Multiple pathologies in dementia : correlations with clinical diagnoses

MULTIPLE PATHOLOGIES IN DEMENTIA: CORRELATIONS WITH CLINICAL DIAGNOSES

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CHAPTER 1
General introduction

Clinical characteristics of dementia

Dementia is a clinical syndrome characterized by an acquired and persistent impairment in multiple cognitive domains that is severe enough to interfere with everyday functioning (DSM IV) (American Psychiatric Association, 1994). In most patients, it is chronic, progressive, and irreversible. Subtypes of dementia, like Alzheimer´s disease (AD), vascular dementia (VaD), frontotemporal lobar dementia (FTLD), Lewy bodies dementia (LBD) and Creutzfeldt-Jakob disease (CJD), can be defined by progressive cognitive impairment and clinical signs, as well as by their typical pattern of neuropathological changes. Standardized clinical and neuropathological criteria for different subtypes of dementia have been proposed in consensus meetings.

The following are the *clinical* features of the most common types of dementia:

- AD is a progressive disorder characterized by an impairment of recent memory and of at least one other cognitive domain, such as language, visuospatial function or executive function.
- The clinical characteristics of LBD are dementia associated with any two of the following three core features: fluctuating cognition or level of consciousness, visual hallucinations, and spontaneous parkinsonian motor signs.
- Three different subtypes of FTLD have been described: one behavioural variant of frontotemporal dementia and two language variants, semantic dementia and progressive non-fluent aphasia. These three subtypes overlap clinically with motoneuron disease (FTD-MND), corticobasal degeneration (CBD) and progressive supranuclear palsy (PSP) (Neary et al., 1998; Seelaar et al., 2011). CBD is characterized by rigidity with focal cortical signs, such as apraxia and aphasia, and often by frontal-type dementia. PSP is an atypical parkinsonian disorder associated with progressive axial rigidity, vertical gaze palsy, dysarthria, dysphagia, and often dementia.
- CJD is typically associated with a rapidly progressive course over a period of months from symptom onset to death. CJD patients show global cognitive deterioration, significant psychiatric symptoms and prominent motor symptoms, including signs of pyramidal, cerebellar, and extrapyramidal involvement.
- Vascular dementia (VaD) is the second most frequent cause of dementia after AD. These patients most often have a dysexecutive syndrome characterized by reduced mental processing speed, decreased working memory, and impairment of abstract reasoning (Chui et al., 2007).

Although *in vivo* criteria for types of dementia are based on clinical features, data on abnormal biomarkers, when available, are important to support the early diagnosis. The known biomarkers are based on findings of structural neuroimaging studies with magnetic resonance imaging (MRI) and molecular neuroimaging with positron emission tomography (PET), as well as cerebrospinal fluid analysis of proteins such as amyloid beta or tau proteins (Dubois et al., 2007).

Neuropathological characteristics of dementia

The definitive diagnosis of dementia types relies on neuropathological examination of the brain. Dementia syndromes, and neurodegenerative conditions in general, are defined as disorders with progressive loss of neurons showing a distinct anatomical distribution characteristic of the different dementia types. Research has identified a spectrum of proteins that are immunohistochemically detectable in the central nervous system and can serve as a basis for protein-based disease classification. This is the reason why certain dementia types are also described as protein misfolding or conformational diseases.

Types of dementia (or neurodegenerative disorders that may cause dementia), are characterized by the accumulation of certain proteins (Chui et al., 2007; Kovacs et al., 2010).

In AD, one of the two key proteins is beta amyloid, which is found in amyloid-positive deposits distributed both diffusely and as dense-core plaques. The other important protein is phosphorylated tau (p-tau), which accumulates within neurons in the form of neurofibrillary tangles (Braak & Braak, 1991). These two proteins are believed to interact and contribute to cognitive impairment (Desikan et al., 2012).

Phosphorylated tau is also the main protein that accumulates in some forms of FTLD, in CBD and PSP, being found in round tau-positive cytoplasmic inclusions in the behavioural variant of FTLD, in astrocytic plaques in CBD and in tufted astrocytes in PSP. In some forms of FTLD, however, tau negative and TDP 43-positive inclusions are present (Mackenzie et al., 2010). The key protein in LBD is alpha-synuclein, which is found in neuronal inclusions, while in CJD, it is the prionic protein (PrP), the disease being characterized by fine and coarse granules, as well as plaques, that stain positive for this protein.

VaD may result from various forms of cerebrovascular injury, including post-stroke syndromes (O´Brien et al., 2003). The challenge of identifying the pathological substrates for VaD is complicated by the heterogeneous nature of cerebrovascular disease and the coexistence of other pathologies, including Alzheimer-type lesions. It has been suggested that subcortical ischemic vascular dementia (SIVD), which is associated with small-vessel disease, lacunar infarcts, and deep white matter changes, is the most important subtype of vascular cognitive impairment (Roman et al., 2002; Kalaria et al., 2004).

Overlap between dementia subtypes

Thorough analysis of the morphological and biochemical markers of proteins (such as tau, beta amyloid, synuclein and TDP 43) enables classification of the vast majority of cases of dementia. On the other hand, there can be considerable overlap in the patterns of accumulation of different proteins, and this often leads to a diagno-

sis of co-occurrence of different subtypes of dementia (Kovacs & Budka, 2010; Kovacs et al., 2010). This raises the question as to whether the mere detection of a particular aggregated protein means that it is really the substrate for the dementia. Several studies comparing clinical and neuropathological diagnoses (Brunnstrom & Englund, 2009; Jellinger, 2009) have found a relatively low mean rate of agreement for the purely clinical diagnoses, which is mainly attributable to the high degree of comorbidity in neuropathological disorders (Jellinger, 2006; Kovacs et al., 2008; Richards & Brayne, 2009; Brunnstrom & Englund, 2009). Diagnosing dementia types is difficult for clinicians and researchers alike, especially in later stages of the disease, when comorbidity is more likely to occur (Richards & Brayne, 2009). This implies that the neuropathological diagnosis of "comorbidity of particular types of dementia" may in some cases be a suitable diagnosis in itself. Since medical treatment for patients with dementia is only indicated when the clinical diagnosis is clearly established, cases of comorbidity might be problematic. Besides, the prevalence of cases showing comorbidity is not clear, nor is it clear whether there is a particular clinical cognitive pattern that can be attributed to comorbidity, and what this clinical pattern could be. The present thesis proposes a new way of approaching dementia diagnosis, from a multidimensional point of view.

Aim and outline of the thesis

This thesis considers dementia as a multifactorial disease and examines the correlations between clinical, neuropathological and neuroimaging changes in demented patients. The main aim of the research underlying this thesis was to correlate clinical and neuropathological findings in dementia. The specific research questions were therefore related to (1) correlations between clinical and neuropathological diagnoses and (2) clinical features of the dementia syndrome.

The questions relating to (1) included "Are dementia types different diseases or should a dementia syndrome be understood as part of a spectrum of diseases?" and "What is the relationship between current clinical and neuropathological diagnoses in dementia?"

The questions relating to (2) included "Are there any clinical features common to all types of dementia or can different profiles be defined?" and "Are there differences in dementia types regarding the presence of vascular risk factors or associated symptoms such as neuropsychiatric symptoms?"

The research was based on three different databases. The first was that of the Maastricht Aging Study (MAAS), carried out at Maastricht University in the Netherlands (Jolles et al., 1995), while the second and third were composed of data from the Netherlands Brain Bank (NBB) (Amsterdam) and the Brain Bank of Navarre (Pamplona, Spain). The present thesis brings together five studies that made use of these three data sources.

In order to answer the first question, we investigated the rate of agreement between clinical and neuropathological diagnoses of dementia, as is reported in Chapter 2. Chapter 3 focuses on the relationship between the most prevalent pathological changes co-occurring in the same brain, namely Alzheimer disease (AD)-related, Lewy body (LB)-related and vascular pathology.

The second main question, “Are there any clinical features common to all types of dementia or can different profiles be defined?”, was addressed by studying cases with confirmed neuropathological diagnoses, as is discussed in Chapters 4 and 5. In Chapter 4, the role of vascular risk factors in dementia is explored by assessing the prevalence of a range of risk factors in cases of dementia with neuropathologically confirmed AD, vascular dementia (VaD), or mixed AD plus vascular pathology. Chapter 5 then reports on an investigation of the prevalence of neuropsychiatric symptoms (NPSs) associated with dementia in cases with neuropathologically confirmed AD and VaD.

Results concerning the MRI markers of early stage AD are presented in Chapter 6. Previous neuropathological studies have reported the parahippocampal gyrus to be the first structure to be affected in AD, but neuroimaging studies have been focusing on hippocampal volume as a biomarker for the early stages of AD. We assessed the role of changes in the parahippocampal gyrus as a neuroimaging biomarker for early stage AD, by measuring the volume of this structure in healthy volunteers as well as in patients with amnestic mild cognitive impairment (aMCI) and mild AD. The power of the parahippocampal gyrus volume to discriminate healthy participants from individuals with mild AD was compared with results using hippocampal volume.

The overall conclusions of this thesis are presented in the chapter entitled “Conclusions”.

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CHAPTER 2

Co-occurrence of different pathologies in dementia: implications for dementia diagnosis

Carmen Echávarri, Saartje Burgmans, Maria Cristina Caballero, Federico García-Bragado, Frans R.J. Verhey and Harry B.M. Uylings. Co-occurrence of different pathologies in dementia: implications for dementia diagnosis. Journal of Alzheimer´s Disease (2012) 30 (4): 909-917.

The standard for differentiating between dementia subtypes is currently based on neuropathological changes, and follows traditional nosological classifications. However, the high incidence of comorbid neuropathologies complicates the differentiation between dementia diagnoses in the clinic. The aim of this study was to investigate the grades of agreement between clinical and neuropathological diagnoses in neurodegenerative disorders, to compare them with rates found in previous studies, and to propose implications for dementia diagnostics. 200 patients who donated their brains to the Brain Bank of Navarre (Pamplona, Spain). All patients had been diagnosed with a neurodegenerative disorder during life (clinical diagnosis) and post-mortem (neuropathological diagnosis). We studied a sample of patients with a short average time interval between the last clinical assessment and death (4.6 months). Overall, there was a mean grade of agreement of 44.0% between the clinical diagnosis and the pure neuropathological diagnosis (i.e. without co-morbid neuropathological disorders). This grade of agreement differed between dementia subtypes: e.g. 85 % for prion disease, 49 % for Alzheimer´s disease, and 0 % for Lewy body dementia. Our data confirm that co-occurrence of multiple neuropathological disorders is very common in individuals with dementia, and that the underlying neuropathology often differs from the neuropathology implied by the clinical diagnosis. These findings support a multidimensional approach to diagnosing dementia, in which dementia syndromes are not categorized into diagnostic subtypes, but are seen as syndromes characterized by a combination of various neuropathological dimensions.

Introduction

Dementia is a syndrome resulting from neuropathological changes in the brain (Kovacs et al., 2012; Uylings et al., 2002), and dementia subtypes are currently classified according to the pattern of these changes. Whereas the pathological diagnoses of neurodegenerative disorders usually follow traditional nosological classifications, previous research suggests that the clinicopathological correlation is weak. Several studies have compared clinical and neuropathological diagnoses (Brunnstrom et al., 2009; Galasko et al., 1994; Gay et al., 2008; Jellinger, 2009; Lim et al., 1999; Massoud et al., 1999; Nelson et al., 2012; Sabbagh et al., 2009; Victoroff et al., 1995) showing a relatively low mean rate of agreement for the pure diagnoses (see Figure 1). This is mainly due to the high degree of comorbidity of neuropathological disorders (Brunnstrom & Englund, 2009; Gay et al., 2008; Victoroff et al., 1995; Jellinger, 2006). Hence, the traditional nosological classification approach as the gold standard for differentiating between dementia subtypes has been called into question (Richards &, Brayne, 2009).

Further investigation is necessary for several reasons. First, most previous studies focused mainly on Alzheimer's disease (AD) and vascular dementia (VaD) (Galasko et al., 1994; Gay et al., 2008; Massoud et al., 1999), and failed to take other, less frequent neurodegenerative disorders into account. Second, previous studies reported contradictory results regarding the agreement between clinical and neuropathological diagnoses (Kovacs et al., 2008). Third, the time interval between the clinical and neuropathological diagnoses in reported investigations tended to be rather long. For instance, Brunnstrom et al. (2009) reported that 35% of the patients had not visited a physician within the last 2 years before death (Brunnstrom & Englund, 2009). This likely affects the concordance between the clinical and neuropathological diagnoses, since neuropathological findings may change in the course of the illness.

The main aim of the present study was to investigate the rates of agreement between clinical and neuropathological diagnoses in neurodegenerative disorders, to compare them with previous studies, and to propose implications for dementia diagnostics. We investigated a more extensive spectrum of neurodegenerative disorders, and included 200 patients with a short average interval between the clinical and neuropathological diagnoses (an average of 4.6 months).

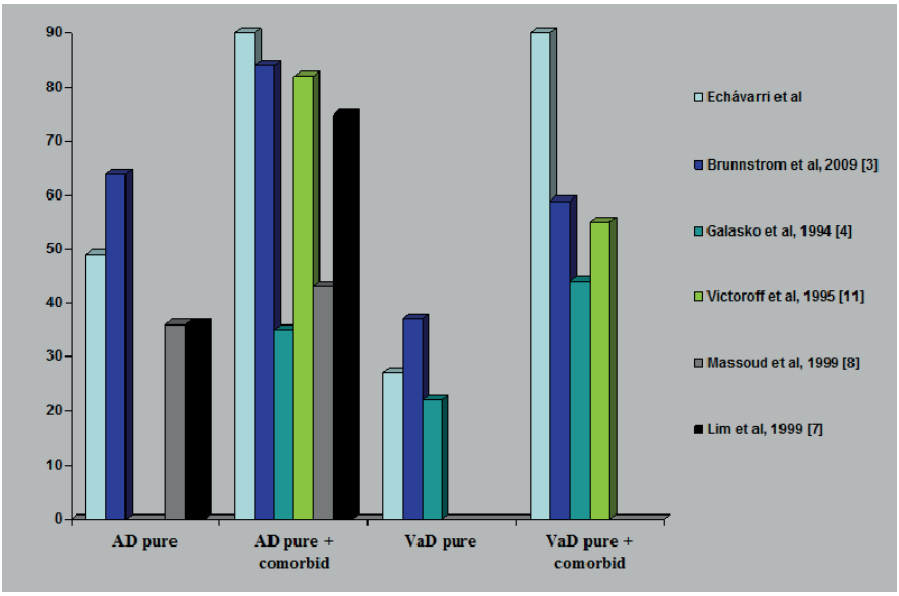

Figure 1.
Note. Rates of agreement (%) between neuropathological and clinical diagnoses of AD and VaD in different studies. The diagnoses on the x-axes represent the neuropathological diagnoses.

Materials and Methods

Sample

The study included all the 231 participants who donated their brains to the Brain Bank of Navarre (Pamplona, Spain) between 2004 and 2011. 31 control participants, defined as subjects without clinical symptoms and without neuropathological relevant findings, were excluded from the study. Finally, the sample consisted of 200 patients. All of them had been diagnosed with a neurodegenerative disorder during life (clinical diagnosis) as well as post-mortem (neuropathological diagnosis). The criteria used for the clinical diagnoses were derived from the literature, as follows: Alzheimer´s disease (AD) (McKhann et al., 1984), Lewy body dementia (LBD) (McKeith et al., 1996), Mixed Dementia (MD; i.e. Mixed AD and VaD) (Roman et al., 1993), frontotemporal lobar degeneration (FTLD) (Neary et al., 1998), progressive supranuclear palsy (PSP) (Litvan et al., 1996), corticobasal degeneration (CBD) (Boeve et al., 2003), motor neuron disease (MND) (Cairns et al., 2007), prion disease (Budka et al., 1995), Parkinson´s disease (PD) (Samii et al., 2004), vascular dementia (VaD) (Roman et al., 1993), Huntington disease (HD) (Vonsattel et al., 1985) and multisystem atrophy (MSA) (Gilman et al., 2008). The criteria used for neuropathological diagnoses were derived from the literature as follows: AD (Braak & Braak, 1991), LBD (McKeith et al., 1996), MD (Zekry et al., 2003), FTLD (Cairns et

al., 2007), PSP (Dickson et al., 2007), CBD (Dickson et al., 2002), MND (Cairns et al., 2007), prion disease (Budka et al., 1995), PD (Braak et al., 2003), VaD (Kalaria et al., 2004), HD (Vonsattel et al., 1985), MSA (Trojanowski et al., 2007) and AGD (Saito et al., 2004).

All clinical diagnoses were established by a multidisciplinary team of neurologist and geriatricians at the Navarre Hospitals Complex, who were blinded to the neuropathological data; diagnoses were based on medical history, medical examination of the patient, course of symptoms, and computerized tomography (CT) and/or magnetic resonance imaging (MRI) scans. A neurologist/geriatrician was present at the first clinical interview with the patient, in order to establish the diagnoses, and at most of the follow-up visits. Neuropsychological assessment was additionally performed in a majority of the patients, but not in all. If the dementia diagnosis had been changed during the time the patient was followed, the most recent diagnosis was used for the present clinicopathological comparison. Clinical information was obtained through detailed interviews with the relatives, except for cases in which the clinical diagnoses had been changed; in these cases the patient was re-examined. The interviews were performed during routine visits or when patients were admitted to the hospital for any reason. Only 3 patients couldn't be examined in a follow-up visit. For these 3 patients clinical diagnosis was made then exclusively by interviews with their informants. In our total sample of 200 patients, the average time interval between the last clinical assessment and death was 4.6 (SD 5.7) months. 96% of our patients had their last clinical assessment within 1 year before death, and only 1 patient had a time interval of over 2 years.

Neuropathological diagnoses were established by one neuropathologist of the Brain Bank of Navarre. A neuropathological diagnosis of mixed dementia (MD) was established when vascular components and Alzheimer-related pathology were both considered to be severe enough to contribute to the dementia symptoms. For vascular pathology, we used the criteria described by Kalaria et al. (2004) (Kalaria et al., 2004): (a) a large infarct or several infarcts (> 50 ml tissue loss) or (b) multiple microinfarcts (more than 3 with a minimum diameter of 5 mm) or small vessel disease (hyalinization, amyloid angiopathy, lacunar infarcts, and/or perivascular changes). According to the Kalaria et al. criteria, cases of MD include Alzheimer related pathology at a Braak stage above III (Kalaria et al., 2004).

All patients in the PD group had cognitive impairment, defined as cognitive dysfunction involving memory and/or other domains, but not dementia, which means without evident functional impairment. Demographic data for the patients, by neuropathological diagnosis, are presented in Table 1. Written informed consent was obtained from all patients or their relatives. The study was approved by the local Medical Ethics Committee of the Tissue Brain Bank of Navarre and Navarre Hospitals Complex (Navarre Health Service).

Table 1. Demographic data by neuropathological diagnosis

		Age (years)	Duration of illness (years)	Last assessment-death (months)	Weight (grams)	Sex
	n	Mean (range)	Mean (range)	Mean (range)	Mean (range)	% women
All	200	78.7 (15-100)	6.9 (1-29)	4.6 (1-64)	1080.2 (575-1800)	55
AD	52	82.1 (56-97)	6.5 (1-14)	2.4 (1-15)	1060.3 (850-1800)	69
LBD	1	84.0	21.0	24.0	1150.0	0
MD	31	83.4 (50-100)	5.9 (1-19)	4.5 (1-24)	1116.2 (725-1400)	52
FTLD	4	69.5 (64-73)	10.5 (3-15)	4.3 (1-37)	882.5 (750-980)	50
PSP	11	77.5 (63-92)	5.5 (3-10)	1.1 (1-4)	1111.8 (800-1400)	54
CBD	4	71.2 (56-85)	6.2 (5-7)	16.7 (1-64)	991.2 (840-1200)	25
MND	5	57.5 (49-73)	2.7 (2-3)	2.6 (1-6)	1290.0 (950-1500)	60
Prion disease	12	64.0 (43-84)	3.5 (1-5)	1.4 (1-5)	1177.6 (850-1700)	27
PD	2	78.5 (78-79)	12 (4-20)	3 (1-5)	1212.5 (1150-1275)	50
VaD	4	72.2 (51-84)	9.7 (4-26)	3.4 (1-9)	967.0 (918-1100)	50
HD	4	61.7 (36-76)	23.0 (6-29)	2.6 (1-6)	1026.2 (700-1255)	25
MSA	1	70.0	6.0	2.0	925.0	100
AGD	4	80.7 (71-85)	5.0 (1-14)	3 (1-5)	1199.2 (1125-1328)	50
Comorbid diagnoses	56	83.2 (65-97)	7.8 (1-20)	5.9 (1-23)	1053.6 (650-1350)	59
Other diagnoses	9	59.3 (15-82)	6.5 (1-19)	1.5 (1-4)	1129.3 (575-1390)	44

Note. AD Alzheimer´s disease, LBD Lewy body dementia, MD mixed dementia, FTLD frontotemporal lobar degeneration, PSP progressive supranuclear palsy, CBD corticobasal degeneration, MND motor neuron disease, PD Parkinson´s disease, VaD vascular dementia, HD Huntington disease, MSA multisystem atrophy, AGD argyrophilic grain disease. Comorbid diagnoses include brains showing more than one neuropathological diagnosis, Other diagnoses include 5 cases with a tumor, 1 case with multiple sclerosis (MS), 1 case with both a brain tumor and MS, 1 case with hereditary spastic paraplegia (suspected diagnosis of FTLD) and 1 case with ceroidolipofuscinosis. Duration of illness = years from onset of symptoms to death; Weight = weight of the whole brain (grams)Histopathological procedure

The histopathological procedures were carried out according to standard practice at the Tissue Brain Bank of Navarre. The left cerebral hemisphere was frozen and the right hemisphere was paraffin-embedded for microscopic examination. Before freezing, all findings of the macroscopic inspection of both hemispheres were carefully recorded (atrophy, vascular lesions, etc.). If any suspected pathology was found in the left hemisphere, the left hemisphere was also paraffin-embedded for microscopic examination. Following fixation in formaldehyde (10%) for approximately three weeks, the brain was sectioned. The procedure for fixing and sectioning was that recommended by BrainNet Europe. The macroscopic examination of the brains included photographing, weighing, pH measurement, taking cerebrospinal fluid samples (where possible) and macroscopic description. Twenty-four paraffin blocks were prepared, and at least one slice from each paraffin block was stained with hematoxylin and eosin (H&E). The immunohistochemical stains used

for each protein are described in Table 2. The antibody dilutions were: tau (Mouse monoclonal antibody, Novocastra, NCL-Tau-2, clone Tau 2)1:100, β amyloid (Mouse monoclonal antibodybody, Novocastra, NCL-B- amyloid, clone 6F-3D 1:200, TDP-43 (Abnova Corporation mouse monoclonal antibody) 1:1500, PrP (Mouse monoclonal antibody, Dako Cytomation, clone 3F4) 1:100, α-synuclein (Liquid mouse monoclonal antibody, Novocastra, NCL-L-ASIN, clone KN51) 1:50, ubiquitin (Lyophlized monoclonal antibody, Novocastra, NCL-UBICm, clone FPM1) 1:700, and α- β crystallin (Mouse monoclonal antibody, Novocastra, clone ABCrys-512) 1:100.

Table 2. Immunostains used for the neuropathological diagnoses

REGIONS	α synuclein	ß-Amyloid	Tau	TDP-43	Ubiquitin	α-ß crystalline	PrP
Cingulate gyrus	x	X					
Motor gyrus		X	x				
Insular cortex			x				
Frontal gyrus	x	X	x	x	X	X	
Anterior thalamus		X	x				
Medial thalamus							
Posterior thalamus							
Putamen			x				
Globus pallidus			x				
Hippocampus	x	X	x	x	X		
Frontal cortex	x	X	x	x	X	X	
Temporal cortex	x	X	x	x	X		
Parietal cortex	x	X	x				x
Occipital cortex		X	x				
Frontal white matter							
Substantia nigra	x	X	x				
Pons	x	X	x				
Medulla oblongata	x	X	x				
Amygdala	x	X	x			X	
Caudate-accumbens		X	x	x	X		
Olfactory bulb	x	X	x	x	X		
Nucleus basalis of Meynert	x		x				
Cerebellum (vermis)		X					x
Cerebellum (dentate nucleus)							

Statistical analysis

The rate of agreement between clinical and neuropathological diagnoses was calculated by dividing the number of cases with agreement between the clinical and neuropathological by the total number of clinically diagnosed cases. With Epi Info Statcal program, version 6 (November 1993), we compared these rates of agreement between the diagnostic groups using an exact Fisher test. We performed this comparison only for the most frequent clinical diagnoses (AD, LBD, MD, FTLD, PSP, and Prion disease), since the n of the other diagnoses was not large enough for a reliable statistical comparison.

Results

Prevalences of clinical and neuropathological diagnoses

Table 3 shows the frequencies of the clinical diagnoses. The most frequent clinical diagnosis was AD, with 80 cases (40 %), followed by MD with 20 cases (10 %) and FTLD with 18 cases (9 %). Table 1 shows the frequencies of the neuropathological diagnoses. The most frequent neuropathological diagnosis was AD with 52 cases (26 %), followed by MD with 31 cases (16 %), prion disease with 12 cases (6 %), PSP with 11 cases (6 %) and FTLD with 4 cases (2 %). Comparing Table 1 and 3, it is notable, that the number of cases neuropathologically diagnosed as MD appears to be higher than the number of cases clinically diagnosed as MD. This is in contrast to the other dementias. In addition, fifty-six patients (28 %) had more than one neuropathological diagnosis (see the supplementary table for a detailed overview of the co-occurrence of all neuropathologies). Nine patients had 'other diagnoses', including 5 with a tumor, 1 with multiple sclerosis (MS), 1 with both a brain tumor and MS, 1 with hereditary spastic paraplegia (suspected diagnosis of FTLD) and 1 with ceroidolipofuscinosis.

Dividing the sample into two groups according to age, we found differences between the moderately old (younger than 80 years) and very old (80 years or older) patients. Of the 86 patients younger than 80 years, 20 (23 %) had comorbidity, compared to 36 (32 %) of the 114 patients older than 80 (difference non-significant).

Agreement between clinical and neuropathological diagnoses

Table 3 and Figure 2 present the agreement between clinical and neuropathological diagnoses. With respect to the concordance between the clinical diagnosis and the pure neuropathological diagnosis, we found an overall agreement rate of 44 % (Table 3). The agreement was largest in patients with the clinical diagnoses prion disease (85 %), MND (83.3%) and MD (80%). The agreement was substantially lower in patients with the clinical diagnoses LBD (0 %), FTLD (22 %), PD (22 %), VaD (27 %), and AD (49 %). When we consider the concordance of the clinical diagnosis with

both the pure and comorbid neuropathological diagnosis (i.e. two or more neuropathological diagnoses were established, and one of these neuropathological diagnoses is in agreement with the clinical diagnosis), we found an overall agreement rate of 66 %. The agreement was substantially larger particularly in patients with the clinical diagnosis MD (100%), MND (100%), AD (90 %) and LBD (54 %), see Table 3. In contrast, the agreement in FTLD and PD was still low (28 % and 33 % respectively). Misdiagnoses (i.e. the neuropathological diagnosis is not in agreement with the clinical diagnosis) were most frequent in FTLD (72 %), PD (67 %), and LBD (46 %).

The exact Fisher tests show that AD diagnoses had a significantly higher rate of agreement compared to LBD and FTLD; MD diagnoses showed a significantly higher rate of agreement compared to LBD and FTLD; and FTLD showed a significantly higher rate of agreement compared to PSP and prion disease (see table 3).

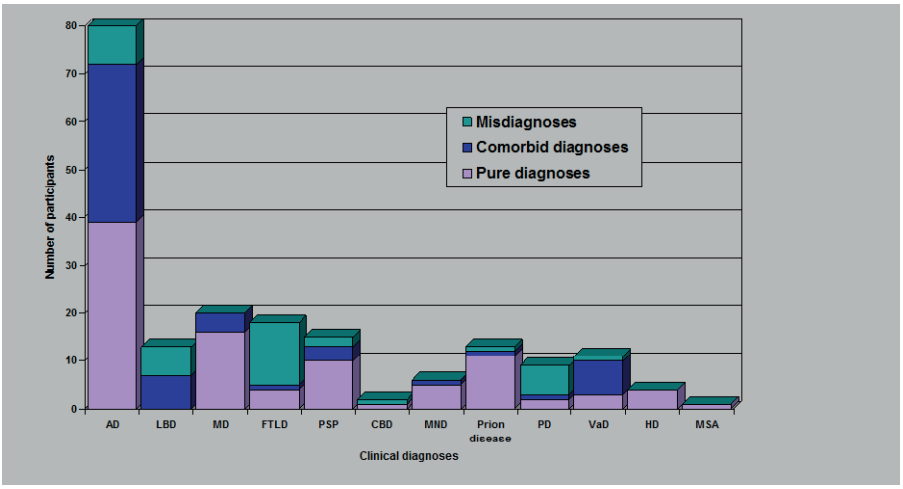

Figure 2. Neuropathological diagnosis for each clinical diagnosis.

Note. AD Alzheimer´s disease, LBD Lewy body dementia, MD mixed dementia, FTLD frontotemporal lobar degeneration, PSP progressive supranuclear palsy, CBD corticobasal degeneration, MND motor neuron disease, PD Parkinson´s disease, VaD vascular dementia, HD Huntington disease, MSA multisystem atrophy, AGD argyrophilic grain disease; misdiagnosis = the neuropathological diagnosis was not in agreement with the clinical diagnosis; comorbid diagnosis = two or more neuropathological diagnoses were established, and one of these neuropathological diagnoses was in agreement with the clinical diagnosis; pure diagnosis = the neuropathological diagnosis was in complete agreement with the clinical diagnosis.

Table 3. Agreement between clinical and neuropathological diagnoses.

Clinical diagnosis		Agreement with pure diagnosis	Agreement with pure and co-morbid diagnosis	Misdiagnosis
	N	n (%)	N (%)	n (%)
All	200	88 (44.0)	132 (66.0)	26 (13.0)
AD	80	39 (48.7[a])	72 (90.0[1])	8 (10.0)
LBD	13	0 (0.0)	7 (53.8)	6 (46.2)
MD	20	16 (80.0[b])	20 (100.0[2])	0 (0.0)
FTLD	18	4 (22.2[c])	5 (27.7[3])	13 (72.3)
PSP	15	10 (66.6[d])	13 (86.6)	2 (13.4)
CBD	2	1 (50.0)	1 (50.0)	1 (50.0)
MND	6	5 (83.3)	6 (100.0)	0 (0.0)
Prion disease *	13	11 (84.6[e])	12 (92.3)	1 (7.7)
PD	9	2 (22.2)	3 (33.3)	6 (66.7)
VaD	11	3 (27.2)	10 (90.9)	1 (9.1)
HD	4	4 (100.0)	4 (100.0)	0 (0.0)
MSA	1	1 (100.0)	1 (100.0)	0 (0.0)
Other **	8	8 (100.0)	8 (100.0)	0 (0.0)

Note. For abbreviations see the legend to Table 1. Pure: the neuropathological diagnosis was in complete agreement with the clinical diagnosis; co-morbid = two or more neuropathological diagnoses were established, and one of these neuropathological diagnoses was in agreement with the clinical diagnosis; misdiagnosis = the neuropathological diagnosis was not in agreement with the clinical diagnosis. * Of the 13 cases with a clinical diagnosis of prion disease, 9 had Creutzfeldt-Jakob disease and 4 FFI. ** Including 5 cases with a tumor, 1 with multiple sclerosis (MS), 1 with both a brain tumor and MS and 1 with ceroidolipofuscinosis.

[1] AD diagnoses had a significantly higher rate of agreement compared to LBD and FTLD (p = 0.003 and 0.000 respectively); [2] MD diagnoses showed a significantly higher rate of agreement compared to LBD and FTLD (p = 0.001 and 0.000 respectively); [3] FTLD showed a significantly lower rate of agreement compared to PSP and prion disease (p = 0.002 and 0.001 respectively)

[a] AD diagnoses had a significantly higher rate of agreement compared to LBD and FTLD (p = 0.000 and 0.000 respectively); [b] MD diagnoses showed a significantly higher rate of agreement compared to LBD and FTLD (p = 0.000 and 0.000 respectively); [c] FTLD showed a significantly lower rate of agreement compared to PSP and prion disease (p = 0.001 and 0.000 respectively); [d] PSP showed a significantly higher rate of agreement compared to LBD (p = 0.001); [e] Prion disease showed a significantly higher rate of agreement compared to LBD (p = 0.000).

Supplementary table. Most frequent clinical diagnoses (rows) and neuropathological diagnoses (columns).

	AD	LBD	PD	AD +LBD	AGD	AD+AGD	FTLD	AD+ alpha syn inclusions	CBD	LBD+CBD	TDP-43 + AGD	AD+TDP-43	AD + TDP-43 inclusions	TDP-43+ LBD+ AGD	MND	AD + CBD	Prion diesease	AD + prion disease	PSP	AD+CBD+PSP	BLD+AGD+PSP	BLD + AGD	AD+PSP	PSP +AGD	MD (AD + VaD)	VaD	HD	MSA	Others	TOTAL
AD	39	1		14	2	3		2	2	1	2#	4													10					80
LBD				6				1				1	1					1							2	1				13
MD	5			4																					11					20
FTLD	6				1		4		1					1		1	1						1		1				1	18
PSP				1	1														10	1	1		1							15
CBD	1								1																					2
MND												1			5															6
Prion disease																	11	1				1								13
PD	1		2	4																		1		1						9
VaD																			1						7	3				11
HD																											4			4
MSA																												1		1
Other																													8	8
	52	1	2	29	4	3	4	3	4	1	2	6	1	1	5	1	12	2	11	1	1	2	2	1	31	4	4	1	9	200

Note. # 1 of these 2 patients showed motor neuron involvement. AD Alzheimer´s disease, LBD Lewy body dementia, MD mixed dementia, FTLD frontotemporal lobar degeneration, PSP progressive supranuclear palsy, CBD corticobasal degeneration, MND motor neuron disease, PD Parkinson´s disease, VaD vascular dementia, HD Huntington disease, MSA multisystem atrophy, AGD argyrophilic grain disease.

Discussion

Our results confirm that the agreement between clinical and neuropathological diagnoses in neurodegenerative disorders is far from optimal. The majority of the clinical diagnoses did not fully correspond with the neuropathological diagnoses. This was mainly due to the fact that co-occurrence of neuropathological disorders was very common, i.e., a large number of patients had multiple neuropathological diagnoses at the post-mortem examination. A possible explanation for the high frequency of co-morbid depositions in dementia is that certain protein deposits

may influence the formation of other deposits (Kovacs et al., 2012). For instance, there is evidence that tau and α synuclein can promote each other's fibrillization (Clinton et al., 2010). This is in line with our finding that LB (Lewy body)-related pathology frequently co-occurred with AD pathology, and vice versa (see Table 3).
Compared to previous studies (Kovacs et al., 2012; Brunnstrom & Englund, 2009; Galasko et al., 1994; Jellinger, 2006; Echávarri et al., 2011), our agreement rates are relatively high. This may be due to the fact that our sample had a relatively short average time interval between the last clinical assessment and death. This relatively high concordance compared to other studies is particularly evident with respect to AD and VaD (See Figure 1). When we included both the pure and comorbid neuropathological diagnoses in our analysis, our sample had the highest agreement rates for AD as well as for VaD, comparing to other studies. When we included only the pure diagnosis, our sample had the second highest agreement rates for AD and VaD (Fig.1). Our results also show that the co-occurrence of neuropathologies is likely to increase with advanced age, as the incidence of comorbidity was higher in the patients above the age of 80. This is in line with other results for community-dwelling older persons and autopsy series (Galasko et al., 1994; Gay et al., 2008; Gilman et al., 2008; Echávarri et al., 2011). Note that the mean age of our sample was similar to those in the studies that we used to compare our concordance rates with.

In our sample, the agreement between clinical and neuropathological diagnoses differed substantially between different neurodegenerative disorders. Patients with a clinical diagnosis of LBD, FTLD, PD, VaD and MD had low agreement rates, while patients with prion disease, HD, MSA or MND had high agreement rates. These data are largely in agreement with previous reports, particularly regarding LBD and VaD (Brunnstrom & Englund, 2009; Gay et al., 2008; Massoud et al., 1999), supporting the view that both diseases rarely appear as pure pathologies but are very likely to be found in association with other pathologies. Another notable finding is the high prevalence of prion disease. Navarre is a region bordering on the Basque Country, where the rate of prion disease is the highest in Europe (Zarranz et al., 2006). The 4 cases diagnosed in our series with fatal familial insomnia (FFI) were members of the same family. They showed a mutation of the prionic protein gene D178N (Trojanowski et al., 2007), which has been shown to be more frequent in Spain (specifically in Basque Country and Navarre) than in other countries in Europe (Iriarte et al., 2007).

The current results indicate that diagnosing patients with the traditional nosological classification approach is rather artificial, since the underlying neuropathology often differs from the neuropathology implied by the dementia subtype. This underlines the necessity of a multidimensional approach as advocated by Richard and Brayne (Richard & Brayne, 2009); that is, dementia, especially in older patients, should not be categorized into isolated subtypes, but should be seen as a diffuse clinical syndrome representing the gradual accumulation of multiple pathologies.

Therefore, a diagnosis of dementia should not be specified by a dementia subtype, but should reflect a spectrum of neuropathological dimensions such as the formation of beta amyloid, phosphorylation of tau, vascular changes and other pathologies. For instance, if a patient is diagnosed with AD in vivo, the likelihood of finding AD-related pathology plus a comorbid pathology - such as Lewy bodies and vascular pathology - in the neuropathological assessment is higher than the likelihood of only finding pure AD.

We think that a multidimensional approach will not only increase the accuracy of dementia diagnoses, but will also lead to a better understanding of neurodegenerative diseases by clinicians, since it stresses that these diseases have a multifactorial nature. An important question is what effect a multidimensional approach has on the diagnosis of dementia. Instead of being diagnosed with a specific dementia subtype, a patient will then receive a less specific diagnosis of 'dementia', including a spectrum of symptoms. This diagnosis is closer to the truth and will probably lead to fewer misdiagnoses. It also takes into account the uncertainties that exist with respect to the role of biomarkers in dementia subtypes. Although many biomarkers, such as white matter lesions and amyloid depositions, are nowadays seen as indicators of specific dementia subtypes, the specificity of these pathological changes is still unclear. Note that we do not want to induce an attitude in clinicians of being oversimplistic by making a generic dementia diagnosis. Instead, we want to encourage clinicians to recognize different neuropathological features and the specific contribution of each of them to the dementing process. We wish they take into account the large spectrum of neuropathological findings and the complexity of neurodegenerative disorders

A few methodological issues need to be mentioned. First, previous studies often used FTLD as an umbrella term to represent a spectrum of pathological entities, including PSP and CBD. In our study we considered FTLD, PSP, and CBD as separate entities, based on the clinical syndromes previously described (Neary et al.,1998; Sha et al., 2006). If we had included CBD and PSP in the FTLD group, the clinicopathological agreement for FTLD would have been higher. Second, the patients in our sample were studied retrospectively and were diagnosed in vivo. Cases of AD, LBD, and VaD met criteria for both possible and probable dementia. It is possible that the use of criteria for probable diagnosis, excluding possible diagnosis according to NINCDS-ADRDA criteria (McKhann et al., 1984), would have resulted in a better concordance. Third, the high prevalence of FTLD and prion disease demonstrates that our sample is not entirely representative of the European population. This is mainly caused by the fact that patients with more exotic neuropathologies are more likely to become Brain Bank donors, and by the high prevalence of prion disease in the region of Navarre and Basque Country (Jicha et al., 2008, Schneider et al., 2009).

To conclude, we believe that these data support the notion that a multidimensional approach to diagnosing dementia would increase the accuracy of clinical diagnoses as well as understanding the complexity of neurodegenerative diseases. In addition, these data underline the importance of neuropathological confirmation of dementia diagnoses in studies about dementia subtypes.

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CHAPTER 3

Associations between Alzheimer pathology, Lewy bodies and cerebrovascular lesions in demented patients

C. Echávarri, S. Burgmans, T. Tuñón, I. Jáuregui, F.R.J. Verhey and H.B.M. Uylings. Associations between Alzheimer pathology, Lewy bodies and cerebrovascular lesions in demented patients. Submitted.

In patients with Alzheimer's disease (AD), the typical AD neuropathological features (neurofibrillary tangles and senile plaques) frequently co-occur with other neuropathological findings, particularly cerebrovascular lesions (CVLs) and Lewy bodies (LBs). The relationship between the severity of AD pathology and these comorbid pathologies is unclear. The present neuropathological study investigated the associations between the degree of Alzheimer pathology (i.e., Braak stage), CVLs and LBs in demented patients. The study included 101 patients who donated their brains to the Brain Bank of Navarre between 2004 and 2011. All patients were diagnosed in vivo with dementia, and received a post-mortem neuropathological diagnosis of AD. The mean time interval between the last in vivo clinical assessment and death was 4.2 months. Patients with large-vessel disease were excluded. With respect to the whole-brain analyses, we found a significant positive relationship between AD Braak stage and CVL severity. As for the regional analyses, we only found a significant correlation between AD Braak stage and CVL severity in the frontal cortex. No relationship was found between Braak and LB stage. Our study supports a positive association between AD pathology and CVLs in demented patients with AD, suggesting that AD and CVLs may have a common aetiopathogenesis. In contrast, we found no evidence to suggest a direct link between AD and LB pathology in these patients.

Introduction

Neurofibrillary tangles (NFTs) and senile plaques, including neuritic plaques (NPs), are considered to be neuropathological hallmarks of Alzheimer's disease (AD) (Braak & Braak., 1990; Braak et al., 2006; Montine et al., 2012). The severity of AD pathology is generally staged according to Braak and Braak criteria, taking into account the amount and site of the deposition of NFTs and NPs (Braak & Braak, 1991). Our data at the Navarre Brain Bank show that most donors who meet the clinical criteria for AD have a high Braak and Braak stage, viz. V-VI (Echávarri, 2011). It is well-known that other neuropathological features frequently co-exist in AD brains. Findings that most frequently co-occur with AD are cerebrovascular lesions (CVLs) and Lewy bodies (LB) (Snowdon et al., 1997; de Leeuw et al., 2004; Schneider et al., 2007; Parkkinen et al., 2008; Foster et al., 2010; Nelson et al., 2010). Furthermore, AD-related pathology can be found together with tauopathies such as corticobasal degeneration (CBD), progressive supranuclear palsy (PSP), or TDP-43 inclusions (Tomlinson et al., 1970; Jellinger, 2006; Nelson et al., 2007; Kovacs et al., 2008; Brunnstrom & Englund, 2009; Echávarri et al., 2011). Comorbid neuropathological processes are thus very common in Alzheimer patients. It is, however, unclear whether there is a relationship between the severity of AD and these comorbid neuropathological features.

To date, studies investigating the relationship between CVLs and Braak stage have produced inconsistent results. Some research has shown a positive relationship, with higher neuritic Braak stages co-occurring with an increased incidence and severity of vascular pathology (Jellinger, 2003; Jellinger, 2008; Jellinger, 2010), while other studies have found an inverse relationship (Goulding et al., 1999; Jellinger, 2000; Sonnen et al., 2011), with more AD pathology correlating with fewer CVLs. Contradictory results were also found when studying the relationship between the severity of AD- and LB-related pathologies. While Oinas et al. (2009) (Oinas et al., 2009) found a positive correlation between Braak stage for AD and the extent of LB-related pathology (Oinas et al., 2009), other researchers have reported the opposite (Selikhova et al., 2009; Compta et al., 2011; Sonnen et al., 2011). All the aforementioned studies were performed post-mortem. Regarding the relationship between AD and CVL severity, the contradictory findings may be explained by the lack of consensus in defining the severity of CVLs. On the other hand, with respect to the association between AD and LB severity, there is a lack of consensus in the classification of the stages of the pathologies, and again this may explain the inconsistent results.

The main aim of the present study was to investigate whether or not there is a correlation between the degree of Alzheimer pathology (i.e., Braak stage) and vascular lesions and LBs. We investigated 101 brains of demented individuals who had donated their brains to the Brain Bank of Navarre (Pamplona, Spain). The patients were diagnosed in vivo with dementia, and post-mortem neuropathological diag-

noses confirmed AD. The sample consisted of 62 cases with pure AD and 39 cases with AD with comorbidity. Comorbidity was defined as a degenerative neuropathological process (sufficiently severe to warrant a separate diagnosis) other than AD. The secondary aim of the study was to add to previous data concerning an important issue namely the distinction between pure AD and AD with comorbidity. These data enabled us to investigate whether the relationship between AD pathology and CVLs is modified by a comorbid degenerative neuropathological process. We hypothesized that, if the severity of AD-related pathology and CVLs were related in patients with AD, the relationship might be mediated by the existence of a comorbid degenerative neuropathological process.

Materials and Methods

Sample

This study included 101 patients who donated their brains to the Brain Bank of Navarre between 2004 and 2011. They were diagnosed with dementia during life (clinical diagnosis) and a neuropathological diagnosis of AD was made at post-mortem. Specifically, of the 296 donors to the Brain Bank, 113 demented patients were found to have AD pathology on post-mortem analysis. Since the present study focused on small-vessel disease, twelve patients with large-vessel lesions (territorial infarcts or lobar bleedings) were excluded. The 101 subjects studied included cases with pure AD and cases with AD plus a comorbid neuropathological process. Comorbid diagnoses included degenerative pathologies other than AD. Neuropathological diagnoses of the 101 patients were as follows: 62 pure AD; 33 AD + LB dementia (LBD); 2 AD + frontotemporal lobe degeneration (FTLD) with TDP-43-positive inclusions; 2 AD + PSP; 1 AD + CBD; 1 AD + LB in the amygdala. Patients with LBs only in the amygdala were given the diagnosis of LBD.

In the last clinical assessment before death, all subjects scored 2 or 3 on the Clinical Dementia Rating (CDR) scale, indicating moderate to severe dementia (Morris et al., 1993). The mean time interval between this clinical assessment and death was 4.2 months. Neuropathological diagnoses were made by the same experienced neuropathologist of the Brain Bank of Navarre (MCC) according to the standard criteria for AD (Braak & Braak, 1991), LBD (McKeith et al., 1996), PSP (Dickson et al., 2007), CBD (Dickson et al., 2002), and frontotemporal lobar degeneration (FTLD) with TDP-43 positive inclusions (Cairns et al., 2007). The severity of small-vessel CVLs (from 0 to 2) was assessed for each case in a semi-quantitative way (see Table 1). In the group of pure AD, 17 cases (22.6%) were found to have at least one non-strategic lacunar infarct, 15 (24%) one strategic infarct (8 in caudate nucleus, 5 in thalamus and 2 in parietal cortex), 28 (45.2%) cortical amyloid angiopathy and 36 (58.1%) diffuse white matter changes. In the group of AD with comorbidity, 7 cases (17.9%) had at least one non-strategic lacunar infarct, 2 (5.1%) one

strategic infarct (both in caudate nucleus), 11 (28.2%) cortical amyloid angiopathy and 22 (56.4%) diffuse white matter changes.

Regional analyses of AD-related tissue were performed, in particular classifying the amount of NFTs semi-quantitatively according to the following criteria (lesions counted within the microscopic field with a diameter of 0.79 mm^2 at x200 magnification): NFT: none = 0; 1 = + (sparse); 2 = ++ (moderate, more than 6 NFT per mm^2); 3 = +++ (frequent, more than 20 NFT per mm^2)

An average score (between 0 and 3) was calculated, representing the density of NFTs in each region studied (hippocampus, amygdala, temporal and frontal cortex).

The LBD cases were classified into three types as in Mc Keith et al. (McKeith et al., 2005) on the basis of the amount of LB and their distribution across brain regions (i.e., into brainstem, limbic and neocortical types). The Braak and Braak stage for AD ranged from III to VI.

Demographic data for the patients are presented in Table 2. Written informed consent was obtained from all patients or their close relatives.

Table 1. Classification of cerebrovascular lesions (CVLs)

0	No concomitant CVLs
1	Minimal CVLs: 1-2 small lacunes, mild-moderate cerebral amyloid angiopathy (CAA), mild CVLs, mild leukoencephalopathy
2	Moderate CVLs: >2 lacunes, severe subcortical lacunar state, severe CAA with moderate CVLs, diffuse white matter lesions

Table 2. Demographical data

	Age (years) Mean (SD)	Gender (% female)	Time from diagnosis to death (years) Mean (SD)	Braak stage (AD) Mean (SD)
AD (n = 62)	82.5 (8.0)	65.0	6.5 (3.3)	5.5 (0.7)
AD + comorbidity (n = 39)	84.0 (7.4)	56.0	8.5 (4.4)	4.5 (1.0)
P	0.342	0.224	0.014	0.000

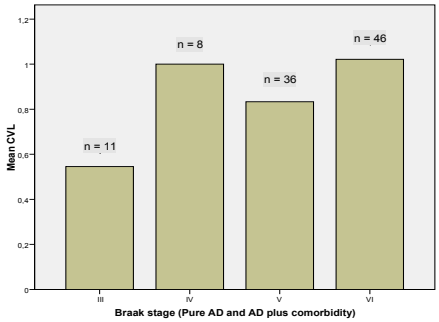
The whole-group analyses revealed a significant positive relationship between AD Braak stage and CVL severity ($r = 0.19$, $p = 0.048$). That is, higher Braak stages were associated with more CVLs. The post-hoc analyses, in which we performed separate calculations for patients with pure AD and patients with AD plus a comorbid neuropathological process, differences did not reach significance. See Figure 1. When LBD stage was included as a covariate in the partial correlation for the whole group of AD participants, the relationship was no longer significant ($r = 0.18$, $p = 0.07$). Figure 2 shows the prevalence of the CVL categories per Braak stage, with the

number of subjects for each Braak stage set to 100%. This demonstrates that the relative number of patients with vascular lesions (CVL score = 1 or 2) increases with higher Braak stages.

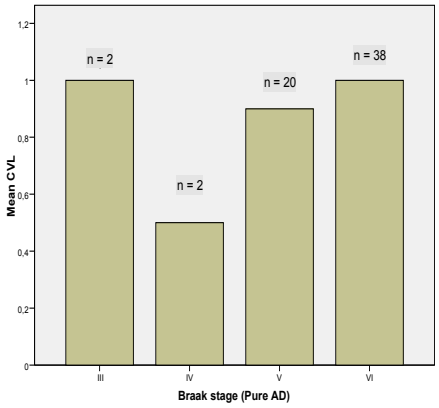
With respect to regional analyses, considering the hippocampus, amygdala, temporal cortex and frontal cortex separately, the only significant correlation was found ($r = 0.21$; $p = 0.034$) between the amount of NFTs and severity of CVLs in the frontal cortex and for the two groups together ('AD pure' and 'AD with comorbidity').

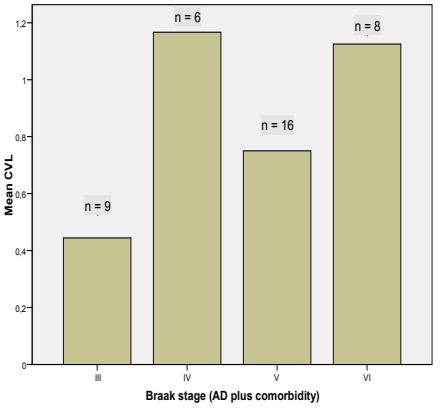
No significant association was found between AD Braak stage and LBD stage, either in the whole-brain analysis or in the regional analyses.

Figure 1. Cerebrovascular lesion (CVL) severity per Braak stage
(see Table 1 for the classification of CVLs)



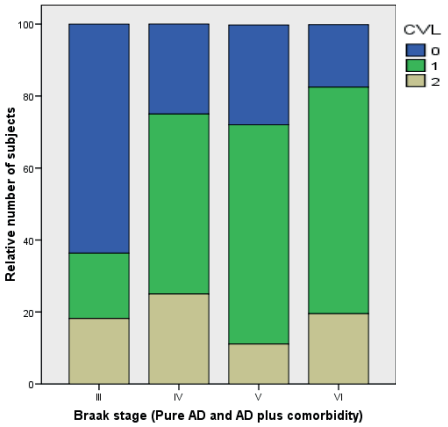
a) Whole group analysis



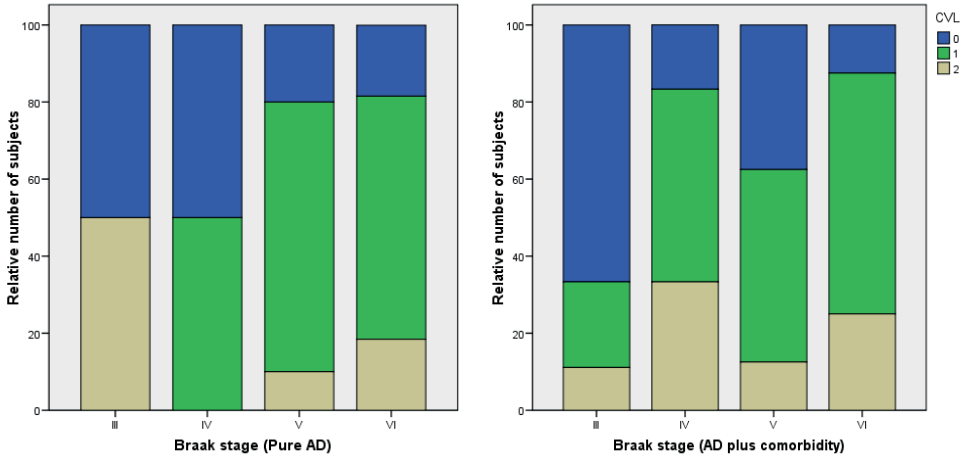


b) Post-hoc analysis in which we analysed the pure AD group (left) and AD with comorbidity group (right) separately

Figure 2. Prevalence of the CVL categories per Braak stage.
In this figure, the number of subjects for each Braak stage was set at 100%. This demonstrates that the relative number of patients with vascular lesions (CVL = 1 or 2) increases with higher Braak stages



a) Whole group analysis



b) Post-hoc analysis in which we analysed the pure AD group (left) and AD with comorbidity group (right) separately

Discussion

We studied a sample of 101 participants with a diagnosis of dementia during life and AD-related pathology in the neuropathological assessment at post-mortem. We found that higher Braak and Braak stages are associated with more severe vascular lesions, in particular in the frontal cortex. This is in line with the report of Jellinger et al. (2008) (Jellinger et al., 2008), who studied a sample of 100 patients

with Parkinson´s disease and 20 patients with LBD and AD-related pathology and found a positive relationship between CVL severity and AD Braak stage. Previous in vivo studies have also shown a positive correlation between AD and CVLs (Kim et al., 2001; Yoshita et al., 2006). Our results are, however, not consistent with two previous neuropathological studies that found a negative relationship between Braak stage and vascular lesions: Goulding et al. in 1999 (Goulding et al., 1999) and Jellinger et al. in 2000 (Jellinger et al., 2000). Both studies were based on very small samples (25 and 27 cases respectively), and although all cases had predominantly AD-type dementia, no other details were provided regarding their diagnoses. This could explain the contradictory results. Indeed, when Jellinger et al. studied a much larger sample in 2008 (120 cases) all cases showed co-occurrence of AD and LB related pathologies, and in this more recent study they found a positive correlation. We therefore conclude that, overall, our positive relationship between AD Braak stages and CVL severity are in line with the other available evidence. This suggests that AD pathology and CVLs have a common aetiopathogenesis in demented patients.

In order to investigate whether this relationship between AD pathology and CVLs is modified by the presence of a comorbid degenerative neuropathological process, we divided our sample into two groups: cases with pure AD and cases with AD plus a comorbid neuropathological process (note that these comorbid diagnoses included other degenerative conditions, but not vascular dementia). Although we did not find any significant associations, this may be due to the small number of subjects in each subgroup, and accordingly we are unable to rule out that the positive association between AD Braak stage and CVLs is mediated by a comorbid degenerative neuropathological process. In fact, since the results were not significant when LBD stage was included as a covariate, we can state that LB pathology is a mediating factor in the positive relationship between severity of CVL and AD-related pathology.

In the present study, we found no correlation between AD Braak stage and Lewy body stage. No prior studies have been performed in samples with comparable characteristics to ours, and research that has been conducted has produced contradictory results (Sonnen et al., 2011; Compta et al., 2011). Sonnen et al. (Sonnen et al., 2011) studied a sample of 336 cognitively normal adults and found an inverse relationship, while Compta et al. (Compta et al., 2011) found a positive relationship in a sample of 56 participants with a diagnosis of Parkinson´s disease, who in most cases had an AD-related tauopathy at a Braak stage of III-IV. We believe that the lack of homogeneity in the clinical characteristics of the participants and the severity of the degenerative pathology may have led to these contradictory results. In any case, we underline that the present data do not support the idea of a direct relationship between AD pathology and LB in demented patients with AD.

With regard to the regional analysis, the only significant correlation was found in the frontal cortex. We hypothesize that AD-related pathology in the frontal cor-

tex may lead to disruption of the fronto-subcortical pathway and make it more susceptible to vascular damage due to small-vessel disease or demyelination of the white matter at the frontal area.

Finally, we address the limitations of our study. First, the small number of participants in each subgroup of AD patients may be responsible for the lack of significant results in the post-hoc analyses. Second, most of our patients were at Braak stages V or VI. This narrow range of Braak stages may have hampered our ability to find robust correlations. Both limitations are not uncommon in post-mortem studies and are a result of the fact that we studied tissue from deceased patients.

In conclusion, our study provides new evidence for a link between AD pathology and CVLs in demented patients with AD, suggesting that AD and CVLs may have a common aetiopathogenesis. Our data do not, however, support the theory of a direct relationship between AD pathology and LB in these patients.

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CHAPTER 4

Vascular risk factors in neuropathologically confirmed Alzheimer´s disease and vascular dementia

C. Echávarri, S. Burgmans, F.R.J. Verhey, H.B.M. Uylings, E. Layana, A.J.M. Rozemuller and W. Kamphorst. Vascular risk factors in neuropathologically confirmed Alzheimer´s disease and vascular dementia. Submitted.

Previous *in vivo* studies have reported various vascular conditions to be risk factors for dementia. Our main aim was to compare vascular risk factor prevalence in neuropathologically confirmed cases of Alzheimer´s disease (AD), vascular dementia (VaD), and mixed AD and VaD (MD). We compare the results with prevalences in elderly local Dutch control populations. The target population of this retrospective descriptive study comprised 120 brains selected from donations to the Netherlands Brain Bank between 1984 and 2010. Forty VaD patients were matched with 40 AD and 40 MD patients. Data from several control groups representing the elderly Dutch population were also included for comparison.

The only differences found were a greater prevalence of history of stroke in VaD and MD patients compared to AD patients and controls, and a greater prevalence of smoking in AD. We argue that none of the risk factors we examined may be related to the presence of dementia, except for stroke history in VaD.

Introduction

Multiple vascular risk factors, such as hypertension, diabetes, history of stroke, atrial fibrillation, hypercholesterolaemia and smoking have been recognised to be associated with dementia, in particular vascular dementia (VaD) but also Alzheimer´s disease (AD) (Cherubini et al., 2007; Henon et al., 2000; Kalaria, 2000; Kivipelto et al., 2001; Skoog et al., 1996). Other studies have failed to distinguish a particular vascular risk factor pattern associated with subtypes of dementia (AD or VaD) (Arvanitakis et al., 2004; Breteler et al., 1994; Luchsinger et al., 2001; Luchsinger et al., 2004; Luchsinger et al., 2005; O´Brien et al., 2003; Ott et al., 1998; Ott et al., 1999; Pohjasvaara et al., 1998; Kivipelto et al., 2001; Knopman et al., 2001; Kuller et al., 2005; Reitz et al., 2008; van Vliet et al., 2010). One possible explanation for this might be the lack of consensus about the clinical diagnoses of VaD, AD and mixed AD and VaD cases (MD). In fact, previous research has shown that clinical diagnoses often have to be revised when neuropathological findings become available (Brunnstrom & Englund, 2009; Kovacs et al., 2008; Echávarri et al., 2012). In addition, AD-related and vascular pathologies commonly co-occur (Parkkinen et al., 2008; Trojanoswki et al., 2003), which complicates the diagnosis of dementia subtypes. The main aim of our study was to compare the prevalence of vascular risk factors in neuropathologically confirmed AD, VaD and mixed AD and VaD (MD). We compared the results with prevalences in several elderly local Dutch control populations. To the best of our knowledge, this is the first combined clinico-neuropathological study on this topic.

Material and methods

Cases

The target population for this retrospective descriptive study comprised 120 brains selected from donations to the Netherlands Brain Bank (NBB) between 1984 and 2010. Necropsy authorization and informed consent were obtained from each donor, relative or caregiver. We included all 40 cases diagnosed with VaD during this period (1984 to 2010), as well as 40 cases of AD and another 40 cases of MD. During the period in which the VaD cases were collected, 660 AD cases were identified at the NBB. Cases of AD, VaD, and MD were matched where possible for age and the Braak stage of AD-related neurofibrillary tangle pathology. The stages, as defined in Braak (Braak et al., 2006), were IV or V for AD cases, III or less for VaD, and IV or V for MD. Brains at Braak stage VI were not included, since they were older than the other cases. More details of the diagnostic criteria used are presented in the next subsection, *Neuropathological assessment*. Brains from the NBB with a mixture of other neurodegenerative disorders or any other structural lesions were also excluded.

The NBB donors were recruited from nursing homes. All demographic information (age, sex, duration of dementia), clinical data including risk factors (hypertension, history of stroke, diabetes, hypercholesterolaemia, atrial fibrillation, smoking) and neuropathological data were collected from patient medical records and NBB records. These data were provided by the patient's family doctor, the doctor at the nursing home, the patient's neurologist and/or psychiatrist. The patients were considered to have had hypertension if they had one of the following: self-report of physician-diagnosed hypertension, use of antihypertensive medication, or blood pressure ≥ 160/95. They were considered to have had hypercholesterolaemia if their recorded total cholesterol ≥ 6.5 mmol/L. History of stroke included transitory ischemic attack (TIA) as well as actual stroke.

As regards hypertension, 67.5% of the patients were reported as having had midlife hypertension and 15.5% as having had late-life hypertension, while no distinction between late-life or mid-life hypertension could be made in 17.5% of the patients. Neuropathological diagnoses were reviewed by an experienced neuropathologist (WK), while the medical records were examined by an experienced neurologist (CE).

Control group data derived from several epidemiological studies involving elderly Dutch populations were compared with the data from the sample of demented cases from the Brain Bank. These subjects from Dutch populations had been selected at random. Most of them were living at home, and a few of them in nursing homes. Data on hypertension were collected from Van Rossum et al. (2000) (Rossum et al., 2000), data on atrial fibrillation from Heeringe et al. (2006) (Heeringe et al., 2006)), data on history of stroke and diabetes from the Nijmegen Continuous Morbidity Register (Van de Lisdonk E. Personal communication, 2012), data on hypercholesterolaemia from the Measuring the Netherlands study (Verschuuren M. Personal communication, 2012), and data on smoking from the Dutch Continuous Survey of Smoking by STIVORO (2010) (Zeegers T. Personal communication, 2012). All but one of the control groups were chosen so as to comprise individuals aged between 75 and 84 years, in order to ensure that they were as similar as possible to the mean age in the demented groups from the Brain Bank. The exception was the control group for hypercholesterolaemia, which included people aged 61 to 70 years.

Neuropathological assessment

Macroscopic assessment of the brain included analysis of photographs. The right hemisphere was fixed in 4% formaldehyde for three weeks, while the left hemisphere was dissected fresh. For diagnostic purposes, the following regions were dissected from the fixed right hemisphere and embedded in paraffin: frontal, cingulate, insular, temporal, parietal and occipital cortices including deep white matter; basal ganglia; thalamus; amygdala; hippocampus and entorhinal areas; mesencephalon including substantia nigra; locus coeruleus and base of pons; medulla

oblongata; and cerebellum and cervical spinal cord (level C1 or C2). If necessary, selected parts of fixed remnants of the left hemisphere were also analysed. Cortical regions were routinely stained with haematoxylin and eosin, Bodian or Gallyas methods (for neurofibrillary tangles), methenamine-silver (for senile plaques), and Congo red (for congophilic plaques and vessels). Immunohistochemistry was performed for alpha-synuclein (to detect Lewy bodies and Lewy neurites), AT8, beta A4, prion protein, ubiquitin and TDP43.

The neuropathological diagnoses in our study were made on the basis of the following criteria. *Vascular dementia* was diagnosed if there was clinical dementia and the neuropathology corresponded to the classification by Brun and Gustafson (Brun et al., 1988) and Brun (Brun et al., 1994). . VaD diagnoses could include Alzheimer-related pathology at a Braak and Braak neurofibrillary tangles stage of III or lower, but no other neurodegenerative disorders (especially Lewy body pathologies). Of the 40 cases of VaD, 16 showed small vessel disease with strategic infarcts (7 in the caudate, 7 in the thalamus and 2 in the parietal lobe), 19 had territorial infarcts (caused by large vessel disease) and 21 had white matter rarefaction and/or lacunar infarcts.

Alzheimer's Disease was diagnosed according to the criteria described by Braak and Braak, based on the presence, density and distribution of cortical neuritic plaques (Braak and Braak, 1997; Braak and Braak, 1990) and tangle pathology (Braak and Braak, 1991; Braak and Braak, 1990). We included only clinically demented cases with Braak stages IV and V for neurofibrillary tangles, without any Lewy bodies. Seven of the 40 cases with AD showed focally reduced white matter density and/or one lacunar white matter infarct. These white matter lesions coexisting with AD were not considered to be severe enough to contribute to the dementia, and these patients were therefore not included in the mixed dementia group.

Mixed dementia was defined as meeting the criteria for VaD, combined with a Braak stage above III. Of the 40 cases diagnosed with MD, 6 showed territorial infarcts caused by large vessel damage, 22 decreased white matter density and/or lacunar infarcts caused by small vessel damage, and 12 a mixture of both large and small vessel damage. No strategic infarcts were found in the MD group.

Statistical analysis

Statistical analyses were performed using two different statistical packages. The Statistical Package for the Social Sciences (SPSS, Chicago, IL) version 16.0 for Windows was used to compare the three groups in our own sample in terms of demographic variables. The following statistical tests were performed: ANOVA (with a general linear model) for continuous variables such as mean age and duration of dementia, and chi-square tests for categorical variables such as sex and the presence of each risk factor. The Statcalc module of Epi Info (version 6, November 1993; CDC, Atlanta, GA) was used to compare risk factor percentages between all

groups (including the control groups) using chi-square tests, and using Fisher's exact test for numerical values. The results were expressed as odds ratios for prevalence, confidence intervals (95%) and p-values.

Results

Demographic variables

The demographic characteristics (age and sex) of the cases and the duration of dementia are summarized in Table 1. No significant differences between AD and VaD were found for these variables. However, the mean age of the MD group was significantly higher than that of the AD and VaD groups. The longest mean duration of dementia was found for VaD (8 years), followed by MD (6.5 years) and AD (6 years), but these differences were not statistically significant.

Table 1. Demographic variables in the three groups of demented cases

	AD n = 40	VaD n = 40	MD n = 40	P
Age in years; mean (SD)	79.7 (3.7)	77.7 (11.1)	82.5 (5)	0.017
Female sex; n (%)	27 (67.5)	21 (52.5)	25 (62.5)	0.171
Duration of dementia in years; mean (SD)	6 (3.2)	8 (4.8)	6.5 (4.6)	0.104

Note. Group differences were assessed with ANOVA (for age and duration of dementia) and chi-square tests (for sex). AD = Alzheimer's disease; VaD = vascular dementia; MD = mixed dementia; p = p value; SD = standard deviation.

Distribution of risk factors

Tables 2 and 3 show the number of participants and prevalence of risk factors in all the groups studied.

Table 2. Number and (%) of participants and distribution of risk factors in the three groups of demented cases

	AD			VaD			MD		
	Total n = 40	Males n =13	Females n =27	Total n = 40	Males n =19	Females n = 21	Total n = 40	Males n = 15	Females n = 25
Hypertension	24 (60%)	6 (46.2%)	18 (66.7%)	21 (52.5%)	10 (52.6%)	11 (52.4%)	22 (55%)	8 (53.3%)	14 (56%)
Stroke history	7 (17.5%)	3 (23.1%)	4 (14.8%)	29 (72.5%)	16 (84.2%)	13 (61.9%)	18 (45%)	7 (46.7%)	11 (44%)
Diabetes	9 (22.5%)	3 (23.1%)	6 (22.2%)	4 (10%)	1 (5.3%)	3 (14.3%)	9 (22.5%)	4 (26.7%)	5 (20%)
Atrial Fibrillation	8 (20%)	3 (23.1%)	5 (18.5%)	9 (22.2%)	4 (21.1%)	5 (23.8%)	4 (10%)	0 (0%)	4 (16%)
Hypercholesterolaemia	8 (20%)	3 (23.1%)	5 (18.5%)	3 (7.5%)	2 (10.5%)	1 (4.8%)	4 (10%)	0 (0%)	4 (15.1%)
Smoking	10 (25%)	4 (30.8%)	6 (22.2%)	3 (7.5%)	2 (10.5%)	1 (4.8%)	3 (7.5%	1 (6.7%)	2 (8%)

Note. AD = Alzheimer´s disease; VaD = vascular dementia; MD = mixed dementia.

Table 3. Number and (%) of participants and distribution of risk factors in the control groups

	Number of cases			Prevalence of risk factors		
	Total N	Males n	Females N	Total n (%)	Males n (%)	Females n (%)
Hypertension[26]	1792	618	1174	811 (45.6%)	225 (36.4%)	586 (50.4%)
Stroke history[28]	583	238	345	110 (18.8%)	51 (21.4%)	59 (17.1%)
Diabetes[28]	583	238	345	107 (18.4%)	42 (17.6%)	65 (18.8%)
Hypercholesterolaemia[29]	1167	580	587	288 (24.7%)	89 (15.5%)	199 (34%)
Atrial fibrillation[27]	1392	492	900	149 (10.7%)	68 (13.8%)	81 (9.2%)
Smoking[30]	959	603	356	95 (9.9%)	61 (10.1%)	34 (9.5%)

Note. Three first columns show total number of cases in each epidemiological group and the three last columns represent number and percentages of cases that had the risk factor. The numbers (26-30) correspond to the references to epidemiological studies from which control group data were obtained.

Figure 1 displays the prevalence of risk factors in each group and the statistical differences between the groups, while Table 4 details differences between the groups, overall and stratified by sex. Hypertension was the most prevalent risk factor in AD and MD groups but not among those diagnosed with VaD. No signifi-

cant differences in hypertension were detected between the groups, though there was a trend towards a higher prevalence of hypertension in AD compared to controls, especially considering female AD cases and controls (Table 4). Similarly, for diabetes no significant differences or trends were found between groups. History of stroke was the most prevalent risk factor in VaD. For this variable, differences between groups were significant in all cases, except for the control and AD groups in which the prevalence was very similar. Regarding hypercholesterolaemia, there was a significant difference between the controls and both VaD and MD groups. Notably, this difference was completely attributable to the female control subgroup in which the prevalence of hypercholesterolaemia was notably higher (compare Fig. 1 with Table 4). Atrial fibrillation was significantly more common in the VaD than the control group and again this was completely attributable to a higher prevalence in the female subgroup (see Fig. 1 and Table 4). A non-significant trend towards a difference was found between female AD cases and controls, with more atrial fibrillation in AD females (Table 4). Finally, the prevalence of smoking was statistically significantly higher in AD cases than controls (Fig 1 and Table 4).

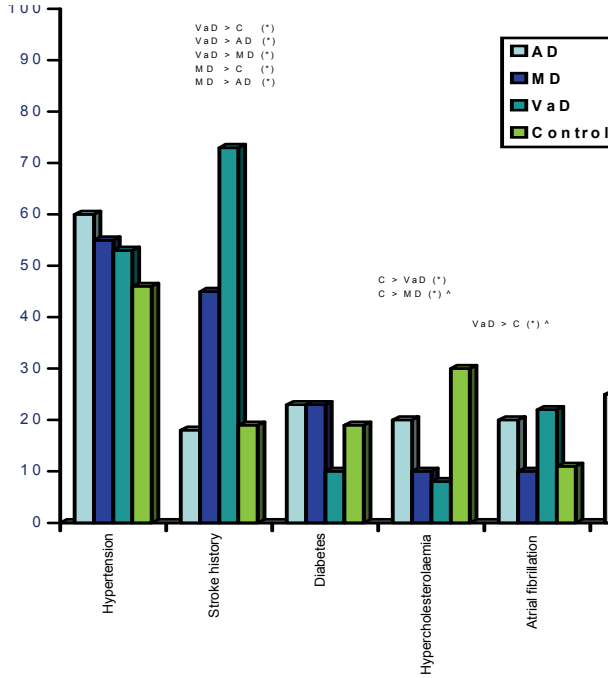

Figure 1. Distribution of vascular risk factors between groups.

Note. The percentage of occurrence of vascular risk factors in the four groups: AD = Alzheimer's disease; MD = mixed dementia; VaD = vascular dementia and corresponding controls. Significant differences (*) are shown above the columns. ^ = the differences in hypercholesterolaemia and atrial fibrillation are solely attributable to the prevalence in the female cases (see Table 4).

Table 4. Differences between groups.

	Hypertension			Stroke history			DM			Hypercholesterolaemia			Atrial fibrillation			Smoking		
	OR	CI	p	OR	CI (95%)	p	OR	CI (95%)	p	OR	CI (95%)	p	OR	CI	p	OR	CI(95%)	p
C vs AD Total	1.8	0.9-3.6	0.064	0.9	0.3-2.2	0.830	1.2	0.5-2.9	0.514	0.7	0.3-1.7	0.498	2.0	0.8-4.8	0.070*	3.0	1.3-6.7	0.006
C vs AD Males	1.5	0.4-5.0	0.563*	1.1	0.2-4.5	0.557	1.4	0.2-5.8	0.421	1.6	0.3-6.8	0.327*	1.8	0.4-7.6	0.407	3.9	1.1-13.2	0.038*
C vs AD Females	2.0	0.8-4.8	0.085	0.8	0.2-2.1	0.505	1.2	0.4-3.3	0.666	0.5	0.1-1.5	0.238	2.3	0.7-6.6	0.096	2.7	1.0-7.1	0.049*
C vs VaD Total	1.3	0.6-2.6	0.364	13.7	6.3-30.2	0.000	0.4	0.1-1.4	0.181	0.2	0.0-0.8	0.012	2.4	1.0-5.4	0.025*	0.7	0.1-2.5	0.435*
C vs VaD Males	1.9	0.7-5.2	0.148	19.5	5.0-88.1	0.000	0.2	0.0-1.9	0.134*	0.6	0.1-2.9	0.429*	1.6	0.4-5.5	0.325*	1.0	0.1-4.6	0.577*
C vs VaD Females	1.0	0.4-2.8	0.822	7.8	2.8-21.9	0.000	1.2	0.4-3.3	0.666	0.1	0.0-0.8	0.015*	3.1	1.2-5.8	0.003	0.4	0.0-3.2	0.373*
C vs MD Total	1.4	0.7-2.9	0.220	4.2	2.1-8.6	0.000	1.2	0.5-2.9	0.514	0.3	0.1-1.0	0.033	0.9	0.2-2.7	0.572	0.7	0.1-2.5	0.435*
C vs MD Males	2.0	0.6-6.2	0.179	3.2	3.9-10.3	0.032*	1.7	0.4-6.1	0.281	0.0	0.0-1.9	0.085	0.0	0.0-2.1	0.111	0.6	0.0-4.7	0.545*
C vs MD Females	1.2	0.5-3.0	0.547	3.8	1.5-9.4	0.002*	1.0	0.3-3.1	0.528	0.3	0.1-0.9	0.044	1.9	0.5-6.1	0.190	0.6	0.0-4.7	0.555*
AD vs VaD Total	0.7	0.2-1.9	0.498	12.4	3.8-42.5	0.000	0.3	0.0-1.1	0.129	0.3	0.0-1.5	0.194	1.1	0.3-3.8	0.784	0.2	0.0-1.0	0.069
AD vs VaD Males	1.3	0.2-6.7	0.723	17.1	2.3-176.3	0.000	0.1	0.0-2.5	0.270*	0.1	0.0-2.5	0.278*	1.5	0.1-12.1	0.618*	0.2	0.0-2.2	0.163*
AD vs VaD Females	0.5	0.1-2.0	0.320	7.3	19.0-48.1	0.000	0.5	0.1-3.2	0.376	0.5	0.1-3.2	0.711*	0.7	0.1-3.5	0.460*	0.1	0.0-1.7	0.117
AD vs MD Total	0.8	0.3-2.1	0.821	3.8	1.2-12.3	0.007	1.0	0.3-3.2	1	0.4	0.1-1.8	0.347	0.4	0.1-1.8	0.347	0.2	0.0-1.0	0.066
AD vs MD Males	1.3	0.2-7.7	0.704	2.9	0.4-21.0	0.254*	0.8	0.1-6.1	0.587*	-	-	0.376	-	-	0.087*	0.1	0.0-2.0	0.152*
AD vs MD Females	0.6	0.1-2.2	0.613	4.5	1.0-21.1	0.020	1.1	0.2-5.2	0.844	1.1	0.2-6.2	0.551*	1.1	0.2-6.2	0.551*	0.3	0.0-1.9	0.251*
VaD vs MD Total	1.1	0.4-2.9	0.822	0.3	0.1-0.8	0.023	2.6	0.6-11.5	0.225	1.3	0.2-8.4	0.500*	0.3	0.0-15.5	0.225	1.0	0.1-5.2	1.000*
VaD vs MD Males	1.0	0.2-4.9	0.760	0.1	0.0-1.0	0.030*	6.5	0.5-175.8	0.145*	0.0	0.0-5.4	0.491*	0.0	0.0-1.8	0.113	1.6	0.1-20.1	0.829*
VaD vs MD Females	1.1	0.3-4.3	0.958	0.4	0.1-1.8	0.360	1.5	0.2-9.4	0.709	3.8	0.3-97.7	0.356	1.2	0.2-7.5	0.525	0.5	0.0-6.8	0.875

Note. C = control group; AD = Alzheimer´s disease group; MD = mixed dementia group; VaD = vascular dementia group. Chi-square tests were performed for comparisons between groups, and when required, Fisher's tests (*). OR = prevalence odds ratio; CI = confidence interval (95%); p = p value; significant differences ($p < 0.05$) are in bold

Discussion

In this clinico-neuropathological study, we compared the presence of vascular risk factors not only between AD, VaD and MD cases (disease groups), but also between the disease groups and elderly Dutch control groups of similar age. The importance of the latter comparison was that it might detect specific causal cofactors (amenable to preventive treatment) for AD and VaD. The main results of the pairwise comparisons between groups are discussed below.

AD vs. control group. Our comparison between the AD patients and the control group (Fig.1, Table 4) revealed no significant differences with respect to the presence of hypertension, stroke history, diabetes mellitus, hypercholesterolaemia or atrial fibrillation, with the notable exception of smoking, which was more common in AD patients. This could indicate that smoking is a causal factor for AD, but smoking behaviour might also be disinhibited in AD, or caregivers might not discourage it in these patients. We were unable to find data in the patient records on smoking load in terms of pack years for the disease period.

VaD vs. control group. the comparison between the VaD group and the control groups (Fig.1, Table 4) shows that a history of stroke appears to be more common in VaD. The high significance level evidently depended on the neuropathological (and clinical) definition of VaD. Previous studies have also found this positive relationship between history of stroke and VaD (Chiang et al., 2007; Erkinjuntti et al., 2007). Hypercholesterolaemia was seen to be more common in the control group than in VaD (and in MD), while atrial fibrillation was more common in VaD cases. The difference in hypercholesterolaemia might be attributable to the female control subgroup, in which the prevalence of hypercholesterolaemia was much higher than in the female VaD and MD groups (compare Fig. 1 with Tables 3 and 4). If this is not a chance observation, we should explore why cholesterol levels are lower in female patients with VaD. Atrial fibrillation was significantly more common in the VaD group than the control group and this was apparently caused by a higher prevalence in the female VaD subgroup (see Fig. 1 and Tables 3 and 4). It remains unclear why it is especially women with VaD who have atrial fibrillation.

MD vs. control group. Our comparison of MD with the control groups produced results similar to the comparison of VaD with its controls, with the exception of the prevalence of atrial fibrillation, which was not significantly different between MD cases and controls.

Comparison of the disease groups with each other did not reveal any significantly different frequencies for any of the risk factors, with the exception, again, as could be expected, for history of stroke. The largest differences in this variable were seen between VaD and controls, and between VaD and AD.

A look at the columns in Table 4 shows that we did not detect any statistically significant differences between the disease groups for the risk factor of hypertension. The prevalence of hypertension was relatively high, between 52.5% and 60.0% in the disease groups and 45.6% in the control group. Although none of the differences reached the significance level, the comparison between AD and control groups showed a trend towards high rates of hypertension in AD, once again attributable to the female subgroup.

Notably, although diabetes mellitus is an important co-determinant of vascular disease, rates of diabetes were not significantly different between the disease groups or between the disease groups and controls, and there was not even any detectable trend. This might be due to the relatively small number of patients in the groups with dementia (see Table 2), but another explanation, which we consider highly plausible, is that diabetes is effectively treated in the Netherlands, which may have prevented strokes.

To the best of our knowledge, our study is the first on this topic (prevalence of several vascular risk factors in AD, VaD and MD) to use a sample of cases with neuropathological confirmation of the diagnoses of dementia. Accordingly, no comparison can be made with previous studies, except with some that were performed *in vivo*. All *in vivo* studies that we found were longitudinal epidemiological investigations, and many of them supported the view that there is a relationship between vascular risk factors and dementia (Pohjasvaara et al., 1998; Kivipelto et al., 2001; Kivipelto et al., 2005; Knopman et al., 2001; Kuller et al., 2005; Reitz et al., 2008; Skoog et al., 1996). In particular, some studies examining the implications of hypertension found an association between this risk factor and late-life VaD (Lindsay et al., 1997) and AD (Skoog et al, 1996). Hypertension in midlife has been associated with VaD and AD and with the number of neuritic plaques and neurofibrillary tangles (Petrovich et al., 2000). The study by Petrovitch et al. examined the relationship between blood pressure levels and the number of neuropathological lesions of AD using counts of neuritic plaques and neurofibrillary tangles as endpoints, not AD, which means that this study is not strictly comparable to ours. Similarly, Launer et al (Launer et al., 2001) found an association between late-life HDL cholesterol levels and the formation of neuropathological lesions of AD, irrespective of dementia. On the other hand, other researchers, whose studies focused on the aetiopathogenesis of dementia, found no evidence of a causal relationship between vascular risk factors and dementia (Launer et al., 2008; Purnell et al., 2009; Richard et al., 2009). Frequent clinical dementia (AD, Lewy body dementia and VaD) cases are known to often comprise mixed conditions with Alzheimer's, vascular and Lewy body pathologies (Echávarri et al., 2012; Jellinger et al., 2003; Knopman et al., 2010). This would not only explain the inconsistent results of *in vivo* studies into the relationship between vascular risk factors and dementia, but also the difficulty of identifying vascular risk factor patterns associated with subtypes of dementia

(AD, VaD or MD). In this respect, a history of stroke is a risk factor for VaD, as has been assumed by other researchers and is supported by our findings (Erkinjuntti et al., 1988).

Some methodological issues have to be addressed. While clinical studies can be strictly controlled and prospective, neuropathological confirmation is often lacking. On the other hand, a clinico-pathological study like ours is necessarily retrospective, meaning that clinical parameters (like total serum cholesterol) are not always available or compiled consistently, and brain bank donation is not strictly controlled. Nevertheless, the availability of a neuropathological diagnosis is a considerable advantage. While Alzheimer's disease is the most frequent cause of dementia, a clinico-pathological study like ours can distinguish between pure AD and VaD cases. It is important to note that establishing a neuropathological diagnosis of VaD is a controversial issue, since no clear criteria have so far been established in the literature (Esiri et al., 1997). In fact, prior studies based on neuropathological series of demented patients have reported low prevalence of pure VaD, between 2 and 10%. The NBB database for 1984 to 2010 includes 660 AD cases but only 40 "pure" VaD cases. This underlines the value of the fact that our study comprised a sample of 40 cases with pure VaD (Jellinger et al., 2007).

A major weakness of our study is, however, the relatively small number of cases (three times n=40), which was determined by the lack of VaD cases. We recognise that these small numbers may explain why we found so few statistically significant differences between groups for the vascular risk factors considered, and many relationships remained unclear, except for some indications from the non-significant trends we detected (see Fig. 1 and Table 4). We could not prove that there are causal relations between risk factors and AD, VaD and MD, but, on the other hand, neither could we disprove them. Therefore, future meta-analytical studies are needed for further statistical testing.

Another weakness of our study is the partial lack of homogeneity in both of the samples (demented and control) we compared. Despite differences in the selection processes of the patients included in the study sample and the lack of neuropathological assessment among the controls, we still consider that our comparison of these two samples has yielded results that are worthy of serious consideration. On the one hand, we assume that the control groups include two different subgroups: a subgroup that will never become demented and a non-demented subgroup that will become demented in the future. On the other hand, all subjects in the disease groups were demented. Thus, we consider that if risk factors play a role in dementia, they would be more concentrated in the disease groups, as the control groups included subgroups of people who would never become demented. Moreover, we used controls of ages similar to those of the disease groups and have also examined men and women separately, making both samples more homogeneous.

We conclude that our clinico-neuropathological study was unable to detect increased prevalences of vascular risk factors in AD except for a higher proportion of

smokers. Among VaD patients, we observed that a history of stroke was more common in the total group (men and women).The rates of hypercholesterolaemia were higher in the female controls than in the female VaD patients, and the rates of atrial fibrillation were higher in the female VaD patients than in the female controls. Overall, however, our approach using neuropathological confirmation of dementia diagnoses seemed to make most of the previously reported group differences in vascular risk factors disappear. We argue that none of the risk factors we examined may be related to the presence of dementia, except for stroke history in VaD.

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CHAPTER 5

Neuropsychiatric symptoms in Alzheimer's disease and vascular dementia

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Neuropsychiatric symptoms (NPSs) have a large impact on the quality of life of patients with dementia. A few studies have compared neuropsychiatric disturbances between dementia subtypes, but the results were conflicting. In the present study, we investigated whether the prevalence of NPSs differs between Alzheimer's disease (AD) and vascular dementia (VaD). The merit of our study is that we used clinical as well as histopathological information to differentiate between dementia subtypes. This retrospective descriptive study comprised 80 brains obtained from donors to the Netherlands Brain Bank (NBB) between 1984 and 2010. These donors were diagnosed post-mortem with AD (n = 40) or VaD (n = 40). We assessed the presence of NPSs by reviewing the information found in the patients' medical files. The most prevalent symptom in the sample as a whole was agitation (45 cases, 57.0%), followed by depression (33, 41.2%) and anxiety (28, 35.4%). Our study tried to contribute to the discussion by including, for the first time in the literature, a sample of AD and VaD patients with neuropathologically confirmed diagnoses. Since no significant differences were found between AD and VaD patients, we suggest that the prevalence of NPSs cannot be predicted from the dementia diagnosis of AD or VaD.

Introduction

Neuropsychiatric symptoms (NPSs) are increasingly recognized as an important and intrinsic aspect of the dementia syndrome [1,2,3,4,5,6]. These symptoms have a large impact on the quality of life of patients with dementia, and have been associated with increased caregiver burden, more rapid progression of cognitive and functional decline, earlier institutionalization and even increased mortality [7]. Several studies have found that NPSs are common in patients with Alzheimer's disease (AD) and vascular dementia (VaD) [8,9,10,11,12,13,14,15], with apathy, depression, anxiety and agitation/aggression having been most frequently reported [16,9,18,4,19,6,20,21,22,23].

It is important to know whether different types of dementia present different neuropsychiatric profiles, because these differences could affect caregiver distress [15]. In addition, this knowledge may lead to more effective treatment, and to further insights into brain-behaviour relationships. A few studies have compared neuropsychiatric disturbances between dementia subtypes, and so far, the results have been conflicting. Some studies found higher prevalences of NPSs in VaD, including affective disturbances such as depression, anxiety or agitation [16,9,24,25,13]; other studies found higher prevalences in AD, including anxiety and agitation [10,18]; and still others found no differences between groups [12,21,14]. This makes it difficult to establish a pattern of NPSs in different subtypes of dementia.

These contradictory results may be due to a lack of accuracy in diagnosing dementia subtypes [26,4,27,20,28,22,29]. Clinical diagnoses made in vivo often differ from the neuropathological diagnoses established post-mortem [30,31]. Hence, comparisons of NPSs between dementia subtypes should preferably include neuropathological information. In the present study, we investigated whether the prevalence of NPSs differs between AD and VaD. We focussed on 12 psychiatric symptoms, which are those assessed by Neuropsychiatric Inventory [32] (delusions, hallucinations, agitation, depression, anxiety, euphoria, apathy, disinhibition, irritability, aberrant motor behaviour, night-time behavioural disturbances and appetite changes). We examined the prevalence of NPSs in 80 patients for whom a neuropathological diagnosis of AD or VaD had been established. The merit of our study is that we used clinical as well as histopathological information to differentiate between dementia subtypes.

Materials and Methods

Participants

This retrospective descriptive study comprised 80 brains obtained from donors of the Netherlands Brain Bank (NBB) between 1984 and 2010. During this period of

time, 660 cases of AD and 40 cases of VaD were diagnosed at the NBB. All 40 VaD cases were included in this study, while 40 AD cases were matched for age with the VaD cases. Demographic data are presented in Table 1. The clinical dementia rating (CDR) score was assessed before death; presence of comorbidity included the presence of any vascular risk factor (hypertension, diabetes, hyperlipidaemia or atrial fibrillation) or any systemic disorder.

Table 1. Demographic data

	VaD (n = 40)	AD (n = 40)	p
Age (years); mean (SD)	77.7 (11.1)	77.8 (2.7)	0.947
Sex (% women)	52.5	60.0	0.254
Duration of illness (years); mean (SD)	8 (4.8)	6 (3.2)	0.037
Number of NPSs; mean (SD)	2.3 (1.6)	2.2 (1.2)	0.836
CDR score prior to death	2.8 (0.3)	2.9 (0.3)	0.251
Prior history of depression n (%)	5 (16.6)	3 (7.5)	0.456
Comorbidity n (%)	38 (95.0)	39 (97.5)	0.556
Nursing home n (%)	14 (35.0)	21 (52.5)	0.114
Medication – sleep disorder n (%)	8 (20.0)	13 (32.5)	0.598
Medication – antidepressant n (%)	9 (22.5)	9 (22.5)	1.000
Medication – behavioural disorder n (%)	17 (42.5)	13 (32.5)	0.355

The group differences (between VaD and AD) were calculated with ANOVA for age and duration of illness, and with Chi-square for sex, previous history of depression, presence of comorbidity, living in a nursing home, antidepressant medication, and medication for sleep disorder or for behavioural disorders. The Mann–Whitney test was used for the CDR score. VaD = vascular dementia; AD = Alzheimer´s disease; duration of illness = years from onset of symptoms to death; p = p-value; SD = standard deviation.

All clinical data were obtained from the patient's GP, the doctor at the nursing home, neurologists or psychiatrists. All patients were diagnosed with dementia, defined as any irreversible and progressive cognitive impairment affecting at least one cognitive domain and interfering with the patient's daily life. Patients were selected according to the neuropathological diagnoses (VaD and AD); all of them had a clinical diagnosis of dementia, but not all neuropathological diagnoses were in agreement with clinical diagnoses. In fact, the clinical diagnoses of the 40 cases with a neuropathological diagnosis of VaD were as follows: 25 (62.5%) VaD, 7 (17.5%) AD, 6 (15%) mixed dementia, 1 (2.5%) frontotemporal lobar dementia and 1 (2.5%) Lewy body dementia, while the clinical diagnoses of the 40 cases with a neuropathological diagnosis of AD were as follows: 28 (70%) AD, 8 (20%) VaD, 3 (7.5%) Lewy body dementia and 1 (2.5%) frontotemporal lobar dementia.

The neuropathological diagnoses were obtained by an experienced neuropathologist. The criteria used for neuropathological diagnoses were as follows: AD was diagnosed according to the criteria described by Braak and Braak, based on the presence, density and distribution of cortical neuritic plaques [33,34] and tangle pathology [35,34]. Braak stages of AD-related neurofibrillary tangle pathology were between IV and VI (8 cases [20%] at Braak stage IV, 14 [35%] at stage V, and 18 [45%] at stage VI). VaD was diagnosed according to the classification of Brun and Gustafson [17] and Brun [36] with a Braak stage for neurofibrillary tangles ≤ III. Of the 40 cases of VaD, 16 showed strategic small vessel infarcts (7 in the caudate, 7 in the thalamus and 2 in the parietal lobe), 19 showed territorial infarcts (caused by large vessel pathology) and 21 showed white matter rarefaction and/or lacunar infarcts. The severity of the vascular pathology (i.e. staging of the vascular lesions) was assessed according to the classification used by Jellinger et al. (see Table 2) [37], ranging from 0 to 3. In our VaD sample, 19 brains had a score of 3, 17 participants a score of 2 and 4 a score of 1. In the AD sample, 38 participants showed no vascular lesions (VL) (score of 0) and 2 a score of 1, but no strategic infarcts were found, and in these two cases VL was considered not to be severe enough to have contributed to the dementia. In order to obtain a more homogeneous distribution of participants in the VaD group, two subgroups were distinguished: 19 brains with large vessel vascular disease (LV VaD) (mean age = 81.3, SD age = 8, percentage women = 52), and 21 brains with small vessel vascular disease (SV VaD) (mean age = 74.4, SD age = 12.6, percentage women = 52.4). LV VaD was diagnosed when the brain showed more than one large vessel infarct; SV VaD was diagnosed when moderate (subjectively assessed) white matter damage and more than one lacunar infarct were present (this group also included vasculitis [n=1], amyloid angiopathy [n=1] and isolated strategic infarcts).

Exclusion criteria were neuropathological diagnoses of other neurodegenerative diseases than AD or VaD (other tauopathies, Lewy body pathology), mixed AD and VaD, and the presence of any tumoural lesion. Written informed consent was obtained from all patients or their relatives.

Neuropsychiatric assessment

We assessed the presence of 12 NPSs – delusions, hallucinations, agitation, depression, anxiety, euphoria, apathy, disinhibition, irritability, aberrant motor behaviour, night-time behavioural disturbances and appetite changes – based on the information in the patients' medical files. The clinical information was collected as follows: symptoms were considered to be present when their presence was recorded in the medical files or in case of any treatments (e.g. antidepressants) for such symptoms, and absent when no mention of the symptom was found in the patient´s file. In many cases, the patient records did not allow a distinction to be made between depressive symptoms and depressive disorders, nor was sufficient information available on the severity of NPSs. Information was collected regarding the frequency of symptoms, the tendency of symptoms to recur or persist, and the medication

used to treat the NPS. These medications included three groups. The first consisted of those used in sleep disorders, including short-acting benzodiazepines and trazodone, which is an antidepressant with sedative properties, while the second group comprised antidepressants, including selective serotonin uptake inhibitors (SSRIs) and others, used to treat depression or depressive symptoms, anxiety and irritability. The third group comprised medications for behavioural disorders, which are neuroleptic (typical and atypical), usually used to treat agitation. Atypical antipsychotic agents (risperidone, olanzapine, quetiapine) were generally used more often than the typical ones. In some cases medication for an NPS was used though the presence of the symptom was not reported the medical file, and no information was provided about the symptom or symptoms that this medication was prescribed for. In view of this, we set the following criteria for determining the presence of a symptom, based on the most common use of medications for NPSs: users of antidepressants were regarded as suffering from depressive symptoms, users of benzodiazepines or trazodone at night were regarded as suffering from sleep or night-time behavioural disturbances, users of benzodiazepines were regarded as suffering from anxiety and users of neuroleptics were regarded as suffering from agitation. A symptom was considered to be recurrent when more than one episode was reported, and was considered to be persistent when the symptom was reported to last for more than one week. The average time between the last assessment of NPSs and death was 5.6 months, with a range of 1 month to 2 years.

Histopathological procedure

Macroscopic assessment of the brain included photographs. The right hemisphere was fixed in 4% formaldehyde for 3 weeks. The left hemisphere was freshly dissected in particular pieces for the Netherlands Brain Bank applicants. For diagnostic purposes, the following regions were dissected from the fixed right hemisphere and embedded in paraffin: frontal, cingulate, insular, temporal, parietal and occipital cortices, including deep white matter; basal ganglia; thalamus; amygdala; hippocampus and entorhinal areas; mesencephalon including substantia nigra; locus coeruleus; medulla oblongata; cerebellum and cervical spinal cord (level C1 or C2). If necessary, selected parts of the fixed remnants of the left hemisphere were also analysed. Cortical regions were routinely stained with HE, Bodian or Gallyas, methenamine-silver, and Congo. Tau (AT 8) immunohistochemistry was applied routinely in every case. Immunohistochemistry for alpha-synuclein was routinely performed to identify Lewy body pathology, while beta A4, prion protein, ubiquitin and TDP43 immunohistochemistry were used when required.

Statistical analysis

Statistical analyses were performed using the Statistical Package for the Social Sciences (SPSS Inc, Chicago), version 16.0 for Windows. Demographic data were compared between groups, using ANOVA (General Linear Model; GLM) for continuous variables such as mean age and duration of illness; chi-square for categorical

variables such as sex, previous history of depression, presence of comorbidity, living in a nursing home, antidepressant medication, medication for sleep disorder or medication for behavioural disorders; and Mann–Whitney for ordinal variables such as CDR score. Logistic regression analysis was used to test the association between each neuropsychiatric symptom (independent variable) and the type of dementia (dependent variable), including duration of illness and age as covariates. Results are presented as odds ratios (OR) with 95% confidence intervals (95% CI). The significance level was set at $p < 0.05$ for all analyses.

Results

The only statistically significant difference in demographic variables between the two diagnostic groups regarded duration of illness ($p < 0.05$). The most prevalent symptom in the sample as a whole was agitation (45 cases, 57.0%), followed by depression (33, 41.2%) and anxiety (28, 35.4%). Figure 1 shows the prevalence of each NPS in the two groups, while the tendency to persist and the need for pharmacological treatment are shown in Table 3. No significant differences between the groups were found, although depression was slightly more frequent in the VaD group (50%, vs. 32.2% in the AD group). The values of OR, CI (95%) and p for differences between groups were: delusions (OR 0.4; CI [95%] 0.0-1.9; p = 0.266); hallucinations (OR 1.1; CI [95%] 0.3-1.9; p = 0.849); agitation (OR 1.6; CI [95%] 0.6-4.1; p = 0.290); depression (OR 0.5; CI [95%] 0.2-1.3; p = 0.202); anxiety (OR 1.5; CI [95%] 0.5-3.8; p = 0.392); euphoria (OR 0.6; CI [95%] 0.0-7.6; p = 0.710); apathy (OR 0.4; CI [95%] 0.1-1.3; p = 0.153); disinhibition (OR 0.1; CI [95%] 0.0-1.0; p = 0.063); irritability (OR 0.4; CI [95%] 0.0-2.3; p = 0.312); aberrant motor behaviour (OR 1.2; CI [95%] 0.3-4.3; p = 0.621); night-time behavioural disturbances (OR 2.7; CI [95%] 0.5-12.7; p = 0.193) and appetite changes (OR 1.2; CI [95%] 0.3-4.3; p = 0.601). When we excluded duration of illness as a covariate from the analysis, we still did not find significant differences. Results regarding the subgroups of VaD showed no differences between the two subgroups (LV VaD and SV VaD) or between each subgroup and AD.

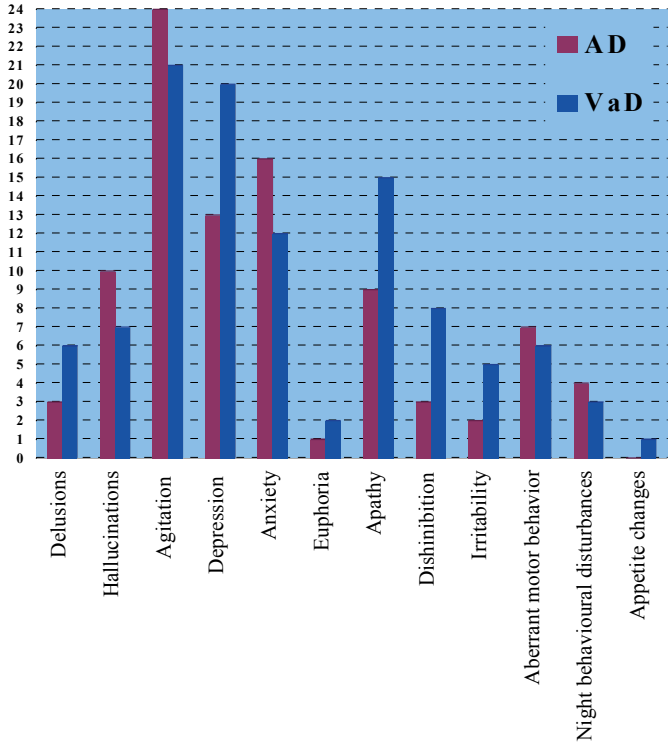

Figure 1. Prevalence of neuropsychiatric symptoms in AD and VaD
Distribution, in numbers, of neuropsychiatric disturbances; AD = Alzheimer´s disease; VaD = vascular dementia.

Table 2. Classification of vascular lesions (VL)

0	No concomitant VL
1	Minimal VL: 1-2 small lacunae, mild–moderate cerebral amyloid angiopathy (CAA), mild VL, mild leukoencephalopathy
2	Moderate VL: >2 lacunae, severe subcortical lacunar state, severe CAA with moderate VL, diffuse white matter lesions
3	Severe VL: (a) Old infarcts, multiple old microinfarcts or haemorrhages, hippocampal sclerosis; (b) acute ischaemic infarcts, haemorrhages (<1-2 days old)

Table 3. Numbers of NPSs, tendency to persist and need for medication in each group.

	AD (n =40)			VaD (n=40)		
	n	Recurrent or persistent cases	Treated with medication	n	Recurrent or persistent cases	Treated with medication
Delusions	3	0	0	6	2	0
Hallucinations	10	6	5	7	4	2
Agitation	24	20	12	21	18	17
Depression	13	6	9	20	10	9
Anxiety	16	14	12	12	9	10
Euphoria	1	1	0	2	0	0
Apathy	9	9	0	15	12	0
Dishinibition	3	2	0	8	1	0
Irritability	2	2	2	5	3	3
Aberrant motor behaviour	7	6	5	6	6	5
Night-time behavioural disturbances	4	4	2	3	3	3
Appetite changes	0	0	0	1	1	0

AD = Alzheimer´s disease; VaD = Vascular dementia. A symptom was considered to be recurrent when more than one limited episode was reported, and was considered to be persistent when the symptom was reported to last for more than one week.

Discussion

Our study did not reveal any significant differences in the prevalence of NPSs between AD and VaD. A few earlier studies have compared NPSs in AD and VaD using the Neuropsychiatric Inventory (NPI). These studies found no consistent differences between dementia subtypes either: Aharon-Peretz et al. [16], Hsieh et al. [25] and Ballard et al. [9] found higher prevalences of apathy, agitation, depression and anxiety in VD; Caputo et al. [18] found a higher prevalence of anxiety in AD; and Fuh et al. [4], Srikanth et al. [21], Johnson et al. [12] and Hargrave et al. [19] found no differences between the two groups. These were all in vivo studies, which did not use neuropathological confirmation of the diagnoses, increasing the risk of misdiagnoses or comorbidity of other pathologies. The added value of the present retrospective study is that, although we could not use standardized tools for assessing the presence and severity of NPSs, we did use neuropathological confirmation of the diagnosis, and can therefore claim to have included “pure” cases of vascular and Alzheimer type dementia. Besides, it is routine clinical practice to ask questions about NPSs in history-taking, which means that the information about the presence or absence of NPSs that we obtained from the patients’ files was, from our point of view, accurate and reliable. It is important to take into account that some symp-

toms are likely to have been gathered with greater reliability than others. In particular, we presume that depression, anxiety and agitation, which are frequent and easily detectable, will have received greater attention from the clinicians in their routine clinical practice than the other symptoms.

Our retrospective study thus had two important limitations, the first one regarding the lack of standardized tools for clinical variables and the second regarding the small number of cases; both could have resulted in a lack of power to observe any differences. Although a trend towards a difference between AD and VaD might be suggested for some symptoms (depression, apathy, disinhibition), Figure 1 shows that the above limitations may explain why we did not find any statistically significant differences. We failed to prove that there are causal relations between NPSs and subtypes of dementia, but neither could we reject them. On the other hand, our study had the advantage of the availability of post-mortem tissue and neuropathological confirmation of the diagnoses.

We would like to draw attention to the higher (though not significantly so) prevalence of depression among the VaD patients in our study. Depression has been attributed to disruptions of cortico-subcortical circuits, involving basal ganglia, thalamus and frontal lobes. Vascular damage can disturb these cortico-subcortical circuits with lacunar lesions and white matter ischaemic injury [9,38,39]. Although these pathological processes are also present in AD, they are more prominent in VaD [40], which might explain why the prevalence of depression was slightly higher in our VaD group.

As regards the VaD group, we had surmised that neuropsychiatric disturbances might depend on the type of VL, but we did not find any differences either between the subgroups of VaD or between these subgroups and the AD group. This lack of significant differences might be due again to the small size of the sample. In view of this, we have to take into account the low prevalence of pure VaD in the general population. The NBB sample included only 40 cases diagnosed with pure VaD, while 660 AD cases were diagnosed in the same period of time. We also note the considerable heterogeneity of VL in our VaD group. Since VaD is quite a rare diagnosis, it was impossible for us to compose more homogeneous vascular groups that were large enough for proper statistical analyses. Therefore, our conclusions apply to the heterogeneous group which is called VaD but which is not a pure nosologic entity. In this respect, our approach is similar to that used in previous studies [40,41].

In conclusion, our data suggests that NPSs are related to dementia in a non-specific way, that is, AD and VaD patients do not differ significantly in terms of the 12 NPSs we included in our analysis. It is therefore important to investigate these NPSs in each demented patient individually, in order to improve the therapeutic approach and decrease caregiver burden as much as possible. We want to stress that previous studies were based on clinical diagnoses, which were probably often not accurate, especially with respect to VaD. Our study is the first one in the litera-

ture to provide an assessment of NPSs in subtypes of dementia based on clinical as well as neuropathological diagnoses.

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CHAPTER 6

Atrophy in the parahippocampal gyrus as an early biomarker of Alzheimer's disease

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The main aim of the present study was to compare volume differences in the hippocampus and parahippocampal gyrus as biomarkers of Alzheimer's disease (AD). Based on previous findings, we hypothesized that there would be significant volume differences between cases of healthy aging, amnestic mild cognitive impairment (aMCI), and AD. Furthermore, we hypothesized that there would be larger volume differences in the parahippocampal gyrus than in the hippocampus. In addition, we investigated differences between the anterior, middle, and posterior parts of both structures.We studied 3 groups of male participants: 18 healthy participants without memory decline; 18 patients with aMCI; and 18 patients with AD. 3T T1-weighted MRI scans were acquired and gray matter volumes of the anterior, middle, and posterior parts of both the hippocampus and parahippocampal gyrus were measured using a manual tracing approach.Volumes of both the hippocampus and parahippocampal gyrus were significantly different between groups in the following order: healthy > aMCI > AD. Volume differences between the groups were relatively larger in the parahippocampal gyrus than in the hippocampus, in particular when we compared healthy controls with individuals with aMCI. No substantial differences were found between the anterior, middle, and posterior parts of both structures. Our results suggest that parahippocampal volume discriminates better than hippocampal volume between cases of healthy aging, aMCI, and AD, in particular in the early phase of the disease. The present results stress the importance of parahippocampal atrophy as an early biomarker of AD.

Introduction

Atrophy in the medial temporal lobe has repeatedly been associated with age-related memory decline, mild cognitive impairment (MCI), and dementia (Burgmans et al., 2009; Hackert et al., 2002; Laakso et al., 2000; Raz et al., 2005; Scher et al., 2007; van de Pol et al., 2006; Visser et al., 2002; Wang et al., 2006). This is particularly true for hippocampal atrophy, which is considered to be one of the best predictors of Alzheimer's disease (AD) (Detoledo-Morrel et al., 1997; Jack et al., 2010; Jack et al.,1997; Pennanen et al., 2004). Recent findings suggest, however, that parahippocampal atrophy might have more potential as a predictor of AD.

Previous histological studies have already shown that the earliest neuropathological changes in AD appear in the entorhinal cortex, which is the anterior part of the parahippocampal gyrus (Braak and Braak 1990; Van Hoesen 1982). At present, several MRI studies suggest that volume measures of the entorhinal cortex provide higher sensitivity than volume measures of the hippocampus when it comes to detecting AD in an early phase of the disease (Detoledo-Morrel et al., 2004; Dickerson et al., 2001; Pennanen et al., 2004). Despite these findings, the parahippocampal gyrus has until now received much less attention than the hippocampus as an early predictor of AD. Moreover, the total parahippocampal volume - including posterior parts of the gyrus - has rarely been investigated in clinical populations. There is only scant evidence that this posterior part is involved in age-related and pathological changes (Burgmans et al., 2009; Insausti et al., 1998; Thangavel et al., 2008; Weniger and Irle 2006).

There is some evidence that the amount of atrophy differs between anterior, middle and posterior areas of the hippocampus and parahippocampal gyrus. One hypothesis is that differences in the amount of atrophy along the longitudinal axis are caused by the way cortical connections are organized. Van Hoesen et al. (1982) and Insausti and Amaral (2008) concluded from nonhuman primate studies that the posterior parahippocampal gyrus plays a key role in the information flow between cortical association areas and the hippocampal formation. There are massive projections from the posterior parahippocampal areas TF and TH to the rostral portion of the entorhinal cortex. Since the entorhinal cortex is reciprocally connected to the anterior hippocampus, we may speculate that damage in the posterior parahippocampal gyrus can lead to tissue changes in the anterior hippocampus. However, more research is needed to reveal the mechanism of differential decline in the medial temporal lobe structures.

In the present study, we compared volume differences in the hippocampus and parahippocampal gyrus in three groups of older individuals: healthy controls, individuals with amnestic MCI, and patients with AD. We hypothesized that volume differences between these groups are larger in the parahippocampal gyrus than in the hippocampus. In addition, we investigated differences between the anterior, middle, and posterior parts of both structures.

Materials and Methods

Participants

Three groups of older male participants were included in this study: healthy participants without memory impairment, patients with amnestic MCI (aMCI) and patients with AD. The healthy participants were recruited by means of advertisements in local newspapers. They were administered an extensive neuropsychological test battery and were included if their performance did not deviate from normal on the Verbal Learning Test (Folstein et al., 1975; Van der Elst et al., 2005). The patients with aMCI or AD were recruited from the Memory Clinic of the Maastricht University Hospital. All the diagnoses were made by a multidisciplinary team under supervision of an experienced neuropsychiatrist from the Memory Clinic (FRJV) according to the Petersen criteria for aMCI (with at least an impairment in the memory domain)(Petersen et al., 2001; Petersen et al., 1999), and according to the DSM-IV and NINCDS-ADRDA criteria for AD (McKhann et al., 1984). The diagnoses were based on medical history, co-morbidity, course, and MRI scan. The MRI scan was used to exclude vascular pathology; levels of atrophy were not used for the diagnosis of aMCI and AD.

The exclusion criteria were: use of psychoactive medication; abuse of alcohol and drugs; other past or present psychiatric or neurological diseases or serious system diseases; and structural abnormalities in the brain that could account for the cognitive decline. Two participants in the control group were excluded because a brain infarct was detected on the MRI scans and two patients in the AD group were excluded because their MRI images showed motion artefacts. The remaining number of participants (after excluding these 4 subjects) was 18 per group. Thus the final population consisted of: 18 healthy male participants (mean age = 64.5 years, SD age = 3.3 years); 18 male patients with aMCI (mean age = 65.11 years, SD age = 4.5 years) and 18 male patients with AD (mean age = 72.2 years, SD age = 9.7 years). A standardized eight-point scale was used to indicate educational level (1=primary school, 8=university). Written informed consent was obtained from all participants and from the primary caregiver of the AD patients. The study was approved by the local Medical Ethics Committee of the Maastricht University Medical Center.

Image acquisition and analysis

MRI scans were acquired with a 3 Tesla Gyroscan NT MRI scanner (Philips, Best, The Netherlands). Structural T1 images were acquired in the sagittal plane using an MPRAGE sequence (TR=8, TE= 3,7 msec, FA= 9, FOV= 240 X 240, matrix size= 240 x 240, number of slices= 180; voxel size = 1 x 1 x 1 mm^3). For data analysis, we used a manual tracing approach. The images were viewed using GIANT (General Image Analysis Tools) (Gronenschild et al., 2010), which is a customized software program that allows tracing of regions of interest in a tri-planar and a rotatable 3D surface-rendered view as well as calculation of volumes of interest. The definitions of

boundaries and divisions of the hippocampus and parahippocampal gyrus were performed according to the criteria described in a previous publication (Burgmans et al., 2009). Volumetric measures were obtained of the anterior, middle, and posterior parts of the hippocampus and parahippocampal gyrus (Figure 1).

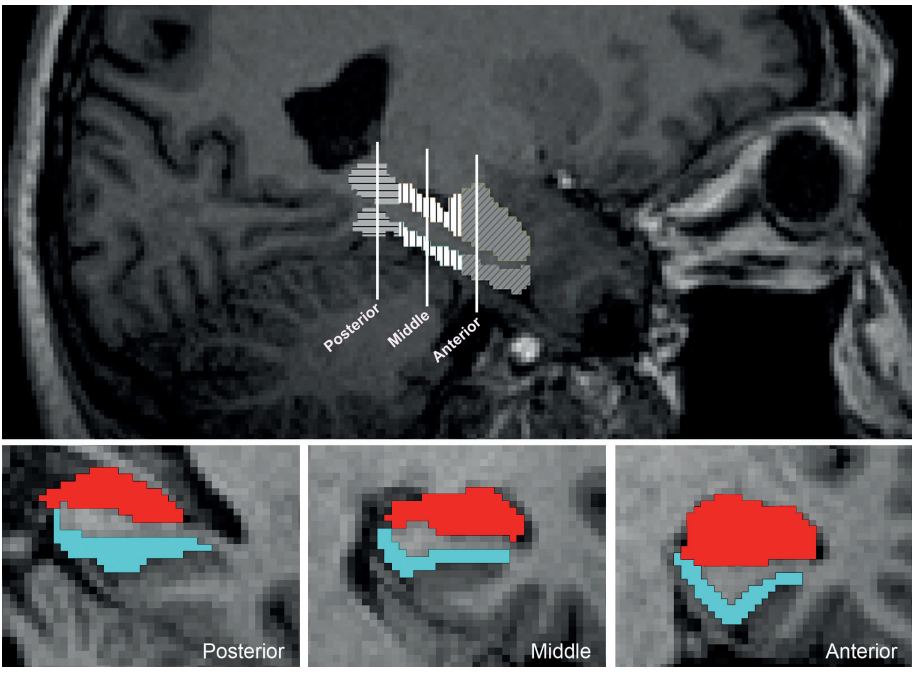

Figure 1.

Note. Patterns in the top panel show the subdivision of the hippocampus and parahippocampal gyrus along the antero-posterior axis. The anterior part included the anterior 35% of the coronal slices (and any rounded off number of slices closest to this cutoff), the middle part comprised 35%, and the posterior part included the remaining 30%. The bottom panel shows the coronal sections of the hippocampus (red) and parahippocampal gyrus (blue) along the anterior-posterior axis.

A single rater (CEZ), who was blind to the demographic and cognitive characteristics of the participants, traced all structures. Intra-rater reliability was determined by the Intraclass Correlation Coefficient (ICC) (Shrout and Fleiss 1979). Ten randomly selected brains were measured twice, and these yielded high test-retest reliability. The ICC of the total hippocampal volume was 0.97 (95% confidence interval= 0.91; 0.99), and the ICC of the total parahippocampal volume was 0.95 (95% confidence interval= 0.84; 0.98). To correct for individual differences in unatrophied brain, intracranial volumes were measured using an automated method (FSL Brain Extraction Tool) developed at the Oxford Centre for Functional MRI of the brain (Smith 2002).

Statistical analysis

Statistical analyses were performed using the Statistical Package for the Social Sciences (SPSS Inc, Chicago) version 16.0 for Windows. To compare the mean age, educational level, and MMSE scores between the 3 groups, ANOVA (General Linear Model; GLM) was performed. For the volumetric comparisons between the 3 groups, ANCOVA (GLM) was used, with volume as dependent variable, group as independent variable, and intracranial volume and age as covariates.

Three main things were tested in the ANCOVA analysis. First, the effect of group was calculated to test the overall difference across the three groups. Second, contrasts were calculated by pairwise comparisons among means to test the differences between two groups: i.e. control vs MCI; control vs AD; and aMCI vs AD. Third, the hypothesis that group differences are larger in the parahippocampal gyrus than in the hippocampus was tested by adding the hippocampus as covariate in the ANCOVA analysis with the parahippocampal gyrus as dependent variable. If group differences are still significant in this analysis, we can state that the parahippocampal gyrus has added value in discriminating between groups on top of the discriminating ability of the hippocampus. For all tests, Pearson's correlation coefficient was calculated as a measure of effect size.

Results

The 3 groups differed significantly with respect to age ($p = 0.001$) and MMSE ($p = 0.000$), but did not differ with respect to educational level ($p > 0.697$). The AD group was older (mean age 72.2, SD 9.7) than the other two groups. Minimental scores differed in the following order: control (mean MMSE score 28.8, range 27-30) > MCI (mean MMSE score 27.6, range 22-30) > AD (mean MMSE score 21.0, range 10-28). 16 of the 18 AD patients were in a mild stage of the disease (mean MMSE score 21.0, range 18-28), and two patients in a moderate-severe stage (MMSE score 10 and 15). The mean educational level in the control group was 4.2 (SD 1.4). The mean educational level in the MCI and the AD group was 3.8 (SD 1.8).

Volumetric comparisons between the 3 groups are summarized in Tables 1 and 2. Age and intracranial volume were included as covariates to correct for possible age and cohort effects. With regard to the main effect of group on the total volumes of the hippocampus and parahippocampal gyrus: differences between the three groups were significant in both volumes (parahippocampal gyrus: $F(2,46) = 24.23$, $p < 0.001$, hippocampus: $F(2,46) = 12.07$, $p < 0.001$). Volumes were significantly different in the following order: healthy > aMCI > AD. ANCOVA analyses demonstrated that the parahippocampal gyrus is better in discriminating between groups than the hippocampal gyrus. When we added the hippocampus as covariate in the ANCOVA analysis with the parahippocampal gyrus as dependent variable, we still found robust significant group differences ($F(2,45) = 11.07$; $p < 0.001$). In contrast, when we added the parahippocampal gyrus as covariate in the ANCOVA anal-

ysis with the hippocampus as dependent variable, the significant group differences disappeared (F (2,45) = 2.42; p = 0.100). This indicates that the parahippocampal volume has added value on top of the discriminating ability of the hippocampus. Furthermore, pairwise comparisons between the healthy and aMCI groups showed a significant difference in the parahippocampal gyrus (12.4%, F (1,29) = 10.46, p = 0.003) but not in the hippocampus (1.7%, F (1,29) = 0.85, p = 0.362). When we compared the control or the aMCI group with the AD group, significant differences were found in both structures (Figure 2).

With respect to differences along the longitudinal axis in the hippocampus and parahippocampal gyrus, the repeated-measures GLM showed a significant group by structure interaction (p = 0.034) in the left parahippocampal gyrus when we compared controls with AD patients. The group difference was larger in the anterior part than in the middle and posterior parts. No other significant differences were found between anterior, middle, and posterior parts. Furthermore, repeated-measures analysis showed no significant differences between both hemispheres (i.e., left-right differences).

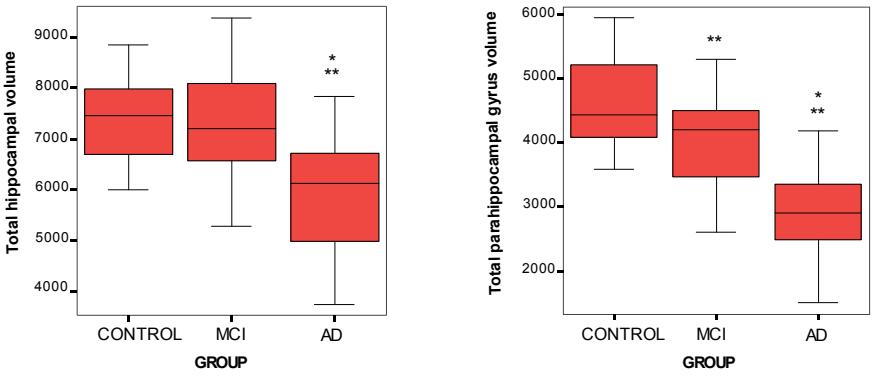

Figure 2. Boxplot of the hippocampal and parahippocampal gyrus volumes in mm^3 in controls, MCI and AD patients.

** = significant difference with control group; * = significant difference with MCI group.

Table 1. Volumetric comparisons between the three groups

Volume in mm^3	Controls (n=18)	aMCI (n=18)	AD (n=18)			
	Mean (SD)	Mean (SD)	Mean (SD)	F(2,46)	r	p
HCG total	7464 (794)	7334 (111)	5922 (122)	12.0	0.1	0.000
HCG ant	2959 (375)	2970 (459)	2452 (500)	8.4	0.4	0.001
HCG middle	2434 (269)	2321 (333)	1915 (394)	10.0	0.5	0.000
HCG post	2100 (359)	2045 (434)	1592 (383)	7.0	0.4	0.002
PHG total	4623 (680)	4047 (732)	3014 (588)	24.2	0.7	0.000
PHG ant	1657 (318)	1389 (326)	954 (303)	16.6	0.6	0.000
PHG middle	1646 (322)	1482 (421)	1074 (298)	15.6	0.6	0.000
PHG post	1.335 (202)	1169 (170)	881 (200)	20.3	0.6	0.000
HCG total L	3773 (344)	3704 (450)	3050 (684)	11.6	0.5	0.000
HCG ant L	1503 (210)	1500 (187)	1241 (156)	7.4	0.4	0.002
HCG middle L	1239 (154)	1194 (154)	994 (217)	11.3	0.5	0.000
HCG post L	1031 (207)	1009 (188)	813 (241)	4.1	0.4	0.023
HCG total R	3719 (575)	3630 (709)	2872 (620)	9.4	0.4	0.000
HCG ant R	1455 (276)	1470 (272)	1204 (287)	5.8	0.3	0.005
HCG middle R	1194 (183)	1123 (216)	920 (198)	11.3	0.4	0.000
HCG post R	1069 (159)	1036 (231)	747 (214)	6.8	0.4	0.000
PHG total L	2321 (487)	2111 (374)	1468 (319)	18.3	0.6	0.000
PHG ant L	843 (212)	705 (203)	436 (184)	11.7	0.6	0.000
PHG middle L	839 (205)	188 (794)	555 (129)	12.7	0.5	0.000
PHG post L	637 (142)	611 (106)	476 (123)	8.3	0.5	0.001
PHG total R	2301 (274)	1936 (429)	1546 (329)	18.9	0.6	0.000
PHG ant R	802 (162)	683 (208)	518 (177)	8.3	0.5	0.001
PHG middle R	812 (100)	693 (159)	586 (140)	11.1	0.6	0.000
PHG post R	687 (130)	558 (122)	441 (115)	15.3	0.6	0.000

Note. The group differences were calculated with ANCOVA (Univariate General Linear Model); HCG = Hippocampus; PHG = Parahippocampal gyrus; ant = anterior; post = posterior; L = left; R = right; SD = Standard deviation; r = effect size by Pearson's correlation coefficient; p = p value; F (2,46) = F-ratio and the degrees of freedom; p, r and F represent group difference.

Table 2. Volumetric pairwise comparisons

	Control and aMCI				aMCI and AD				Control and AD			
Volume in mm3	%	F(1,29)	r	P	%	F(1,29)	r	p	%	F(1,29)	r	p
HCG total	1.7	0.8	0.0	0.362	19.2	12.2	0.5	0.002	20.6	22.7	0.6	0.000
HCG ant	-0.3	0.2	0.0	0.628	17.8	9.2	0.5	0.005	17.1	14.4	0.5	0.001
HCG middle	4.6	3.5	0.1	0.069	17.4	12.5	0.5	0.001	21.3	30.8	0.6	0.000
HCG post	2.5	0.3	0.0	0.559	22.0	8.3	0.4	0.007	24.1	15.3	0.5	0.001
PHG total	12.4	10.4	0.3	0.003	25.5	24.5	0.6	0.000	34.8	36.8	0.7	0.000
PHG ant	16.1	9.4	0.3	0.004	31.3	12.8	0.5	0.001	42.4	25.2	0.7	0.000
PHG middle	13.2	4.9	0.2	0.033	19.3	17.4	0.5	0.000	34.7	25.2	0.7	0.000
PHG post	12.4	9.8	0.4	0.004	24.6	21.6	0.6	0.000	34.0	27.2	0.7	0.000
HCG total L	1,8	1.2	0.0	0.266	17.6	10.9	0.5	0.003	19.1	19.8	0.5	0.000
HCG ant L	0.19	0.4	0.0	0.499	17.2	8.0	0.4	0.008	17.4	11.6	0.5	0.002
HCG middle L	3.6	1.4	0.1	0.233	16.7	12.7	0.5	0.001	19.7	19.7	0.5	0.000
HCG post L	2.1	0.2	0.0	0.654	19.4	5.0	0.4	0.032	21.1	7.6	0.4	0.010
HCG total R	2.3	0.7	0.0	0.379	20.8	10.0	0.4	0.004	22.7	19.7	0.5	0.000
HCG ant R	-1.0	0.0	0.0	0.844	18.9	7.7	0.4	0.009	17.2	10.4	0.4	0.003
HCG middle R	5.9	3.7	0.1	0.063	18.0	8.1	0.4	0.008	22.9	24.4	0.5	0.000
HCG post R	3.08	0.3	0.0	0.580	27.7	8.5	0.4	0.007	30.1	14.1	0.5	0.001
PHG total L	9.04	3.4	0.2	0.071	30.4	36.9	0.7	0.000	36.7	24.4	0.7	0.000
PHG ant L	16.3	4.6	0.3	0.039	38.1	10.9	0.5	0.003	48.2	19.9	0.7	0.000
PHG middle L	17.9	1.1	0.1	0.282	19.3	26.8	0.6	0.000	33.8	17.7	0.6	0.000
PHG post L	4.08	0.8	0.1	0.368	22.0	15.6	0.5	0.000	25.3	10.4	0.5	0.003
PHG total R	15.8	18.4	0.4	0.000	20.1	8.0	0.4	0.008	32.8	35.4	0.7	0.000
PHG ant R	20.4	6.4	0.3	0.016	18.8	4.5	0.4	0.042	35.4	13.9	0.6	0.000
PHG middle R	14.6	10.2	0.4	0.003	15.4	4.1	0.4	0.050	27.8	21.1	0.7	0.000
PHG post R	19.02	11.3	0.4	0.002	20.9	7.7	0.4	0.009	35.8	23.7	0.7	0.000

Note. The groups differences were calculated with ANCOVA (Univariate General Linear Model); HCG = Hippocampus; PHG = Parahippocampal gyrus; % = difference between the two groups (V1-V2 x 100/V1); r = effect size by Pearson's correlation coefficient; p = p value; F(df) = for the covariate effect we give the F-ratio and the degrees of freedom (df)

Discussion

The main aim of the present study was to compare volume differences in the hippocampus and parahippocampal gyrus between healthy controls, individuals with aMCI, and patients with AD. In addition, differences along the longitudinal axis of both structures were investigated. Our results suggest that parahippocampal volume discriminates better than hippocampal volume especially in the early phase of AD. No significant differences were found along the longitudinal axis, except for the left parahippocampal gyrus in which the anterior parts showed significantly larger differences than the posterior parts when controls were compared to AD patients.

Our finding that parahippocampal volume discriminates better than hippocampal volume is in line with the findings of most previous studies on pathological aging in which a comparison was made between healthy and MCI groups (Detoledo-Morrel et al., 2000; Jack et al., 2000; Jauhiainen et al., 2009; Killiany et al., 2002; Pennanen et al., 2004; Xu et al., 2000), between MCI and AD groups (Du et al., 2001; Jauhiainen et al., 2009; Tapiola et al., 2008; Visser et al., 1999; Xu et al., 2000), or between healthy and AD groups (Detoledo-Morrel et al., 2000; Dickerson et al., 2001; Du et al., 2001; Jauhiainen et al., 2009; Killiany et al., 2002; Pennanen et al.,2004). There have been, however, also a few studies that found larger group differences in the hippocampus (Detoledo-Morrel et al., 1997; Jack et al.,1997; Pennanen et al., 2004; Visser et al., 2002). Nevertheless, in these cases a group of AD patients was always involved. Thus, in those cases where the hippocampus was found to be a better discriminator than the parahippocampal gyrus, the group comparisons were always between MCI and AD, or between control and AD, but never between control and MCI. This might indicate that the parahippocampal gyrus is a better discriminator particularly in the early (preclinical) phase of AD. The suggestion that the parahippocampal gyrus in particular is a highly sensitive marker with which to detect AD at a very early stage is in line with earlier reports on the entorhinal cortex (Detoledo-Morrel et al., 2004; Dickerson et al., 2001; Pennanen et al., 2004). The present study adds a new finding as it shows that this is not only the case with regard to the anterior part, but also with regard to the posterior part of the parahippocampal gyrus.

The present data are also partly in line with a previous study by our group (Burgmans et al., 2009) in which healthy, aging individuals with and without memory decline were compared. In that study we also reported larger group differences in the parahippocampal gyrus. However, we found the largest group difference in the posterior part of the parahippocampal gyrus. This is in contrast with the present results, since here we found larger differences in the anterior part of the left parahippocampal gyrus than in the posterior parts of the left parahippocampal gyrus between controls and AD patients. A possible explanation for these different findings could be that atrophy in the posterior medial temporal lobe is more strongly related to healthy aging, whereas atrophy in the anterior medial

temporal lobe is more strongly related to pathological aging. In a recent study, Raji et al., (2009) observed that in normal aging the most affected region was the posterior part of the hippocampus, while in AD patients the most affected regions were the anterior parts of the hippocampus and the parahippocampal gyrus.

There remain a few methodological issues to be addressed. First, our MRI data were acquired at only one time point, which precludes the assessment of actual pathological aging changes in volume, or of causality. Second, we included only male participants in order to decrease interindividual variation. We must therefore be cautious with generalizing our findings to females. Previous studies, however, did not reveal substantial gender-related differences with respect to hippocampal and parahippocampal atrophy in AD. Third, the subdivision applied to the hippocampus and the parahippocampal gyrus was an MRI macroscopical one. We chose to divide both structures into three subregions along the longitudinal axis, because the literature suggests there are important differences in age-related and pathological-related changes between the anterior and posterior areas. However, there are no clear anatomical reasons for the cutoff points used in the present study (i.e., 35% for the anterior volume; 35% for the middle volume; and 30% for the posterior volume). Nevertheless, one of the aims of this study was to explore group differences along the longitudinal axis, which has been made possible by our MRI-based approach. When it comes to investigating the subregions of the medial temporal lobe in more detail, cytoarchitectonic studies are considered more ideal.

In sum, our results suggest that parahippocampal volume discriminates better than hippocampal volume between cases of healthy aging, aMCI, and AD, in particular in the early phase of the disease. Our findings stress the importance of parahippocampal atrophy as an early biomarker of AD. Such predictive biological markers could be of great help for the development of early interventions designed to retard the progression of the disease. A possible implication could be a visual rating scale of the parahippocampal gyrus, similar to the one that exists for the hippocampus (Scheltens et al., 1992), which could help detect the earliest neurodegenerative changes indicative of AD in routine clinical practice.

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CHAPTER 7
Concluding remarks

In this thesis, the dementia syndrome is described from a multidimensional perspective, including clinical, neuropathological, and neuroimaging features of dementia. The main aim of the research underlying it was to investigate the correlation between clinical and neuropathological features of dementia. The specific research questions are therefore related to the correlations between clinical and neuropathological diagnoses and to clinical features of the dementia syndrome. Most of the research reported in this thesis was based on brain tissue from the Brain Bank of Navarre (Pamplona, Spain) and the Netherlands Brain Bank (Amsterdam). All the participants included in the study of the correlations between clinical and neuropathological diagnoses had been diagnosed with a neurodegenerative disorder (most of them with dementia) during life as well as post-mortem. All types of dementia present were included in order to assess the correlation between ante-mortem and post-mortem diagnoses. Clinical features, such as the prevalences of risk factors and neuropsychiatric symptoms (NPSs), were studied in patients with Alzheimer´s disease (AD) and vascular dementia (VaD), which are the most common types of dementia.

Summary of the main findings

Multiple pathologies in dementia

Chapter 2 discusses the rate of agreement between clinical and neuropathological diagnoses of dementia. The data showed low rates of agreement for the most common diagnoses, which is mainly due to the fact that several pathological changes often co-occur in the brains of demented patients. Overall, the agreement between clinical and pathological diagnosis was less than half (44%), confirming that the underlying biological findings often differ from the neuropathological disease suggested by the clinical diagnosis. Along the same line, Chapter 3 focuses on the relationship between the most prevalent pathological changes co-occurring in the same brain, which are Alzheimer´s disease (AD)-related, Lewy body (LB)-related, and vascular pathology (VaD or cerebrovascular lesions (CVL)). The analysis found evidence to support a positive association between AD and vascular lesion severity, suggesting that there may be a common pathogenesis underlying AD and vascular lesions. On the other hand, the results did not support a link between AD and LB pathologies, though it appears that LB pathology is a mediating factor which leads to a positive relationship between the severity of CVLs and AD-related pathology.

Cerebrovascular risk factors in dementia

The study reported on in Chapter 4 explored the role of vascular risk factors in dementia by estimating the prevalence of different risk factors in cases of dementia with neuropathologically confirmed AD, VaD and mixed AD plus vascular pathology.

Previous ***in vivo*** studies have reported various vascular conditions to be risk factors for dementia, and differences in risk factor profile have been found between subtypes of dementia. In order to compare the results of the sample from the Netherlands Brain Bank (NBB) with a more representative sample of the Dutch population, data from several control groups representing the elderly Dutch population were also included. Comparison of the three disease groups did not reveal significant differences in the prevalence of any of the vascular risk factors considered, except for a greater prevalence of a history of stroke in VaD and MD. Compared to controls, the only differences found were that smoking was more common in AD cases and that more VaD cases had a history of stroke. Our data show that the use of neuropathological confirmation of dementia diagnoses led to the disappearance of most of the previously reported differences in vascular risk factors between AD, MD, VaD, and controls.

Neuropsychiatry in vascular and degenerative pathology

Chapter 5 discusses the presence of NPSs associated with dementia in patients with neuropathologically confirmed AD and VaD. The most common symptom in the sample as a whole was agitation (45 cases, 57%), followed by depression (33, 41.2%) and anxiety (28, 35.4%). No significant differences between groups were found regarding the prevalences of NPSs, suggesting that NPSs are related to dementia in a non-specific way, that is, AD and VaD patients do not differ significantly in terms of NPSs. Our results suggest that the prevalence of these NPSs cannot be related to the dementia diagnosis of AD or VaD. We want to stress that previous studies were based on clinical diagnoses, which were probably often not sufficiently accurate, especially those for VaD. Our study is the first one in the literature to provide an assessment of NPSs in types of dementia based on clinical as well as neuropathological diagnoses.

Early stages of dementia

Chapter 6 discusses magnetic resonance imaging (MRI) markers of early stage AD. Previous neuropathological studies have identified the parahippocampal gyrus as the first structure to be involved in AD (Braak & Braak, 1991), but neuroimaging studies have been focusing on changes in the hippocampus as a biomarker for early stage AD. The role of changes in the parahippocampal gyrus as a neuroimaging biomarker for early stage AD was explored by measuring the volume of this structure and of the hippocampus in healthy volunteers, and in patients with amnestic mild cognitive impairment (aMCI) and mild AD. The results suggest that parahippocampal volume is better able to discriminate between cases of healthy ageing, aMCI, and mild AD than hippocampal volume, especially in the early stages of the disease. These findings underline the importance of parahippocampal atrophy as an early biomarker of AD, which had not been demonstrated before.

Methodological considerations

There are some methodological issues that need to be addressed as regards the research underlying this thesis, mainly arising from the characteristic limitations of a retrospective neuropathological study not based on a population series but on a series of donors from brain banks.

First, as is inherent in all clinicopathological studies, the data used for our thesis are subject to selection bias. Such selection bias most commonly results from referral bias, which is influenced by the severity of the disorder and the management provided by the caregiver or caregivers of the demented person (Zaccai et al., 2006). In fact, patients with rarer neuropathological conditions are more likely to become brain bank donors (Jicha et al., 2008; Schneider et al., 2009). For instance, there is a particularly high prevalence of FTLD and prion disease cases in the sample from the Brain Bank of Navarre. Besides, donors to brain banks are usually elderly patients in the later stages of their diseases. Since our samples from the brain banks are thus not entirely representative of the European population, our findings may have been affected by selection bias, so they must be interpreted very cautiously, especially when it comes to assessing demographical or epidemiological variables.

Second, the relatively small number of participants in our clinico-neuropathological studies could have resulted in low statistical power and, hence, failure to find significant results. Furthermore, most of the donors to the brain banks were in the later stages of their diseases; for instance, most individuals with AD were at Braak stages of V or VI. This narrow range of Braak stages may have hampered the process of finding robust correlations, especially when studying the relationship between the severity of AD pathology and CVLs. These results have to be interpreted by taking into account that they apply mostly to the later stages of the dementia syndrome. We have no neuropathological findings for the early stages of the disease, and consequently have no information about the way pathology spreads through the brain until its later stages.

Third, there was a time lag between clinical assessment and pathological data collection in our studies. In addition, clinical data were not collected prospectively, but in a retrospective way from patients' medical and brain bank records. Although clinical studies can be strictly controlled and prospective, clinical information is often lacking at the moment of neuropathological analysis. The research for this thesis found that clinical parameters, such as the presence of vascular risk factors or NPSs, were not always available or had not been compiled consistently. Indeed, no standardized tools were used for assessing the presence and severity of NPSs. On the other hand, the availability of a neuropathological diagnosis is a considerable advantage. In a clinico-pathological study like ours, pure AD and VaD cases can be distinguished. Only 40 pure VaD cases were diagnosed in the NBB sample, while 660 pure AD cases were diagnosed in the same period of time. As mentioned

above, there was a high degree of heterogeneity in terms of the vascular lesions in the group. Accordingly, with pure VaD being such a rare diagnosis in the available sample, it was impossible to establish more homogeneous vascular subgroups that were large enough for robust statistical analyses. Therefore, the conclusions apply to the heterogeneous group which is referred to as VaD, but which is not a pure nosological entity. Note that this approach is similar to that adopted in previous studies (Kalaria et al., 2004; Pantoni et al., 2006; Alafuzoffa et al., 2012).

Implications

What do our findings mean for diagnosis, for doctors who look after people with this syndrome, for pathologists, for researchers, and for patients? This thesis raises several important questions, and an attempt will be made to answer these questions, approaching them from different perspectives, i.e. from both a clinical and a neuropathological point of view, as well as from the perspective of researchers, brain banks, and of course patients and their caregivers.

Nosological implications: multiple pathologies in dementia

The criteria for differentiating between dementia types are currently based on neuropathological changes, and follow traditional nosological classifications. However, it is known that clinical diagnoses often need to be revised when neuropathological findings are taken into account (Braak & Braak, 1991; Kovacs et al., 2008; Brunnstrom & Englund, 2009; Echávarri et al., 2012; Alzheimer´s Association, 2012), since the high incidence of comorbid neuropathologies complicates the differentiation between dementia diagnoses in clinical practice. The findings presented in the present thesis show that the co-occurrence of neuropathological changes is likely to increase with advanced age, as the incidence of comorbidity was higher in patients over the age of 80. This is in line with other results for community-dwelling older persons and autopsy series (Galasko et al., 1994; Echávarri et al., 2011). This thesis supports the idea of a multidimensional approach to diagnosing dementia, in which dementia syndromes are not categorized into diagnostic types, but are seen as one clinical syndrome representing the gradual accumulation of multiple pathologies. This multidimensional approach takes into consideration that categorical diagnoses made in vivo often do not correspond to the multimorbid pathology, and may thus be oversimplified. In agreement with Richard and Brayne (Richards & Brayne, 2009), we claim that diagnostic classifications made on the basis of assumed clinicopathological correlations should be changed so as to arrive at a global approach. Thus, this thesis underlines that a multidimensional approach would not only increase the accuracy of dementia diagnoses, but also lead to a better understanding of neurodegenerative diseases among clinicians.

Cerebrovascular risk factors in dementia

Previous studies of the relationship between vascular risk factors and dementia have produced inconsistent results. Although data from longitudinal epidemiological studies have supported the view that there is a relationship between vascular risk factors and dementia (Pohjasvaara et al., 1998; Knopman et al., 2001; Kivipelto et al., 2001; Kivipelto et al., 2005; Kuller et al., 2005; Reitz et al., 2008; Schneider et al., 2009; Knopman & Roberts, 2010), this was not confirmed by some other researchers (Launer et al., 2008; Purnell et al., 2009; Richard et al., 2009). In agreement with other studies, this thesis notes that common clinical dementia (like AD, Lewy bodies dementia or VaD) cases are often in fact mixed conditions, with Alzheimer's, vascular and Lewy body-related pathologies (Dickerson et al., 2001; Jellinger & Attems, 2003; Trojanowski et al., 2003; Parkkinen et al., 2008; Knopman & Roberts, 2010; Echávarri et al., 2012). This would explain the failure to identify vascular risk factor patterns associated with types of dementia (AD, VaD, or MD). In Chapter 4, we argued that vascular risk factors may indeed not be related to the presence of dementia, except for stroke history in VaD. Specifically, no differences were found regarding the distribution of vascular risk factors in AD, VaD, and mixed dementia, suggesting that the burdens caused by vascular and AD type lesions are independent of each other. On the other hand, Chapter 3 presents evidence indicating a relationship between degenerative and vascular lesion severity, which is consistent with a synergic effect of the two types of lesions in dementia (Launer et al., 2000). From a clinical perspective, bearing in mind all the aforementioned findings, this thesis suggests that the presence of vascular risk factors should be taken into account in demented patients. That is, it is essential to consider the vascular component of degenerative dementias to provide better treatment options for demented patients; the data reflect how important it is to prevent vascular risk factors throughout the course of dementia, in order to minimize the severity of the disease.

Neuropsychiatry in vascular and degenerative pathology

In agreement with prior studies, our study of the NBB sample suggests that pure VaD cases are rather rare, making it difficult to attribute different patterns of symptoms associated with dementia specifically to AD or VaD (Launer et al., 2008). Our results suggest that the prevalence of these NPSs cannot be related to the underlying (and pathologically verified) dementia diagnosis of AD or VaD, which means that NPSs in dementia do not differ with the subtype of dementia. A few previous studies have compared NPSs between dementia subtypes, with conflicting results. Some studies found higher prevalences of NPSs in VaD, including depression, anxiety, or agitation, while others found higher prevalences in AD, including anxiety and agitation, and still others found no differences between groups (Ballard et al., 2000; Caputo et al., 2008; Johnson et al., 2011). Again, these contradictory results may be due to a lack of accuracy in diagnosing dementia subtypes. Our study is the only one performed on this topic which provides a sample with neuropathological con-

firmation of clinical diagnoses. Although no differences regarding NPSs were found between the AD and VaD groups, we stress the high prevalence of NPSs in demented patients. Thus, it is important to investigate these NPSs in each demented patient individually, to be able to improve the therapeutic approach and decrease caregiver burden as much as possible.

Early stage dementia

Results presented in this thesis suggest that changes in the parahippocampal gyrus are a highly sensitive marker for detecting AD at a very early stage. This is in line with earlier reports on the anterior part of the parahippocampal gyrus, the entorhinal cortex (Reitz et al., 2008; Echávarri et al., 2011). The present thesis adds to the existing body of knowledge in that it shows that this is also true for the posterior part of the parahippocampal gyrus. The findings underline the importance of parahippocampal atrophy as an early biomarker of AD, which has never been shown before. In fact, there have been no previous studies focusing on the role of the parahippocampal gyrus as a neuroradiological biomarker of AD. Such predictive biological markers could be very valuable for the development of early interventions designed to delay the progression of the disease. An implication is that it might be useful to develop a visual rating scale of the parahippocampal gyrus, similar to the one that exists for the hippocampus (Scheltens et al., 1992), as this could help detect the earliest neurodegenerative changes indicative of AD in routine clinical practice.

Directions for the future

The high dependency associated with dementia results in very high costs to society (Alzheimer´s Association, 2012), which makes research into the aetiology and pathogenesis of late-life dementia a high priority. Beyond the pathological confirmation of diagnoses, working with neuropathological findings allows a range of other issues to be investigated. These include identification of the brain lesions that best correlate with cognitive decline, and the assessment of links between pathological findings and potential risk factors. This thesis highlights the role of brain banks in research in neurosciences, in particular in dementia; the study of brain tissue is essential since it provides useful feedback to clinical teams and may also lead to further improvements in the diagnostic procedures (Brunnstrom & Englund, 2009). Further systematic comparisons of clinical and pathological dementia diagnoses are needed.

The potential to establish an early diagnosis, to predict the underlying pathology during life, and to stage disease progression will all be required for trials of disease-specific treatments in the future. This approach highlights the importance of brain lesions as substrates for the decline noted in normal ageing, in comparison to dementia. It is possible to test hypothetical semi-quantitative

thresholds definable in terms of a staging system to classify clinical dementia and shed light on the critical steps in the neurobiological progression of cognitive decline through states such as MCI. Thus, identifying neuropathological markers relevant to cognitive function is useful for both the diagnostic process and our understanding of the molecular and clinical aetiologies of various types of dementia, and can ultimately be expected to result in the development of better treatments.

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SUMMARY

Dementia is a clinical syndrome characterized by an acquired and persistent impairment in multiple cognitive domains that is severe enough to interfere with everyday functioning. Although standardized clinical and neuropathological criteria for different subtypes of dementia have been proposed in consensus meetings, correlations between clinical and neuropathological symptoms and diagnoses are far from optimal. The research reported on in this thesis investigated this relationship between the clinical and neuropathological approaches to dementia. The general introduction (***Chapter 1***) provides background information regarding clinical and neuropathological characteristics of dementia subtypes, and outlines the main aim and research questions of this thesis.

In ***Chapter 2*** we investigate the level of agreement between clinical and neuropathological diagnoses in neurodegenerative disorders. We found a mean agreement of 44.0% between the clinical diagnosis and the purely neuropathological diagnosis. This level of agreement differed between dementia subtypes, e.g. 85% for prion disease, 49% for AD and 0% for Lewy bodies dementia. Our data confirmed that co-occurrence of multiple neuropathological disorders is very common in individuals with dementia, supporting a multidimensional approach to diagnosing dementia, in which dementia syndromes are characterized using a combination of various neuropathological dimensions.

Chapter 3 discusses the associations between the severity of Alzheimer pathology (i.e., Braak stage), cerebrovascular lesions (CVLs) and Lewy bodies in demented patients. Cases of pure AD and AD with comorbidity were analyzed together and also separately, in both groups. For the group as a whole, we found a significant positive relationship between AD Braak stage and CVL severity, which was not found when analyzing pure AD and AD plus comorbidity groups separately. No relationship was found between Braak and LB stages. Our study supports a positive association between AD pathology and CVLs in demented patients with AD, suggesting that AD and CVLs may have a common pathogenesis.

Chapter 4 compares the prevalences of vascular risk factors in neuropathologically confirmed cases of AD, vascular dementia (VaD), and mixed AD and VaD (MD). It compares the results with published prevalence data for elderly local Dutch control populations. Comparing the three disease groups did not reveal significant differences in prevalence for any of the risk factors considered, except for a greater prevalence of a history of stroke in VaD and MD. Compared to controls, the only differences found were that smoking was more common in AD cases and more VaD cases had a history of stroke. We argue that risk factors may not be related to the presence of dementia, except for stroke history in VaD. Although differences in vascular risk factors between AD, MD, and VaD had previously been reported from ***in vivo*** studies, we demonstrate that these differences disappear when using neuropathological confirmation of dementia diagnoses.

Chapter 5 discusses the prevalence of NPSs associated with dementia in cases with neuropathologically confirmed AD and VaD. Our study failed to find differ-

ences between groups regarding these symptoms, suggesting that AD and VaD patients do not differ significantly in terms of NPSs. This is the first study on this topic to use neuropathological confirmation of the diagnoses.

Chapter 6 examines the role of the parahippocampal gyrus as an early neuroimaging biomarker of AD by measuring the volume of this structure and the hippocampus in healthy participants and participants with amnestic mild cognitive impairment (aMCI) and mild AD. Our results suggest that parahippocampal volume discriminates better than hippocampal volume between cases of healthy ageing, aMCI, and mild AD, especially in the early stages of the disease. Our study is this first one to demonstrate the importance of parahippocampal atrophy as the earliest neuroradiological biomarker of AD.

Chapter 7 presents some concluding remarks and summarizes the main findings of this thesis, comparing them with those previously reported in the literature. It also presents some methodological considerations and discusses implications of our results in relation to clinical practice and further research.

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I have learned many things about neurosciences, research, brain banks, and about other countries…, and in fact about life, during this journey. The main benefit I got out of it is to know how pleasant and worthwhile it is to live with hopes and to work hard to realize these hopes!. My wish is that I will be able to continue living like this.

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CURRICULUM VITAE

Carmen Echávarri Zalba was born 17 April 1976 in Pamplona, Spain. She studied medicine at the University of Navarra, Spain (1994-2000), continuing her training in neurology at the Hospital of Navarra in Pamplona, Spain (2002-2006). She has worked as a general neurologist and as a neurologist specializing in psychogeriatry and dementia in various places: the Department of Neurology at the Hospital of Navarre, the Psychogeriatric Clinic Josefina Arregui in Alsasua (Navarra), the Memory Clinic at the ACE foundation in Barcelona (Spain), and at the hospitals of Harrogate and Scarborough (United Kingdom). She completed a three-year training period in neuropsychology (organized by the Spanish Society of Neurology in Barcelona). She further developed her skills in neuropathology and brain bank management at various hospitals: the Department of Pathology of the Complex of Hospitals of Navarra under Drs Teresa Tuñón and Federico García-Bragado, the Department of Neuropathology at the University Hospital of Belltvitge (Barcelona, Spain) under Professor Isidro Ferrer, the Department of Neuropathology and Brain Bank of Cambridge at Addenbrooke´s Hospital (United Kingdom) under Dr John Xuereb and the Department of Neuropathology and Brain Bank of the Netherlands (at the VU Medical Center) under Professor Annemieke Rozemuller and Dr Wouter Kamphorst.

She completed her doctorate courses in neurosciences at the University Clinic of Navarre (2003-3006) under Professor Jose Masdeu, and started her PhD project at the Department of Neuropsychiatry and Psychology at Maastricht University under the supervision of Professor Frans Verhey, Professor Harry Uylings and Dr Saartje Burgmans.

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