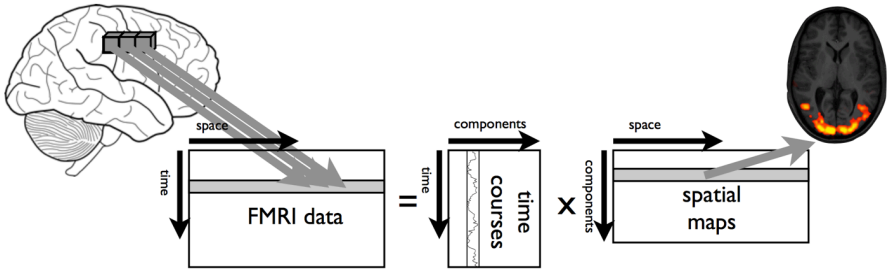


Figure 12. Beckmann CF (2005) *Neuroimage*; Calhoun VD (2001) *Human Brain Mapping*

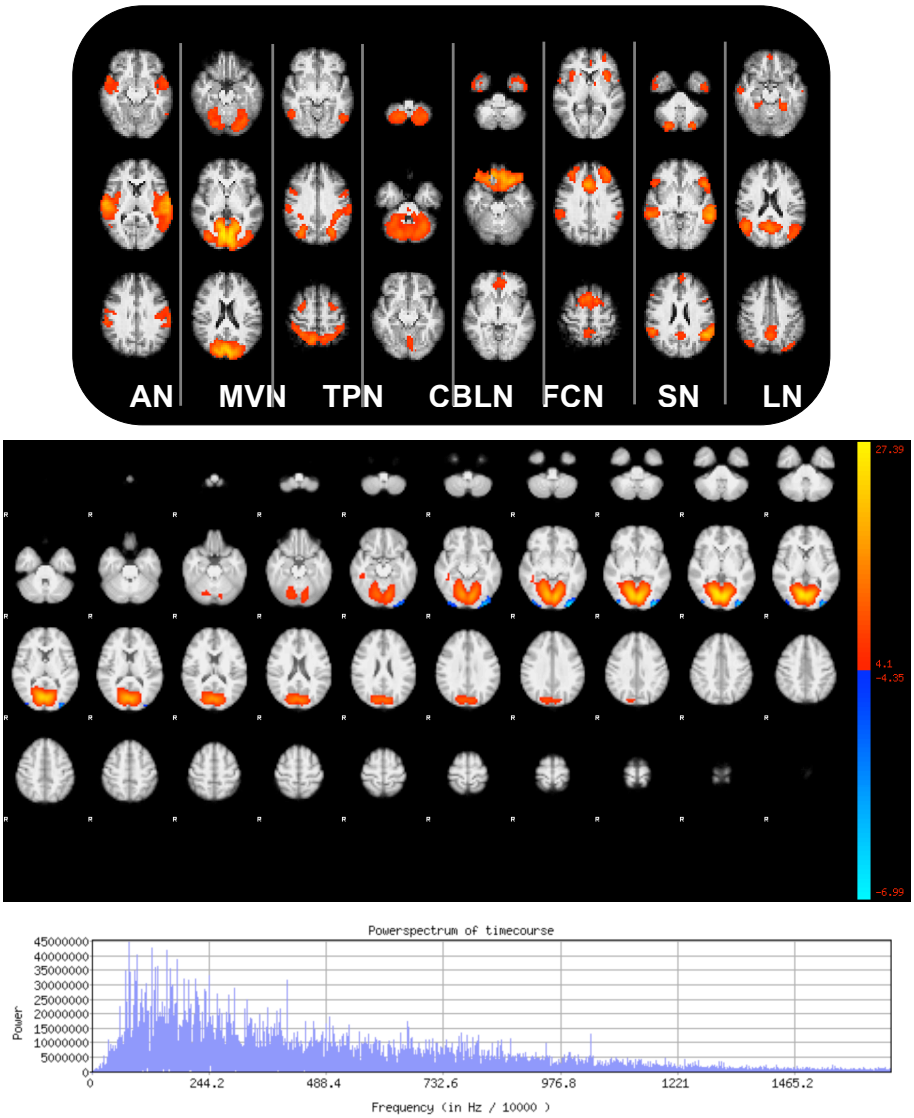
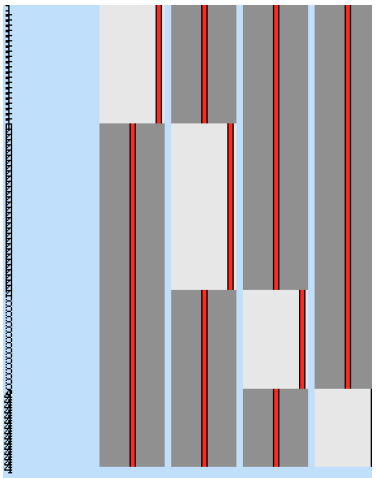


Figure 13: Example of an IC component that is also a resting state network (RSN). RSN ICs are typically characterized by predominantly low frequency power and 'blob-like' spatial maps primarily covering grey matter areas.



		AD	HC	M	VD
C1	HC-AD	-1	1	0	0
C2	HC-VD	0	1	0	-1
C3	AD-HC	1	-1	0	0
C4	VD-HC	0	-1	0	1
C5	AD-VD	1	0	0	-1
C6	VD-AD	-1	0	0	1
C7	HC-M	0	1	-1	0
C8	M-HC	0	-1	1	0

Figure 14. Design matrix of our study

Dual regression—evaluation of group differences within the RSNs

A non-parametric permutation test, referred to as "dual regression" technique, was then applied to compare group-specific FC maps for each independent spatial component. In particular with this analysis differences between HC, AD, VaD ad M groups were tested using 8 different comparisons, which we will refer to as contrasts (C1: HC-AD; C2: HC > VaD; C3: AD > HC; C4: VaD > HC; C5: AD > VaD; C6: VaD > AD; C7: HC > M and C8: M > HC) of the desing matrix reported in Figure 15) The dual regression algorithm allows for between-subjects analysis by carrying out a voxel-wise comparisons of the resting FC (Filippini et al., 2009). In this work the dual regression analysis was carried out on the total ICs using age, gender and education level and normalised grey matter ratio as additional covariates in the permutation tests (http://fsl.fmrib.ox.ac.

uk/fsl/fslwiki/DualRegression).

In detail, spatial ICs were used in a linear model fit against each individual rs-fMRI data set (spatial regression), to create matrices that described the temporal dynamics for each component and subject separately. These matrices were used in a linear model fit against the associated subject's rs-fMRI data set (temporal regression), to estimate subject-specific spatial correlation maps. Subsequently spatial maps of all subjects were collected into single 4-dimensional files for each original independent component and tested voxel-wise for statistically significant differences between groups using nonparametric permutation tests (10,000 permutations) (Filippini et al., 2009; Binnewijzend et al., 2012). The resulting statistical maps were family-wise error(FWE) corrected for multiple comparisons. A further threshold-free cluster enhancement (TFCE) correction was also applied to remove false positive voxels of FC alteration. Voxels that survived a statistical threshold of $p \leq 0.05$ were considered significant (Binnewijzend et al., 2012) and were saved as *tstatFC* maps.

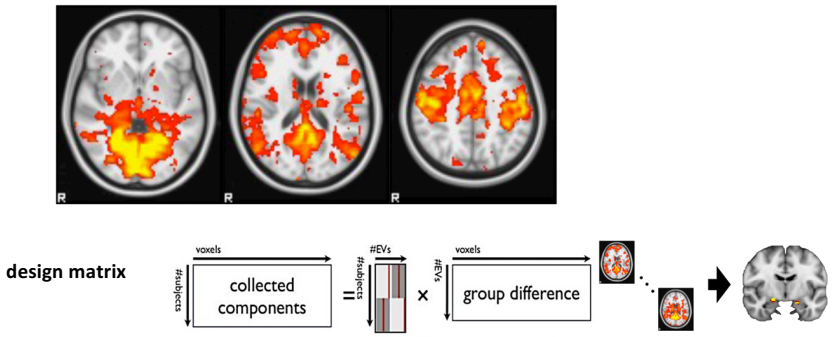


Figure 15. Picture showing the details of the group-comparison analysis operated by the dual regression method on the ICA resulting ICs.

Non-Imaging Statistics

All other statistical analyses were performed using SPSS (version 19.0; SPSS, Chicago, IL, USA). For continuous measures, differences between groups were assessed using one-way analysis of variance (ANOVA). We performed a $\chi 2$ test to compare frequency distributions of age, gender and education level.

Results

Resting State Networks Identification

In this rs-fMRI investigation, 19 RSNs were identified. RSN alterations in VaD and AD and M compared to HC were observed in several networks. The ICA analysis resulted in 46 independent components, 19 of which were recognized as part of 15 full RSNs based on their frequency spectra and spatial patterns (Beckmann et al., 2005; Damoiseaux et al., 2006; Smith et al., 2009; Cole et al., 2010).

The 19 RSNs were: executive control network (*ECN),* auditory right and left network (*AN*), lateral visual network (*LVN*), sensory motor right and left network (*SMN*), medial visual network (*MVN*), task positive network (*TPN*), right and left ventral attention networks (*VAN*), default mode network (*DMN*), salience network (*SN*), cerebellar network (*CBLN*), basal ganglia network (*BGN*), pre-cuneus network (PCN), frontal cortical network (*FCN*), Occipital visual network (OVN), working memory network (WMN), task-positive network (TPN), language network (*LN*).

The *AN* is responsible for auditory processing and is located in the bilateral superior temporal gyrus, involving the primary and secondary auditory cortices. The *SMN* is responsible for sensory-motor processing and includes the primary somatosensory, primary motor, premotor, and supplementary motor cortices as well as the cerebellum. The *LVN* and *MVN* are the networks responsible for visual processing, and are located in the lateral and medial parts of the occipital lobe, respectively, involving secondary and primary visual cortex, and also involve the cerebellum. The *TPN* is activated during task-oriented behaviours and includes bilateral dorsolateral and ventral prefrontal cortex,

insula and primary sensory-motor areas. The *ECN* is responsible for executive processing, including perception, action selection, memory retrieval and emotional evaluation: it includes bilaterally the superior, middle and ventrolateral prefrontal cortices, the anterior cingulate, and the para-cingulate gyri. The *VAN* is the networks involved in the attentional processing: *VAN (left and right)* is largely lateralized in the temporal-parietal junction, in the ventral frontal cortex, in the insula and in the contralateral cerebellum. The *SN* mediates the function of other networks and includes bilaterally the anterior cingulate cortex, premotor, supplementary motor areas, and anterior insula (Bonnelle et al., 2012). The *DMN*, which is active during episodic and autobiographical memory retrieval and shows decreased activity during cognitive tasks demanding attention to external stimuli (Raichle et al., 2001; Greicius et al., 2003, 2004), includes the bilateral inferior parietal cortex, the pre-cuneus, anterior and posterior cingulate cortex, mesial-temporal structures including dorsolateral prefrontal cortex, thalamus and cerebellum. The *BGN* is involved in emotional processing and includes the amygdala, striatum and lateral globus pallidus, mammillary bodies, hypothalamus, and the ventral tegmental area of midbrain. The *FCN* is involved in core mental functions at the basis of human social behaviours (Spreng et al., 2009) and includes the bilateral anterior cingulate cortex and boundary areas between prefrontal cortex (middle and inferior frontal gyri) and orbito-frontal cortex (Janes et al., 2012). The *AIN* is involved in cognitive control and orienting attention (Corbetta et al., 2002) and (Cole and Schneider, 2007) includes bilaterally the areas of insula, inferior frontal gyrus, anterior cingulate, the superior temporal gyrus, and cerebellum. The CBLN network is intrinsic to cerebellum and includes bilaterally large areas centred in crus I and crus II, cerebellar tonsil, the IV, V, VI, VIII, and IX lobes, dentate, declive, vermis, culmen, uvula, tuber, pyramis, medulla, and nodule. Finally, the LN involves the bilateral post cerebellum and inferior parietal lobule, the left inferior temporal gyrus and precuneus, as well as the inferior, middle, and superior frontal gyri. *LVN*, *MVN*, *AN*, *TPN*, and *SMN* are networks directly related to sensory-motor processing, while *DMN*, *SN*, *FCN*, *ECN, AIN*, *BGN*, *VAN,* and *LN*, are prominently associated with higher cognitive functions. The cerebellum showed

components not only in the intrinsic *CBLN* but also in *SMN*, *MVN*, *LVN, DMN*, *AIN,VAN*, *LN* in line with the involvement of cerebellum both in sensory-motor and cognitive processing (Schmahmann et al., 1999; D'Angelo and Casali, 2012; Stoodley, 2012; Stoodley et al., 2012).

For each contrast, we generated a global map of FC alteration by putting all together the tstatFC maps of each identified RSN. This step enabled us to identify the global profile of FC impairment for each pathological group (AD, VaD and M). A detailed description of the areas involved in the global map of alteration for each contrast are here reported:

- Contrast C1: in the global network involving the 1st contrast, testing areas of decreased FC in AD vs HC (HC > AD or HC - AD), we observed 8 different clusters involving the first one: Left Cerebrum, Frontal Lobe, Subcallosal Gyrus, White Matter, Right Cerebrum, Sub-lobar, Lentiform Nucleus, Gray Matter, Lateral Globus Pallidus, Pallidum and putamen.

- Contrast C2: ss regard to the 2nd contrast, testing areas of decreased FC in VaD vs HC (HC > VaD), we observed other 8 different clusters in which the main areas were: Left Cerebrum, Limbic Lobe, Posterior Cingulate, White Matter, Cingulate Gyrus, Gray Matter, Brodman area 23, Cingulum.

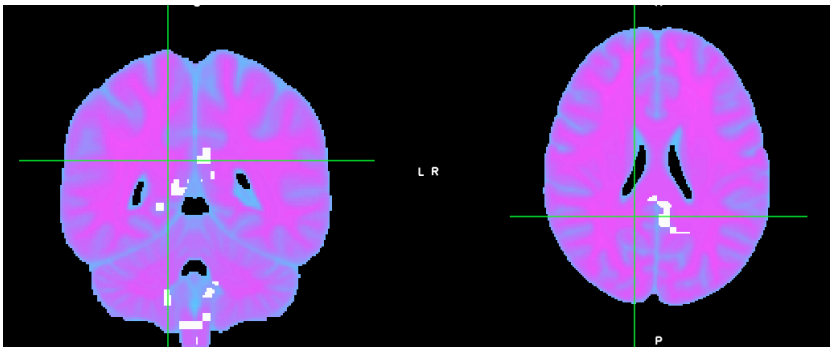
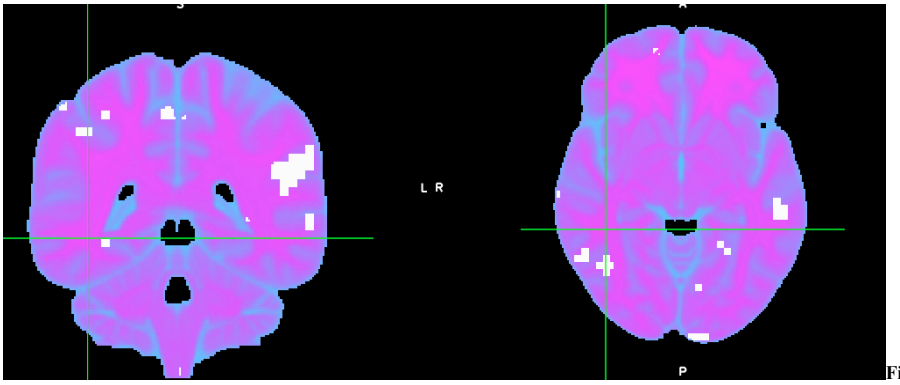


Figure 16. Global C1-C2 contrast

- Contrast C3: for the 3rd contrast, testing areas of increased FC in AD vs HC (AD > HC), we find out mainly the following areas involved: Right Cerebrum, Frontal Lobe, Middle Frontal Gyru, Gray Matter, brodmann area 10.

- Contrast C4: As regard 4th contrast, testing areas of increased FC in VaD vs HC (VaD > HC), we observe other 25 different clusters in which the main areas were: Right Cerebrum, Frontal Lobe, Sub-Gyral, White Matter Parietal Lobe, Inferior Parietal Lobule brodmann area 9 Limbic Lobe, Cingulate Gyrus, White Matter, Cingulum antero-lateral.



Fi

gure 17. Global C3-C4 contrast

- Contrast C5: ss regard the 5th contrast, we tested the areas of increased FC in AD compared to VaD (AD > VaD) and we identified 4 clusters involving the following areas: Right Cerebrum, Frontal Lobe, Medial Frontal Gyrus, Gray Matter, Brodman 8 area.
- Contrast C6: ss regard the 6th contrast, we tested the areas of increased FC in VaD compared to AD (VaD > AD) , identifying 30 clusters involving the following areas: Left Cerebrum, Occipital Lobe, Lingual Gyrus, White matter, Lingual left, Gray Matter, Brodman area 18, Left Cerebrum, Limbic Lobe, Parahippocampa Gyrus Sub-lobar, Thalamus, Gray Matter, Ventral Posterior Medial Nucleus, right Thalamus.

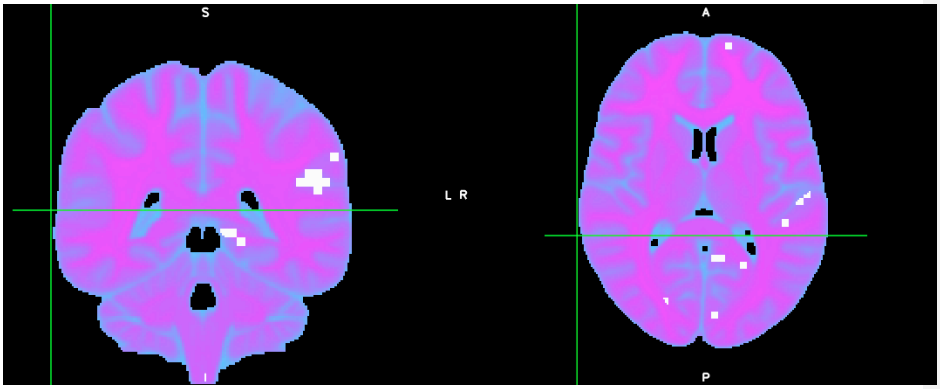


Figure 18. Global C5-C6 contrast

- *Contrast C7:* with the 7th contrast, we tested the areas of decreased FC in patients presented a combined cognitive impairment (M patients) compared to HC (HC > M), identifying 28 clusters. The main involved areas were: Right Cerebellum, Cerebellum Posterior Lobe, Cerebellar Tonsil, Cerebellum right, Uvula, Frontal Lobe, Inferior Frontal Gyrus, Frontal inferior left lobe, Temporal Lobe, Sub-Gyral, White Matter,

temporal inferior right lobe.

- *Contrast C8:* when testing the areas of increased FC in M subjects compared to HC (M > HC), we found out 42 clusters, involving the following areas: left and right cerebellum, Cerebellum Posterior Lobe and Cerebellar Tonsil, Right Cerebrum, Frontal Lobe, paracentral Lobule, White Matter, Medial Frontal Gyrus, Gray Matter, Brodman area 9, Frontal and Superior Medial lobe.

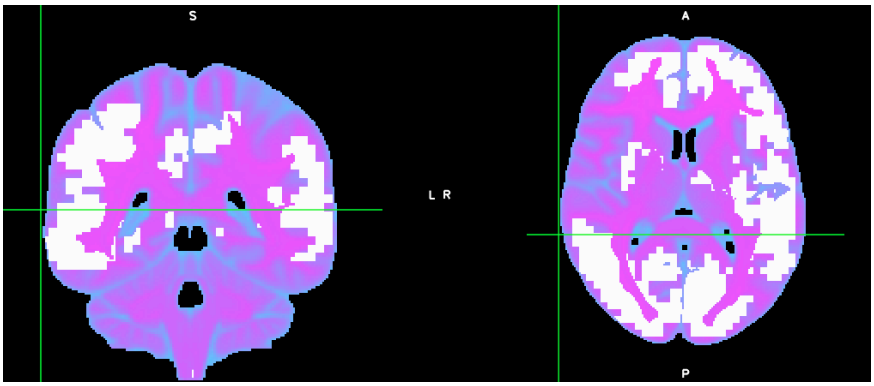


Figure 19. Global C7-C8 contrast

Discussions

We used rs-fMRI with the aim to discriminate main RS network between VaD, combined cognitive impairment and AD patients.

Our study outlines that: i) multiple RSNs are modified in AD and VaD compared to HC; ii) VaD patients, compared to AD, are more functionally impaired in the attentional and executive RSNs, including DMN, VANs and ECN; iii) FC impairments tend to be more asymmetric in VaD than in AD, mainly afflicting the left prefrontal cortex and the right PCC. By comparing the different cerebral areas matched to every contrast and in particular comparing C1 and C2, the global map showed below was obtained.

The picture shows the difference between the areas involved in the two different RS network between HC and AD patients. The MRI image involving the AD patients presented a specific posterior and frontal lobe's involvement, while the healthy subjects show a primarily frontal contrast representation.

We found different contrast comparing the AD patients' and vascular ones. The one with vascular dementia presented a particular involvement of the frontal and DMN areas while in patients affected by vascular disease the contrast were located in the cerebellum and DMN areas.

Comparing, on the contrary, the AD and VaD population we obtained a most involvement of the frontal areas for the AD groups and a more diffuse frontal involvement but also posterior in the vascular disease patients. That could be explained on the different etiopathogenesis of the disease that can result from various causes and resulting in a larger and different spectrum of maps.

As regard the last contrast we have a combined involvement of different areas, both fronto-temporal and posterior cerebral areas. As far as the last contrast is concerned, we have obtained a representation of larger areas with fronto-temporal and posterior bilateral location; this could probably depend on the different etiopathy of the combined forms of cognitive impairment that can be caused by various pathologies.

The preliminar analysis revealed that VaD and AD showed abnormal regional brain activation as well as large-scale brain networks. VaD patients showed hypoactivation in default, frontoparietal, and visual networks relative to healthy controls, whereas AD-

related hypoactivation mainly located in visual, default, and ventral attention networks relative to healthy controls. Both VaD-related and AD-related hyperactivation fell in frontoparietal, ventral attention, default, and somatomotor networks relative to healthy controls. VaD and AD presented different pathological, so the system-level findings are helpful to link the fundamental declines of cognitive tasks to brain networks in VaD and AD. As regard the combined cognitive impairment patients the results suggest that this type of neurodegenerative disease is associated with an alteration of large-scale functional brain networks, which extends well beyond the DMN. We find out that a medial parietal RS fMRI signal change seems to be present since the early phase of cognitive impairment. Resting state functional connectivity alterations are noted during initial stages of cognitive decline in AD, even when there are no significant white matter microstructural changes. It is therefore possible that the RS networks obtained are so different in relation to the non-defined cognitive impairment etiology, which therefore makes more difficult to identify the underlying disease in the early clinical stage of onset.

In light of the data so far obtained, fMRI may be useful in evaluating the areas attributed to each pathological group, but it is not consistent in determining the underlying pathology. This is because it is not strictly specific in differentiating the diagnosis but provides a useful tool for distinguish the main areas involved in the single pathology and in particular AD and vascular dementia. For these reasons it will provide new investigative techniques, so called machine learning, that could become more and more useful to classify and specify the disease with an unclear etiology. The processes on which this methodology is based consist in a very complex statistical analysis processes, which may now allow us to distinguish the pathological groups more accurately and in a predictive way.

Conclusions

We have successfully developed a strategy to assess multiple RSNs in terms of their FC changes (both in magnitude and spatial extension) as well as to evaluate contributions of different cortical regions to FC alterations. The picture that emerges from these rs-fMRI results suggests that MCI and VaD FC changes occur in hubs laying at the intersection of multiple RSNs (all the 19 RSNs identified in the study), rather than just in the DMN, and encompass both decreased and increased FC. These hubs are mostly located in the prefrontal and parieto-temporal cortices. The cerebellum is also involved in several changes, which might suggest that it participates as well as various cortical areas to compensatory pro- cesses, although the hypothesis of reduced mutual information cannot be discarded, especially in the advanced stage of the dis- ease such as in AD. Longitudinal studies on larger patients' groups are needed to investigate physiological meanings of changes and the correlation between RSN changes and disease development.

The resting state analysis is useful to highlight the functional alterations of AD and VaD's patients, already diagnosed. In the future we could use the combination of ICA and dual regression analysis on a larger sample of AD and VaD patients to better define global maps used as example for these diseases. However, the FC alteration can be able to tell us if these are more common in AD patients than the VD patients. However, we could use our results to combine these FC (obtained from RSNs) with innovative technique (machine learning techniques) to make predictions of the prevalent disease. So network analysis can not be used to diagnose the specific disease, but the machine learning combined with rs-fMRI analysis can figure out whether combined cognitive impairment is similar to AD or VaD neuro-imagin before became pathological. The study presented here, though, thoroughly demonstrated that the assessment of alterations in FC of multiple extended large-scale networks of the cerebral cortex and cerebellum using rs-fMRI deserves further attention and may provide new cues to examine the pathophysiology of neurodegenerative.

In Alzheimer's disease (AD), magnetic resonance imaging (MRI) is essential for early diagnosis, differential diagnosis, and evaluation of disease progression. In structural MRI,

the automatic diagnosis of atrophy by computers, even when it is not visually noticeable, is possible in daily clinical practice. Furthermore, subfield volumetric measurements of the medial temporal structures, as well as longitudinal volume measurements with high accuracy, have been developed and are useful for calculating the needed sample size in clinical trials. In addition to detecting local atrophy, graph theory has been applied to structural MRI for evaluation of alterations of the brain networks potentially affected in AD.

Several limiting factors need to be addressed. The somewhat small sample size in this study may have been an issue. Additionally, a possible overlap between early AD and VaD in our patients cannot be completely excluded. Since VaD and AD are both common in old age, they can co-occur and may have among our patients. Although exploring the nature of abnormal functional connectivity in VaD, AD and combined cognitive impairment brains will need more direct evidence, our findings suggest that rs-FC can offer some neurobiological information about all this patients and that this study was helpful for increasing the understanding of the neural mechanisms in neurodegenerative diseases.

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