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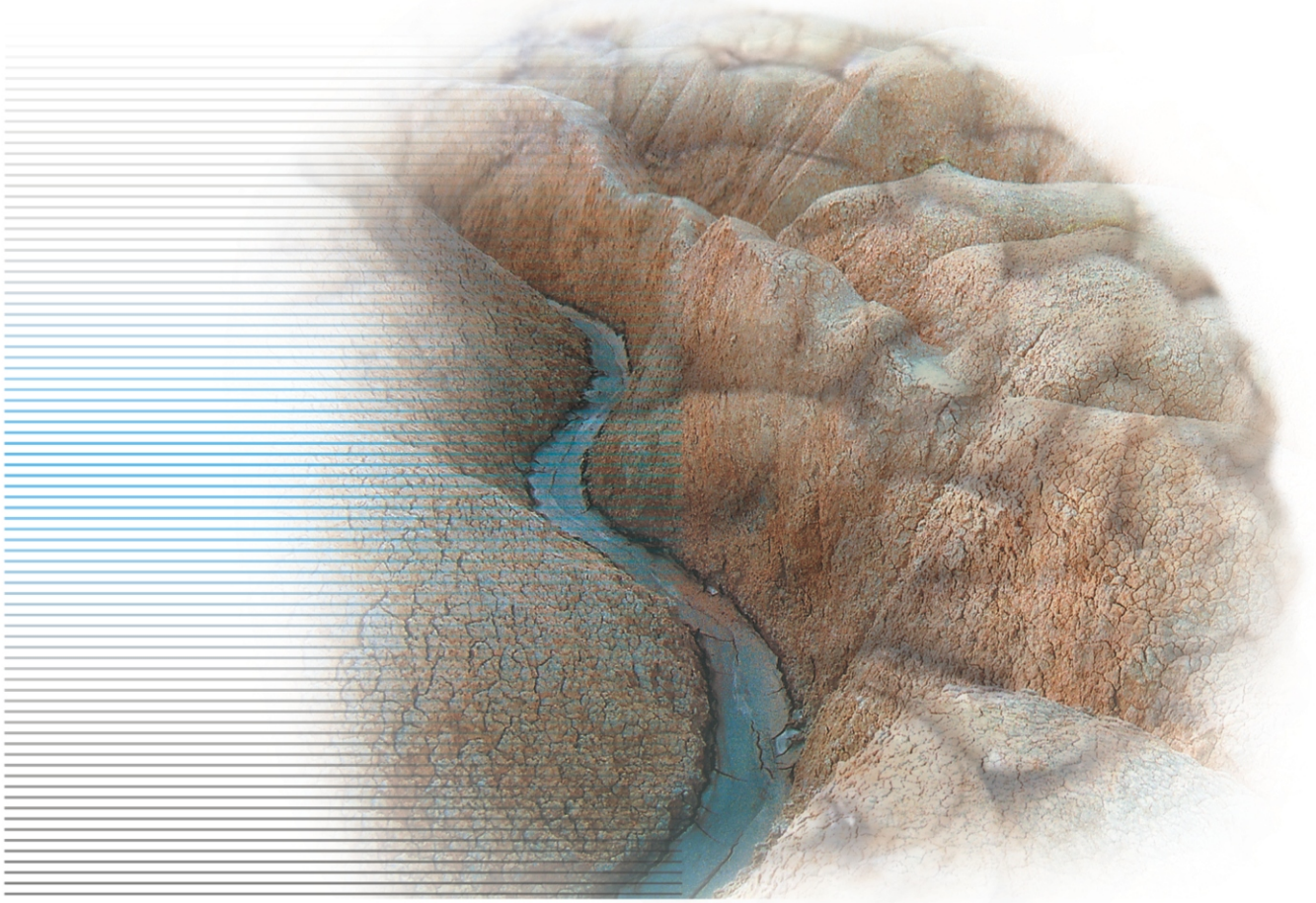

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Treatment Strategies in Frontotemporal Lobar Degeneration

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Abstract

Frontotemporal lobar degeneration (FTLD) is a new pathoanatomical designation for what is called Pick's disease in orthodox terminology1,2. It is the most frequent cause of presenile dementia next to Alzheimer's disease (AD). At our memory clinic, the FTLD/AD prevalence ratio is approximately 1/6. Considerable efforts are made to identify and treat patients with Alzheimer's disease (AD), even in "preclinical" stages. Less attention has been paid to these issues in FTLD. This is due in part to clinical underrecognition of FTLD. In fact, FTLD is usually diagnosed clinically as AD3. Also, less is known about pathogenesis in FTLD than in AD. This review deals with tentative lines of evidence in treatment and management of patients with FTLD.

Keywords: Frontotemporal lobar degeneration, Alzheimer disease

Clinical variety of FTLD

It is widely appreciated that FTLD cases may differ significantly in clinical picture. This is important when it comes to treatment. Clinical presentations result from different "atrophic foci" (Germ. *Schrumpfungszentren*)[2]. They may also reflect differences in pathogenesis, as argued in a recent study[4]. Anatomically, these foci cluster around the entrance of the Sylvian fissure. Atrophic foci also occur atypically in the inferior parietal lobule and the superior frontal gyrus[2]. Disruption of social cognition has long been recognized in FTLD. It is associated with atrophy of the orbitofrontal region and the temporal poles[2,5,6]. This deficit, along with executive dysfunction, is subsumed under the label *frontotemporal dementia* in the strict sense[1]. Some FTLD cases have prominent atrophy of the frontal convexity; here, executive dysfunction and apathy prevails. Other patients have marked aphasia. Of these, some lose the conceptual structures that are crucial for language and object recognition. This syndrome, intensely studied in recent years, is called *semantic dementia*[1]. It is associated with atrophy of the neocortical infratemporal region, an anatomical pattern described in detail long ago[2]. Others show nonfluent aphasia with oral apraxia or acquired stuttering. This is referred to as *nonfluent progressive aphasia*[1]; such patients have prominent left perisylvian atrophy.

Pharmacotherapy

FTLD has been construed as a tauopathy. This contrasts with AD, where abnormal amyloid metabolism is implicated. However, the pathogenesis remains unknown in most FTLD cases[7]. Many patients, perhaps temporal lobe cases in particular, show tau-negative

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but ubiquitin-positive inclusions and neurites on neuropathological examination[4] (Fig. 1). The ubiquitin protein is part of an evolutionarily conserved pathway for regulated protein turnover whose function is essential for neuronal viability. It helps to mediate proteolytic reactions and attaches to the paired helical filaments in neurofibrillary pathology. These findings underscore the relationship between FTLD and motor neuron disease and open an interesting field for approaching fundamental pathological processes in FTLD. Ubiquitin metabolites may be a future target for monitoring the disease process and treatment.

Pending such progress, symptomatic and neuroprotective pharmacotherapy should be considered. A number of neurotransmitter systems modify states of processing in the cerebral cortex. They are involved in the control of behavior and are targeted by various psychopharmacological agents. Serotoninergic, dopaminergic, and glutamatergic deficits have been demonstrated in FTLD[8,9]. *Cholinergic* drugs are used in the treatment of AD. The use of such drugs in FTLD has been rejected, as there is no evidence for a significant cholinergic deficit in FTLD. Cholinergic drugs actually may exacerbate abnormal behavior in these patients[10]. *Selective serotonin reuptake inhibitors* have been tried in FTLD. These agents may be useful in reducing impulsive or compulsive behavior as well as hyperphagia[11]. To complicate matters, many patients with FTLD are both disinhibited and apathetic, and a minority may be only apathetic. Apathy and impaired attention is likely to respond better to *catecholaminergic* agents. Such drugs include reuptake inhibitors (reboxetine, bupropion), α_2-adrenergic antagonists, and dopamine receptor agonists. A selective α_2-adrenergic antagonist improved the performance of patients with frontotemporal dementia on computerized tests of planning, sustained attention, verbal fluency, and episodic memory[12]. Spatial working memory worsened with treatment, however. Like patients with Lewy body disease, FTLD patients may tolerate *antipsychotics* poorly[13,14]. *Tianeptine*, the selective serotonin reuptake accelerator, has antidepressant and neuroprotective properties[15]. Whether it is useful in FTLD is unknown. The same holds true for the neuroprotective agent *piracetam*[16]. It facilitates callosal transfer and might be useful in aphasic FTLD. *Memantine* is a glutamate receptor antagonist. It may be of use in AD, vascular, and mixed dementia[17]. It too remains untried in FTLD.

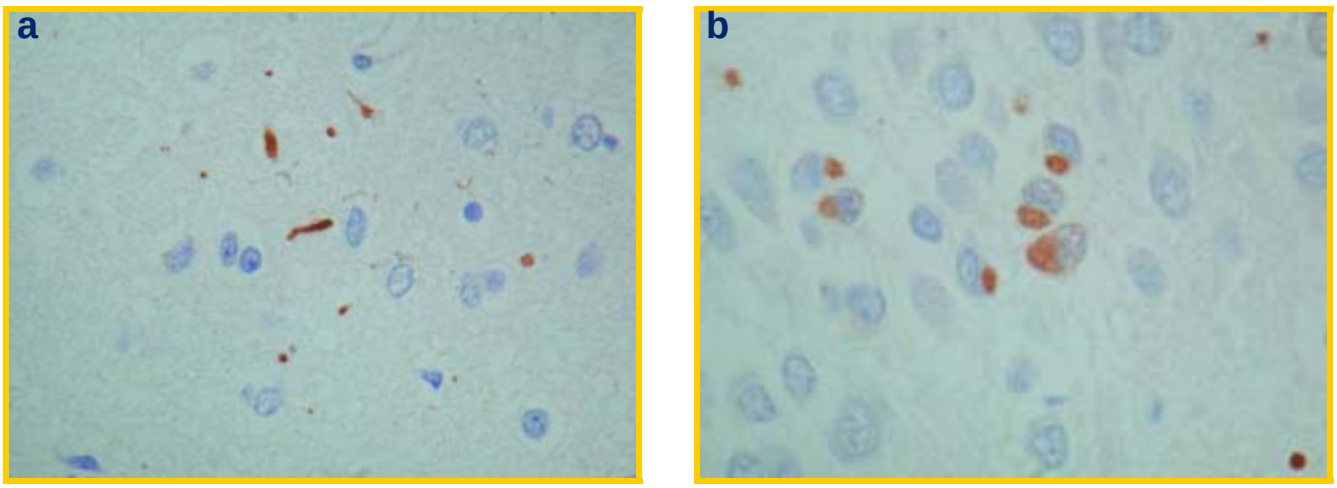

Figure 1. Ubiquitin-positive neurites (*a*) and intraneuronal inclusions (*b*) in the hippocampal dentate gyrus in FTLD (semantic dementia). Neither dystrophic neurites nor neuronal inclusions were immunoreactive for antibodies against tau. *(By courtesy of Dr. Nenad Bogdanovic, Brain Bank, Huddinge University Hospital, Stockholm, Sweden.)*

Behavioral intervention in FTLD

Well-defined aphasic disorders in FTLD might be targets for behavioral intervention. The effects of aphasia therapy in stroke rehabilitation remain unclear. This is due mainly to the lack of studies that meet modern standards[18]. Unsurprisingly, even less is known about aphasia treatment in FTLD. There is convincing evidence for preserved learning in semantic dementia[19]. Relearning of verbal concepts through definitions has proved possible for some time in semantic dementia[20]. Verbal plus gestural matrix training improved sentence production in nonfluent progressive aphasia[21]. Nonfluent progressive aphasics achieved improved phonological skills through the use of acoustically modified speech stimuli[22]. This study was based on the controversial hypothesis that phonological disorders arise from failures in rapid auditory processing[23].

Challenging behaviors in FTLD may be controlled or eliminated by intervention. For instance, redirecting patients' initiative to well-preserved activities of neutral or positive value (blood pressure measurement, singing) ameliorated disruptive compulsions in a ward[24]. Existing compulsions may be rechanneled into less disturbing variants[25]. Caregiver education obviously is crucial in the management of FTLD.

Table 1. Some psychopharmacological agents of interest in the treatment of FTLD.

Generic name	Main pharmacological action(s)	Target symptom(s)
Citalopram, escitalopram, fluoxetine, fluvoxamine, paroxetine, sertraline	Selective serotonin reuptake inhibitor	Disinhibition, compulsions, hyperphagia
Reboxetine	Selective noradrenaline reuptake inhibitor	Apathy, impaired attention
Bupropion	Selective catecholamine reuptake inhibitor	Apathy, impaired attention
Mirtazapine, mianserine	α_2 (+ α_1) adrenergic antagonist, indoleamine antagonist (H_1, 5-HT_{2+3})	Apathy, impaired attention; insomnia?
Trazodone	Serotonin antagonist (5-HT_{1A}, 5-HT_{1C}, 5-HT_2) and reuptake inhibitor	Agitation? Insomnia?
Moclobemide	Reversible monoamine oxidase A inhibitor	Broad range of symptoms?
Buspirone	Presynaptic 5-HT_{1A} agonist, presynaptic D_2 antagonist	Agitation? Aggression? Neuroprotective?
Tianeptine	Selective serotonin reuptake enhancer	Apathy? Neuroprotective?
Piracetam	Uncertain; GABA derivative	Aphasia? Neuroprotective?
Memantine	Glutamate receptor antagonist	Cognitive and behavioral

Conclusion

The traditional aim of AD treatment in clinical trials has been to improve cognitive abilities. FTLD differs from AD in pathology as well as in clinical symptomatology. Expanding knowledge of the etiopathogenesis of FTLD and its neurobiological substrates will give us the possibility to develop new treatment strategies. So far trials with improvement of the cholinergic system like in AD have not been successful. While cognition has been previously viewed as the primary measure of efficacy, areas such as behavioral problems, psychiatric symptoms, aphasia, social interaction, functional abilities, and quality of life should be assessed to fully evaluate treatment effects in FTLD. We have outlined some currently available and visionary intervention strategies that could be symptomatic but also disease-modifying through a neuroprotective mode of action. If these drugs could postpone or slow decline in any of the areas it may represent an important benefit both for patients, caregivers, and society.

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Biomarkers for Alzheimer Disease in the Cerebrospinal Fluid: Tau, Hyperphosphorylated Tau and Amyloid Beta Protein

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Abstract

The current therapeutic compounds for treatment of Alzheimer disease (AD) e.g. acetylcholine esterase inhibitors and tomorrow's compounds such as γ-secretase inhibitors demand improving of the diagnostic accuracy of AD. One diagnostic tool is biomarkers in the cerebrospinal fluid (CSF). These would be especially valuable early in the course of the disease, when correct diagnosis is difficult, but where the therapeutic compounds might have their greatest potential of being effective. This paper is a review of CSF biomarkers for AD, with focus on their role in the clinical diagnosis. Increased CSF levels of total tau (T-tau) and reduced CSF levels of the 42 amino acid form of Aβ (Aβ42) in AD have been found in numerous studies, with sensitivity above 85%, but with lower specificity against other dementias. By adding phosphorylated tau (P-tau) the specificity increases, since normal levels are found in both frontotemporal, Lewy body dementia, and cerebrovascular disease. Recent studies suggest that these CSF markers perform satisfactory enough to be added in the clinical work-up of patients with dementia if used together and with other clinical information and brain-imaging techniques.

Keywords: Alzheimer disease, MCI, tau, PHF-tau, β-amyloid, biochemical markers, cerebrospinal fluid, diagnosis.

Introduction

Alzheimer disease (AD) is the most common form of dementia. The vast majority has no clear family history and is classified as sporadic AD[1]. The cost for the society is substantial and for Sweden the direct cost is estimated to around 0.4 Billion Euro per million inhabitants[2]. If nothing is done this cost will continue to increase, due to the rapid increase in the elderly population. At the same time the progression of dementia will have substantial impact on the family caregiver and the entire health system and AD has become one of the most costly affections for the modern society.

The ante mortem diagnosis is based on clinical and neuropsychological evaluation and the absence of other known causes of dementia as outlined by the NINDS-ADRDA[3], and diagnosis is only definite at autopsy. The accuracy of the diagnosis are shown to be from 65% to at most 90% with figures emanating from academic centers with special interest of AD and are based on patients in the later stages of the disease who were followed for several years before the confirming autopsy[4-6].

At the level of primary care and in general hospitals, the diagnostic accuracy rate is probably lower[7], and this may especially be the case in the early phase of the disease where symptoms are vague and indistinct.

Previously, with no medical treatment of dementia, the need for an exact diagnosis was less important. Many patients were moved to different forms of sheltered accommodation without any, or an inadequate, clinical involvement. Today, with existing[8] and emerging therapeutic compounds (vaccination, β or γ -secretase inhibitors, or stations and more) the situation is totally different and we have a great need for a reliable diagnosis. This is not least important early in the course of the disease, before neurodegeneration is too severe and widespread. In this stage of the disease the diagnostic problems however are the most difficult.

Furthermore, early diagnosis would give families more time to plan for proper care of the AD patient. It is assumed that the clinical phase is preceded by a 15-30 year

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preclinical period. Currently no single peripheral biomarker specific for AD has been found[9-11]. Therefore, finding reliable biochemical diagnostic markers for the diagnosis, especially for Alzheimer disease (AD) is one of the most pressing tasks for making an early diagnosis possible.

The pathology of AD is restricted to the brain, and since the cerebrospinal fluid (CSF) is in direct contact with the extracellular space of the brain, biochemical changes in the brain may be reflected in the CSF. For this reasons the CSF would be an obvious source of biomarkers for AD. A diagnostic marker for AD should reflect the central pathogenic process of the disease, i.e. the degeneration of the neurons and their synapses and the defining lesions senile plaques (SP) and neurofibrillary tangles (NFT)[12].

Suggested biomarkers for these pathogenic processes are, respectively, normal (total) tau protein (T-tau), Aβ42, and phosphorylated tau protein (P-tau) (Fig. 1).

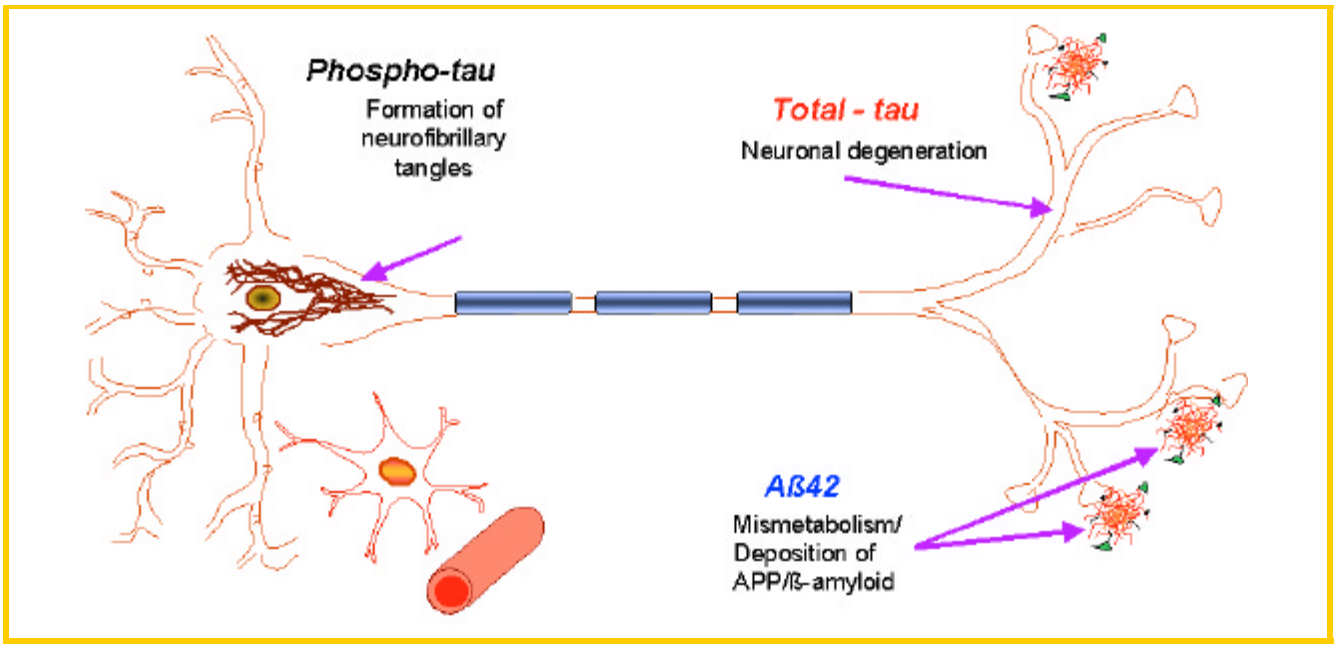

Figure 1 Schematic drawing of a neuron with neurofibrillary tangles in the cytoplasm and three senile (neuritic) plaques near the synapses. The three CSF markers for Alzheimer disease, and the pathogenic process they possibly reflect, are shown.

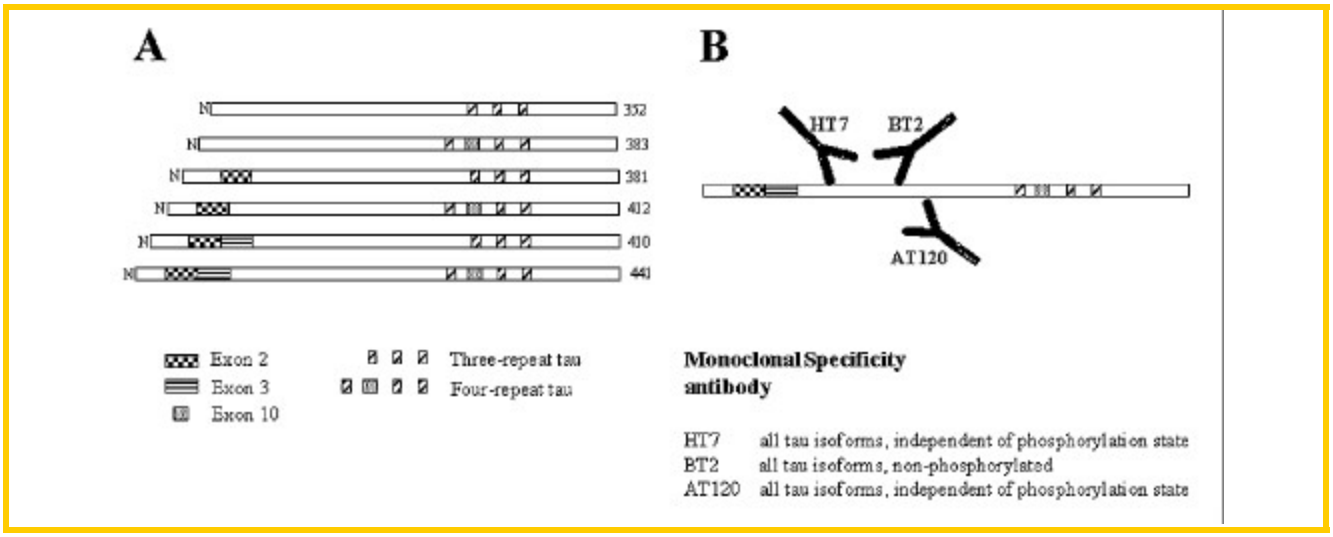

Figure 2. A schematic picture of human tau protein with the six isoforms. Alternatively spliced exons (exon 2, 3 and 10) are shown. At top the smallest tau isoform containing three repeat domains. At bottom the largest tau isoform containing four repeat domains (exon 10 spliced in) and two extra domains from exons 2 and 3.
Principles for an ELISA specific for total tau (Blennow et al., 1995), in which three monoclonal antibodies (AT120, HT7 and BT2) are used. All antibodies recognize tau irrespective of phosphorylation state, and are specific for epitopes outside the alternatively spliced exons, and the ELISA thus recognizes all forms of tau.

Table 1. Alzheimer disease, clinical performance of cerebrospinal fluid biomarkers

Disease	T-tau	P-tau	Ab42
Normal aging	Normal levels	Normal levels	Normal levels
Alzheimer disease	Moderate to marked increase	Moderate to marked increase	Moderate to marked decrease
Frontotemporal dementia	Normal levels to mild increase	Normal levels to mild decrease	Normal levels to mild decrease
Lewy body dementia	Normal levels to mild increase	Normal levels	Mild to moderate decrease
Parkinson disease	Normal levels	Normal levels	Normal levels
Depression	Normal levels	Normal levels	Normal levels
Alcohol dementia	Normal levels	Normal levels	Normal levels
Creutzfeldt-Jakob disease	Very marked increase	Normal levels, but some cases with mild to moderate increase	Moderate to marked decrease
Acute stroke	Increased levels related to the size of the infarct	Normal levels	Normal levels
Vascular dementia	Conflicting data (some studies with normal levels – some with increased)	Normal levels	Normal levels to mild decrease

The table presents a summary on the diagnostic utility of CSF biomarkers for AD based on all published papers up to February 2002.

Total-tau (T-tau)

The abnormal phosphorylated tau is a major part of the paired helical filaments accumulated in the neurofibrillary tangles (NFT) of AD. A correlation has been found between severity and numbers of NFTs in AD brains[13]. Tau is located in the neuronal axons with six different isoforms (Fig. 1) in the human brain[14]. Using monoclonal antibodies that detect all isoforms of tau independent of phosphorylation, ELISAs have been developed for measurement of "total" tau (T-tau) in CSF[15-17], see Figure 2.

An increase in CSF-tau in AD has consistently been found in numerous studies[15-45], with a sensitivity to discriminate between AD and normal aging of approximately 85% and a mean level of increase in AD compared with controls above 300%.

A widespread formation of neurofibrillary tangles consisting of phosphorylated microtubule-associated protein is found in many neurodegenerative diseases known as taupathies. Thus, high T-tau in CSF is found in a proportion of cases with other dementia disorders, e.g. vascular dementia[16,25] and frontotemporal dementia[34-37]. In contrast, in patients with other types of dementias (e.g. alcoholic dementia), chronic neurological disorders (e.g. Parkinson's disease), corticobasal degeneration (CBD)[46] and psychiatric disorders (e.g. depression), elevated CSF-tau levels are found only in occasional cases[16,37,38,47], see Table 1.

If T-tau shall be of value in the diagnosis of AD, especially in the early phase of the disease it is crucial that the value is stable throughout the disease. Some papers have reported an increase of T-tau trough the disease[19,48-53] but other authors report stability[25,26,30,39].

The level of T-tau in CSF probably reflects the degree of neuronal degeneration and damage[16]. Thus, a transient increase in T-tau is found after acute stroke, with a positive correlation between T-tau and infarct size measured by CT[54]. A very marked increase is also found in Creutzfeldt-Jakob disease, with very rapid degeneration[55].

Aβ42

Senile plaques consist of a central amorphous proteinaceous core surrounded by a variety of neuronal and glial processes[56]. The main protein in the core structure is a 4 kDa protein called β-amyloid protein (Aβ or β/A4 protein)[57-59]. Aβ (β-amyloid) is a cleavage product from the amyloid precursor protein (APP) (Fig. 3). In the production of Aβ, APP is first cleaved after position 671 by β-secretase, resulting in the release of a large N-terminal derivative called β-secretase cleaved soluble APP (β-sAPP), and in a second step by the γ-secretase complex releasing free Aβ (Fig. 3).

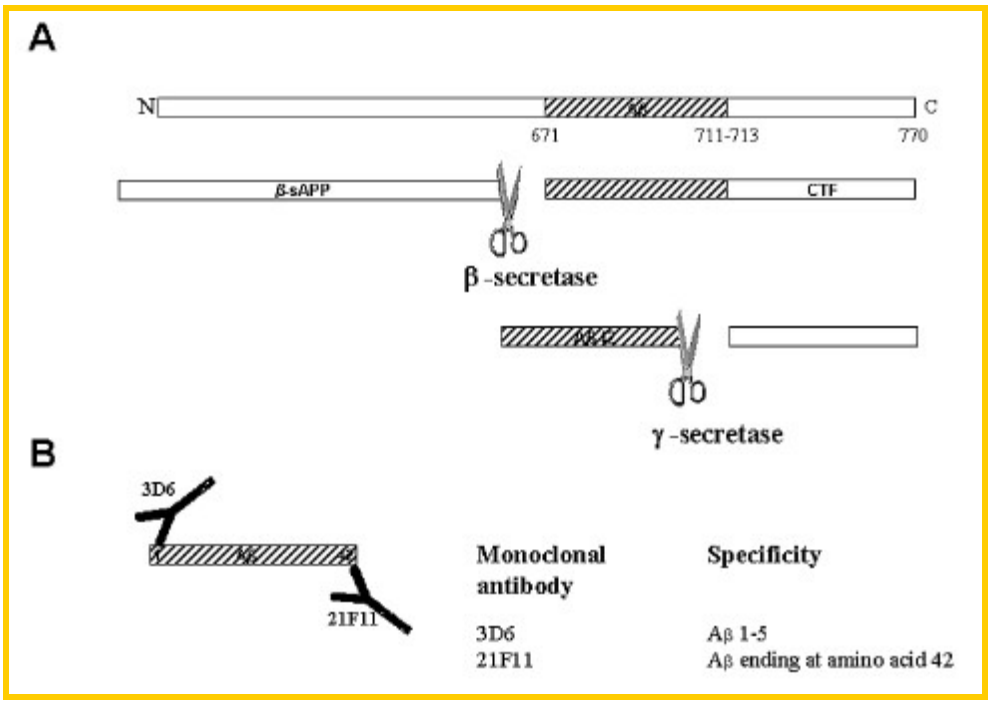

Figure 3. A schematic picture of amyloid precursor protein (APP) and the generation of free β-amyloid (Aβ). Aβ is generated through cleavage by two proteases. In the first step, APP is cleaved by β-secretase, resulting in the release of a large N-terminal fragment (β-sAPP). In the second step, the C-terminal fragment (CTF) is cleaved by γ-secretase, releasing free Aβ, which is secreted to the CSF. Principles for an ELISA specific for Aβ42 (Vanderstichele et al, 1998), in which capture antibody (21F12) is specific for the C-terminus of β-amyloid and the detection antibody (3D6) specifically recognizes the N-terminus of β-amyloid (Aβ1).

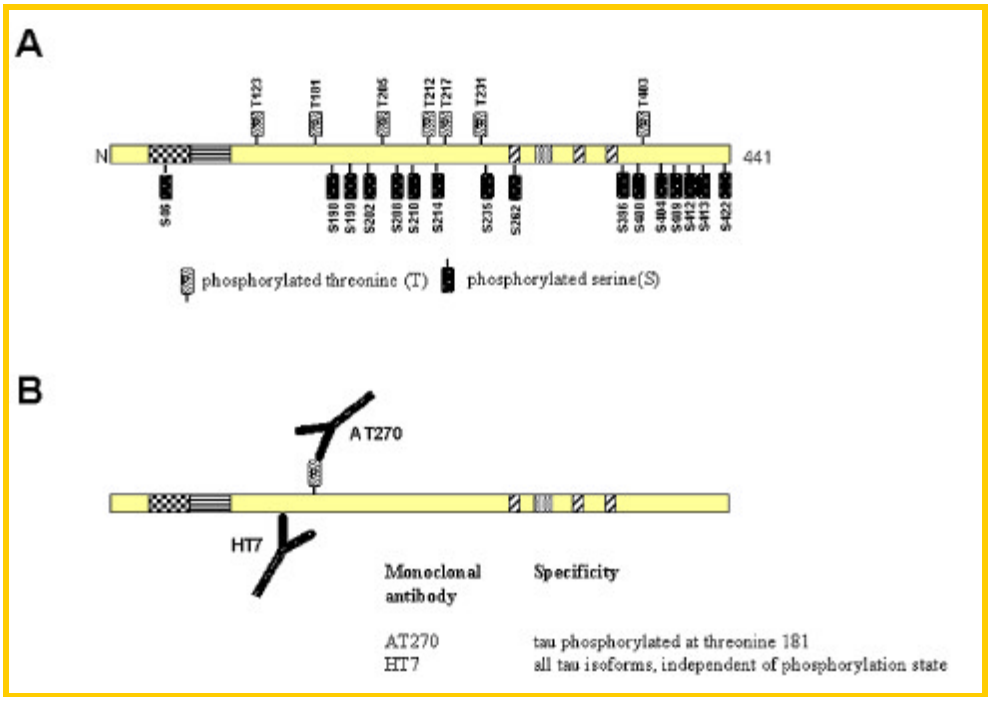

Figure 4. A schematic picture of the largest tau isoform (tau 441), with phosphorylation sites, either threonine (T) or serine (S). Principles for an ELISA specific for phospho-tau (Vanmechelen et al, 2000), in which the capture antibody (HT7) recognizes all forms of tau and the detection antibody (AT180) specifically recognizes tau phosphorylated at threonine 181.

Several ELISA methods have been developed that is specific to Aβ42[60-65], the principles for such an ELISA[63] is given in Figure 3.

Several studies have consistently found a marked (≈50% of control levels) decrease in Aβ42 in AD[27,28,36,41,43,51,52,60-63,65-76, for review 77], with high sensitivity figures for the discrimination between AD and normal aging. One study found an increase in Aβ42 in AD, which may be due to methodological differences (e.g., assay specificity for mono- versus oligomers) or differences in patient and control materials.

Data on the ability of CSF-Aβ42 to distinguish AD from other dementias and neurological disorders are limited. Low levels are also found in Lewy body dementia[27,75], a disorder also characterized by the presence of senile plaques. Further, low CSF-Aβ42 is found in a relatively large percentage of patients with frontotemporal dementia and vascular dementia[36,43].

The reduction in CSF-Aβ42 in AD was initially hypothesized to reflect a deposition of the peptide in SP, with lower levels diffusing to the CSF[60]. However, a marked reduction in CSF-Aβ42 is also found in Creutzfeldt-Jakob disease, and in amyotrophic lateral sclerosis[78], also in cases without Aβ positive plaques[72,79], and in a percentage of patients with frontotemporal dementia and vascular dementia[36,43]. This implicate that an alternative explanation for the reduction of Aβ42 in CSF that is secondary to a disturbance in the metabolism of APP and Aβ, secondary to neuronal dysfunction. After acute stroke, there is a marked increase in CSF-total tau, which correlates to the size of the infarct, while CSF-Aβ42 does not change[54]. This finding show that CSF-Aβ42 is not simply a marker for neuronal damage, as CSF-total tau, but suggest that the level of Aβ42 in CSF may reflect the metabolism of Aβ in the brain. A third possible explanation is that the reduction in CSF-Aβ42 may be due to that large Aβ aggregates, found in CSF in AD[80], are not recognized, or quantified, in the same way as Aβ monomers by the antibodies used in different ELISAs. These findings question the putative relation between low CSF-Aβ42 and formation of SP.

Phosphorylated tau (P-tau)

The human tau has numerous possible phosphorylation sites (Figure 3). Hyperphosphorylation of tau, which are found in AD, promotes aggregation of tau with subsequent formation of NFT[14]. The level of phosphorylated tau (P-tau) in CSF may thus be a biochemical marker for AD.

The principles for an ELISA[81] for measurement of tau phosphorylated at threonine 181 (P-Tau$_{181}$) are given in Figure 2. Several other ELISAs have been developed for different phosphorylated epitopes of tau, including threonine 181 and 231 (P-tau$_{181+231}$)[16], threonine 231 and serine 235 (P-tau$_{231+235}$)[82], serine 199 (P-tau$_{199}$)[82], and threonine 231 (P-tau$_{231}$)[83].

An increased level of P-tau in CSF in AD has been found using all the different assays[16,45,81-86]. The sensitivity for P-tau to discriminate between AD and normal aging is about the same as for T-tau and Aβ42, around 85%. Interestingly, the specificity for P-tau to differentiate AD from other dementias seems to be higher than for T-tau and Aβ42. Normal levels of P-tau in CSF are found normal in VAD and in frontotemporal dementia[45], and in Lewy body dementia[87], suggesting that this analysis may help in the discrimination between AD and these dementias.

Further, after acute stroke, there is a marked increase in CSF-total tau, while CSF-phospho-tau does not change[88]. This finding suggest that CSF-phospho-tau is not simply a marker for neuronal damage, as CSF-total tau, but suggest that it specifically reflects phosphorylated tau, and thus possibly the formation of NFT.

CSF Markers in mild cognitive impairment and in EARLY AD

Several studies have found high T-tau and low Aβ42 in CSF in early AD, i.e. in AD patients with high (>23-25) Mini-Mental State Examination scores, but with dementia[21,23,26,29,36,67,69,73]. As for more severely demented AD cases, the sensitivity figures are about 80-90%, suggesting that these CSF markers are positive early in the disease process.

High T-tau and low Aβ42 are also found in CSF in patients with mild cognitive impairment (MCI) without dementia, that later developed AD with clinical dementia[27,50,70]. High T-tau was also found to discriminate MCI patients that later progressed to AD from those that did not progress with high sensitivity and specificity [27,41]. In the same way, high P-tau in CSF is found in a high proportion of MCI cases[86,89,90]. These findings suggest that these CSF markers may be of use in the clinical identification of AD in the very early phases of the disease.

CSF Markers for AD in clinical PRACTICE

In two studies, the performance of T-tau[26] and the combination of T-tau and Aβ42[27] was evaluated on prospective patient samples and ELISA assays were run each week in clinical neurochemical routine. The analytical variation and stability (analyzed during one year) for these CSF analyses were adequate. Also in clinical practice, the ability of CSF-tau[26] and the combination of CSF-tau and CSF-Aβ42[27] to differentiate AD from normal aging, depression and Parkinson's disease was high, while the specificity against other dementias was relatively poor.

However, the addition of P-tau increases the specificity, since normal P-tau levels are found in most cases with frontotemporal and Lewy body dementia, and also in cerebrovascular disease[54]. Thus, the combination of several CSF markers (T-tau, Aβ42 and P-tau) will increase the specificity for the diagnosis of AD.

As CSF biomarkers require an invasive technique (lumbar puncture), it has faced a certain resistance. The incidence of the main complication after lumbar puncture, post-lumbar headache (PLPH), is clearly age-dependant, with a marked reduction after 60 years of age[91]. In prospective studies on patients admitted for investigation of dementia, the incidence of PLPH has been even lower, less than 2%[27,92]. This suggests that lumbar puncture can be included in the routine investigation of patients with dementia, with only minor risk for complications. One might find the resistance of the procedure of LP a bit overreacting as nobody would avoid LP for analyzing the CSF if one suspects for example multiple sclerosis or meningitis and therefore analyzing the CSF when one suspects an early AD should be just as naturally a thing to do.

The suggestion therefore is, that in clinical practice, CSF markers may be used together with the cumulative information gained from the clinical history and examination, EEG findings and brain-imaging techniques (e.g. SPECT and MRT scans), helping to increase the diagnostic accuracy of AD.

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New Aspects about APP-signaling

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Abstract

Alzheimer's disease (AD), the most common cause of dementia in the elderly, is a progressive neurodegenerative disorder characterized by impaired memory and cognition. Although several genetic defects have been identified in patients with a family history of this disease, the majority of AD cases is late-onset (over the age of 65) and involves individuals with no known genetic predisposition. The amyloid precursor protein (APP) and its related catabolic products have been implicated in the pathogenesis of AD. APP fragments, including β-amyloid and APP-C-terminal fragments (CTFγ), have been reported to cause apoptosis. The present study aimed at gaining more insights into the APP-signaling mechanism and describes for the first time a new cellular model, expressing a tumor-necrosis-factor-receptor-(TNFR)-APP-GFP-fusion-protein, that is suitable to study APP-signaling in a classical 'ligand-receptor-system'. Besides, we characterized neuronal expression of CTFγ57/59. Additionally, we found that the 'Sunday-driver' SYD2 is a new APP-interacting protein pointing to a pivotal link between intracellular apoptotic signaling pathways and microtubule-dependent transport.

Keywords: apoptosis, cell death, differentiation, nerve growth factor, neurodegeneration

Introduction

The treatment of Alzheimer's disease (AD) remains a major challenge because of the incomplete understanding of the triggering events that lead to the selective neurodegeneration characteristic of AD brains. The disease is characterized by the presence of neuritic amyloid plaques, cerebrovascular amyloidosis and neurofibrillary tangles. Proteolytic processing of the amyloid precursor protein APP generates the amyloid-beta peptide (Aβ) and has been implicated in the pathogenesis of AD[1]. Mutations in the APP encoding gene and also in presenilin-1 and presenilin-2 (PS-1/2), which lead to early-onset AD, are associated with excess cytotoxic Aβ deposition in the brains of AD patient suggesting that the deposition of Aβ is the central disease-causing event[2].

However, the physiological function of APP, whether this function is related to the proteolytic processing of APP, and where this processing takes place in neurons in vivo remain unclear. Previously, it has been shown that the axonal transport of APP in neurons is mediated by the direct binding of APP to the kinesin light chain subunit of kinesin-I, a microtubule motor protein. Thus, APP may function as a kinesin-I membrane receptor mediating the axonal transport of beta-secretase (BACE) and PS-1. The data also point to a role for APP as a kinesin-I membrane receptor needed for BACE and PS-1 transport in an axonal membrane compartment that may also contain Gap43, synapsin-I, and the neurotrophin (nerve growth factor (NGF)) receptor TrkA. Additionally, it raises the possibility that axonal damage might induce APP proteolysis to release the C-terminus, which might then transmit an injury signal to the cell body[3-6].

Three proteolytic activities have been identified that bear on the production of Aβ from APP. The predominant cleavage event, by α-secretase, occurs in the extracellular domain of APP within the Aβ sequence, precluding its formation. Alternatively, the extracellular domain of APP can be cleaved at the amino terminus of Aβ by the β-secretase BACE.

The carboxyl terminal of APP undergoes intramembrane proteolysis by a presenilin-dependent γ-secretase activity, releasing Aβ, as well as the small cytoplasmic fragment of APP (APP-CTFγ57 and APP-CTFγ59, reflecting the predominant cleavage sites of γ-secretase in APP). γ-secretase cleavage of APP may contribute to AD-related neurodegeneration in two ways: release of Aβ, and liberation of a bioactive C-terminal domain (CTFγ57/59) from membrane bound APP[2,7].

The predominant cytoplasmic fragment generated by γ-secretase cleavage of APP is predicted to be CTFγ59. It has been hypothesized that this results in nuclear signaling and

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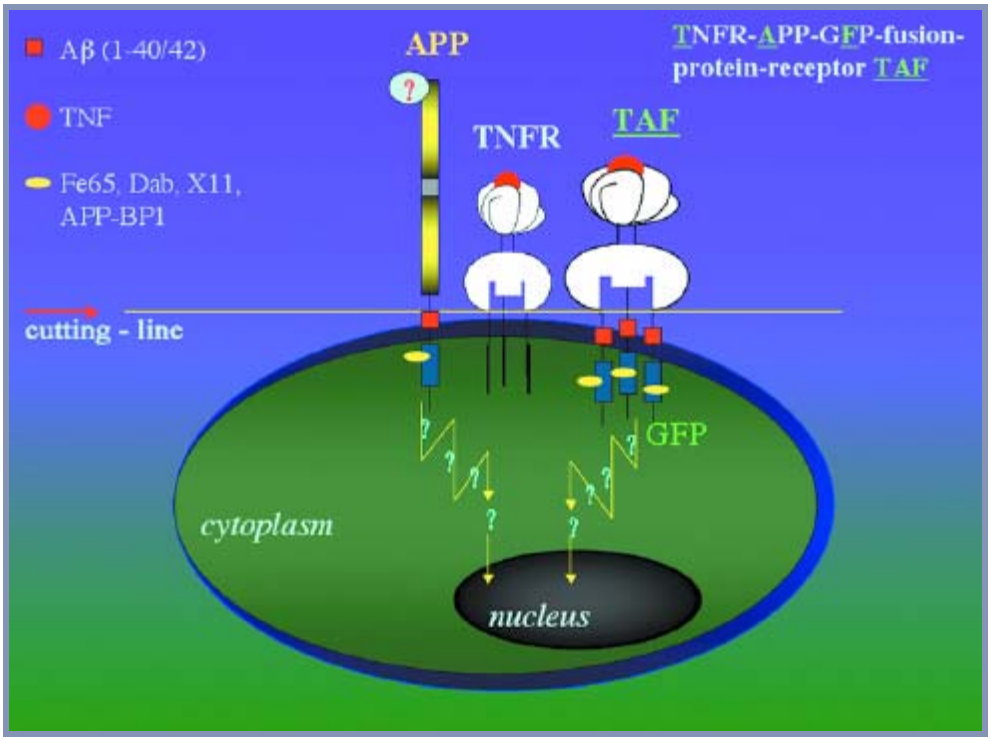

Figure 1. Schematic illustration of TAF signaling. The fusion protein TAF consists of the extracellular part of TNFR, the transmembrane/intracellular part of APP and is connected at its C-terminus with GFP as described in Materials and Methods.

the activation of genes required for APP-mediated cellular processes. A consequence of, for instance, PS mutations that cause familial AD may be an increase in the alternative CTFγ57 which accompanies the production of the more toxic 42 amino-acid Aβ peptide Aβ (1-42).

To further explore the cause of neuronal degeneration in AD we started to analyze the signaling of APP by different approaches.

Formed on the analogy of the fusion protein created by Canossa & Rovelli et al., (1993/1996)[8,9], we have constructed a fusion protein TAF consisting of the extracellular domain of the human TNF receptor and the intracellular part of human APP connected to the green fluorescent protein GFP. After transfecting a rat neuronal cell line this fusion protein can be activated specifically by human TNF-α as illustrated in Figure 1.

In addition, this model can be used to study the subcellular translocation of TAF/CTF by using fluorescent light microscopy as it has been described recently as a novel visual classification approach[10].

Moreover, we have expressed the C-terminal fragments of APP, CTFγ57 and CTFγ59, according to Ebinu and Yankner (2002)[7], to show their different effects on neuronal survival.

Furthermore, we applied the yeast-two-hybrid-system screening analysis - which is an *in vivo* yeast-based system that identifies the interaction between two proteins (X and Y) by reconstituting an active transcription factor - to find new APP-interacting proteins and describe here for the first time the 'Sunday-driver' SYD2[11,12] as a new APP-interacting protein.

Materials and Methods

Reagents

Unless indicated, all reagents used for biochemical methods were purchased from Sigma-Aldrich (Tokyo, Japan).

Cell culture

PC12 cells were propagated in Dulbecco's Modified Eagle Medium (D-MEM)/F12 (1:1) containing N2-supplement and 10 % fetal calf serum (FCS, Gibco BRL, Grand Island, NY, USA) at 37 °C in humidified 5% CO_2/95% air. For induction of neurite outgrowth PC12 cells were stimulated with 50 ng/ml NGF (Invitrogen, Tokyo, Japan).

Reverse transcription-polymerase chain reaction (RT-PCR) / Sub-cloning

The RT-PCR method was used for generation of the fusion-protein TAF according to Rovelli et al., 1993 and Canossa et al., 1996[8,9]. Human TNFRII or human APP was subjected to PCR amplification (25 µl reaction-volume) as described previously[10]. [Human APP (AccessNo.: A31584): from: nt2013-nt2310=297nt=99aa; human tumor necrosis factor receptor II (AccessNo.: M32315): from: nt90–nt860=771nt=257aa (Invitrogen, Tokyo, Japan); First: P1 plus P2 (for TNFRII-N-terminus) and P4 plus P5 (for APP-C-terminus); second: P1 plus P3 and P6 plus P5; finally: P1 plus P5;
(primers: P1: 5'-gccatggcgcccgtcgccgtctggg-3',

P2: 5'-gtcgccagtgctcccttcagctgggggctggggcccattgg-3',
P3: 5'-ggaattctgcatc/gtcgccagtgctcccttcagctgggggct-3',
P4: 5'-gcactggcgac/gatgcagaattccgacatgactca-3'
("/" = indicates: TNFRII/APP-overlap),
P5: 5'-tgttctgcatctgctcaaagaactt-3' (without stop-codon for expressing as a GFP-fusion-protein),
P6: 5'-gccccccagctgaagggagcactggcgacgatgcagaattccga-3');
the fusion protein contains the extracellular part of human TNFRII and the transmembrane/intracellular part of human APP including the Aβ (1-42) peptide that starts from: '-DAEFRHDSGYEVHHQKLVFFAEDVGSNKGAIIGLMV GGVVIA-TVIVITLVMLKKKQ…——> C-terminus of APP'.]. The numbers of cycles used to amplify each cDNA were chosen to allow the PCR to proceed in a linear range according to the Elongase™ enzyme mix-protocol (Gibco BRL, Grand Island, NY, USA). The amplification steps involved denaturation at 94°C for 1 min, annealing for 15 s at 65°C (AnnT) with specific primers and extension for 0.5 or 1 min at 68°C (AnnT: 65°C/24 cycles). The PCR reactions were analyzed by electrophoresis in 1.5% agarose gels.

Finally, an expression construct was generated by inserting the fusion-protein in-frame with the green fluorescent protein (GFP) of pcDNA3.1CT-GFP-TOPO® (Invitrogen, Tokyo, Japan) at the C-terminus of TAF (Fig. 1) and the fusion-protein has been confirmed by full-length-sequence-analysis.

According to Ebinu and Yankner (2002)[7], CTFγ57 and CTFγ59 were subcloned into pEB6CAGMCS-IRES-EGFP (kindly provided by Ms. T. Yokota at BF Research Institute, Inc., Suita, Osaka, Japan; based on the expression-vector constructed by Tanaka et al. 1999[13]) to co-express (co-translate) CTFγ57 or CTFγ59 with GFP from the same mRNA (pCTFγ57/59-IRES-GFP).

ProQuest™ Two-Hybrid System with Gateway™ Technology

The two-hybrid system is an in vivo yeast-based system that identifies the interaction between two proteins (X = APP and Y = Sunday-driver) by reconstituting an active transcription factor. The active transcription factors are formed as a dimer between two fusion proteins, one of which contains a DNA-binding domain (db) fused to the first protein of interest (db-X; also known as the "bait") and the other, an activation domain (ad) fused to the second protein of interest (ad-Y; also known as the "prey" or "target protein"). db-X:ad-Y interaction reconstitutes a functional transcription factor that activates chromosomally-integrated reporter genes driven by promoters containing the relevant db binding sites. When a selectable marker such as *HIS3* is used as a reporter gene, two-hybrid-dependent transcription activation can be monitored by growth of cells on plates lacking histidine, thereby providing a means to detect protein:protein interactions genetically. This method can be used to test whether two known proteins interact with each other or detect an unknown protein encoded by a cDNA library that interacts with a protein (APP) of interest[14].

As recently described, the two-hybrid-system analysis was performed according to the manufacturer's protocol (Invitrogen, Tokyo, Japan)[15]. Briefly, human *APP* was sub-cloned from pENTR/D-TOPO®- into the pDEST™32-vector (Invitrogen) containing the GAL4 DNA binding domain. pEXP-AD502 was used as an activation domain expression vector containing the ProQuest™ two-hybrid human brain cDNA library (Invitrogen). The used yeast strain for ProQuest™ system was MaV203.

For selection three reporter genes were used: A single copy of each of three reporter genes (*HIS3*, *URA3* and *lacZ*) are stably integrated at different loci in the yeast genome. The promoter regions of *URA3*, *HIS3*, and *lacZ* are unrelated (except for the presence of GAL4 binding sites). In the ProQuest™ two-hybrid system, in comparison to standard two-hybrid systems, false positives are reduced because three independent transcription events (from distinct promoters) must occur at independent chromosomal loci. Induction of the *HIS3* and *URA3* reporter genes allow two-hybrid-dependent transcription activation to be monitored by cell growth on plates lacking histidine or uracil, respectively. Induction of the *lacZ* gene results in a blue color when assayed with X-gal (5-bromo-4-chloro-3-indolyl-β-D-galactopyranoside). Moreover, two-hybrid-dependent induction of *URA3* results in conversion of the compound 5-fluoroorotic acid (5FOA) to 5-fluorouracil, which is toxic. Hence, cells containing interacting proteins grow when plated on medium lacking uracil, but growth is inhibited when plated on medium containing 5FOA.

This system therefore reduces false positives by providing four phenotypes [His^+ ($3AT^R$), β-gal, Ura^+ and $5FOA^S$] for assessing true interactors and by using low-copy-number (ARS/CEN) vectors that reduce expression levels and toxicity.

Positive clones were confirmed by retransformation assay: Yeast cells containing potentially interacting proteins harbor both db-human *APP* and ad-Y (Y= e.g.: Sunday-driver). Plasmid DNA isolated from yeast cells containing db-human *APP* and ad-Y (Y= e.g.: Sunday-driver) was introduced into E. coli by electroporation and transformants containing ad-Y (Y= e.g.: Sunday-driver) were selected with ampicillin (or db-human *APP* with gentamicin). The plasmid DNA ad-Y (Y= e.g.: Sunday-driver) from these E. coli cells was transformed into MaV203 together with pDBLeu or db-human *APP* and tested for induction of the reporter genes. True positives induced the reporter genes with pdb-human *APP* but not with the pdbLeu control vector alone.

Transfection and Survival-Assay

PC12 cells were transiently transfected with TAF, CTFγ57/59, GFP (Clontech, Tokyo, Japan) expression vector or empty plasmid (controls) using SuperFector transfection

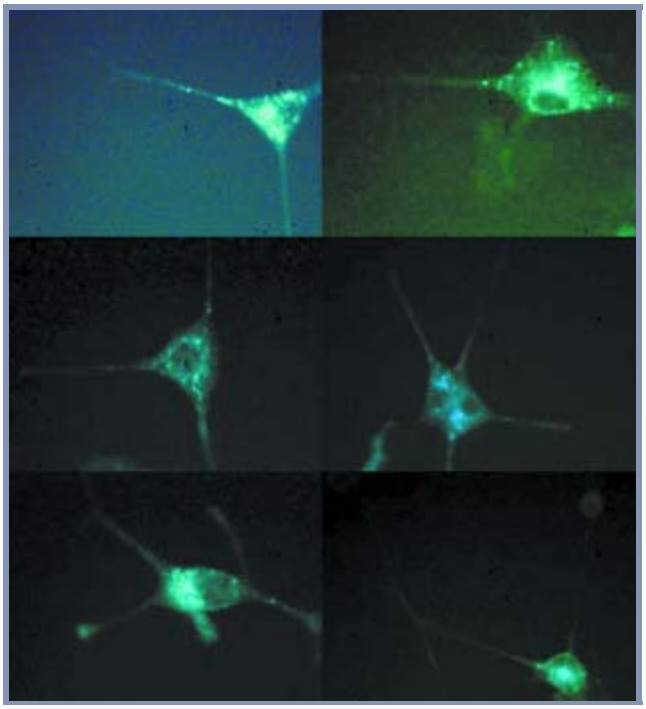

Figure 2. Rat neuronal PC12 cells expressing TAF and stimulated with NGF

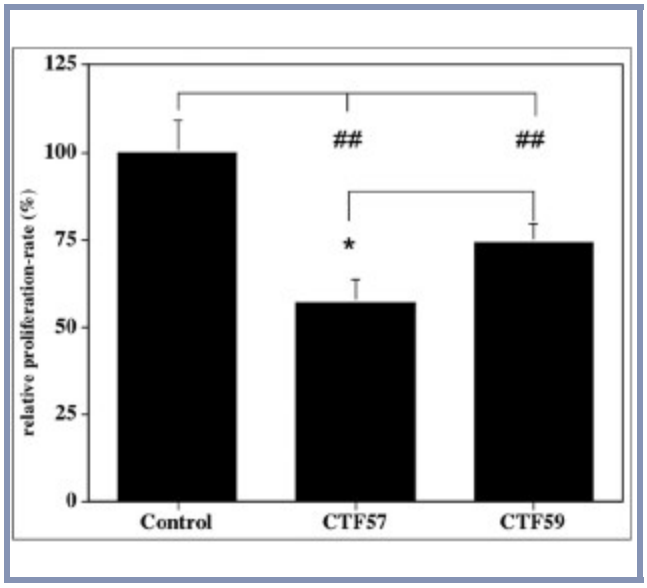

Figure 3. Effect of CTFγ57 and CTFγ59 on survival of PC12 cells. ELISA-Cell-Titer 96® AQ_{ueous} Assay (Promega) 48 hrs post transfection. Cells were incubated as described in Materials and Methods. Data are shown as mean±S.E.M. of eight independent experiments, each done in duplicate [**P*<0.05, ##P<0.01, (controls = GFP-transfected cells), ANOVA].

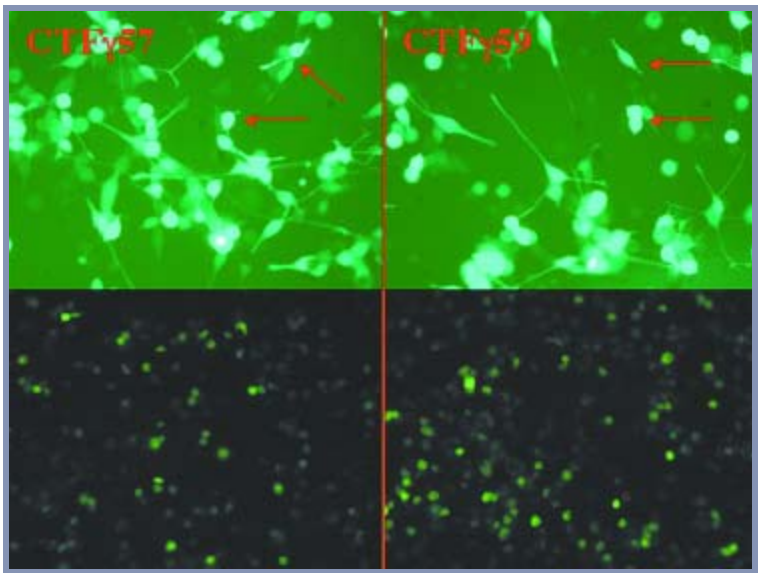

Figure 4. Rat neuronal PC12 cells co-expressing CTFγ-57 or CTFγ-59 plus GFP and stimulated with NGF.

Table 1. The yeast-two-hybrid-system analysis reveals the 'Sunday-driver' as a new APP-interacting protein. * = indicates the strength of the interaction, according to Heese et al., 2002[15].

Protein name	LocusID/GeneAccessNo	Beta-gal-activity
Sunday-driver (SYD2)	# 23162 / NM_015133	****

reagent (according to the manufacturer's protocol; B-Bridge, San Jose, CA, USA) and maintained in D-MEM)/F12(1:1)/N2 medium containing 10 % FCS (Gibco) at 37 °C in humidified 5% CO_2/95% air[15]. Additionally, transfection efficiency (about 50-60 %) was always confirmed by co-transfection of GFP reporter transcripts (with or without TAF or CTFγ57/59) with p53-/PKC-DsRed1 (Clontech, Tokyo, Japan) transcripts as reported recently[16,17]. Cell survival of TAF and CTFγ57/59 positive cells was assessed 48 hrs post transfection by fluorescence microscopy (Olympus IX70, Olympus, Tokyo, Japan). Additionally, 24 hrs after transfection cells were stimulated with NGF (murine NGF 2.5S, 50 ng/ml; Invitrogen) for 120 hrs. Thereafter, cell survival was measured using the Cell-Titer 96® AQ_{ueous} Assay (Promega, Madison, WI) and examined by fluorescence microscopy[15,17].

Results

As illustrated in Figure 1, we have constructed the fusion-protein TAF consisting of the extracellular part of human TNF-receptor, the transmembranar/intracellular part of human APP and the GFP protein. Transfection of this protein TAF into rat neuronal PC12 cells shows a similar TAF-protein distribution upon stimulation with NGF (Fig. 2) as demonstrated by Ando et al. (1999)[18] who transfected wild type APP (*wt*APP) into PC12 cells.

PC12 cells expressing CTFγ57/59 show a reduced proliferation rate in comparison to control cells (Fig. 3) and, after stimulation with NGF, CTFγ57/59-positive cells indicate, rather, a slight arrestive effect on neurite outgrowth (Fig. 4), because GFP itself does not affect neurite outgrowth[15]. Most interesting, CTFγ57 reveals a stronger inhibitory effect on neuronal survival than CTFγ59 (Fig. 3).

In search of new APP-interacting associates with the two-hybrid-system, we could detect the 'Sunday-driver' SYD2 as such a new protein (table 1).

Discussion

TAF expressing PC12 cells represent a physiological cellular model to study APP-mediated signaling, because – similar to *wt*APP[1] – TAF itself does not induce apoptosis. Using this cellular model, the C-terminus of APP can be activated specifically with an extracellular ligand and APP-mediated signaling can be characterized as a classical 'ligand-receptor-signaling-system'. Thus, introducing a familiar AD-related APP mutation or co-transfection of TAF expressing PC12 cells with mutated PS-1/2 (*mu*PS-1/2) this system might be a suitable model to study familiar AD-related APP-signaling and to disclose the physiological and pathophysiological meaning of APP signal-transduction.

APP-derived CTFγ57, that showed a more pro-apoptotic feature in our cellular model, could potentially contribute to AD pathology through pro-apoptotic signaling or by negatively interfering with normal CTFγ59 signaling mechanisms. Alternatively, CTFγ57 may not be efficiently - as CTFγ59 – processed to the smaller and more active fragment CTFγ50, which is probably the final active C-terminal signaling product of APP[7].

Several reports suggest that APP-CTF can translocate to the nucleus and play a role in cellular signaling, for instance, by showing the interaction with Fe65 and histone acetyltransferase Tip60 to mediate transactivation of a reporter gene or by controlling retinoic acid-responsive gene expression[19-21]. The generation of nuclear signaling proteins such as APP-CTFγ57/59 by regulated intramembrane proteolysis (RIP) is a new paradigm of signal transduction[7].

The protein encoded by the 'Sunday-driver' (SYD) gene, which is a member of a conserved family of proteins found in *C. elegans*, drosophila, mice, and humans, is required for the functional interaction of kinesin-I with axonal cargo[11,12,22]. The SYD protein exhibits a tripartite structure, consisting of an N-terminal domain (cytoplasmic) possessing two predicted coiled-coil regions, a central transmembrane domain, and a C-terminal (cytoplasmic) domain. In mammalian tissue culture cells, SYD was localized to tubulovesicular Golgi-derived organelles, where it colocalized with conventional kinesin. The analysis revealed that SYD did not associate with the kinesin heavy chain directly; rather, the N-terminus of SYD interacted with the tetratricopeptide domain of the kinesin light chains (KLC). Thus, SYD functions as a membrane-associated receptor for the axonal transport of post-Golgi vesicles and possibly other SYD binding proteins by interacting with the TPR domain of the KLC subunit of kinesin-I. Taken together, these results suggest that SYD attaches kinesin to exocytic organelles through the motor's light chains.

SYD probably has another function beyond its proposed role as a kinesin receptor. SYD was recently identified as a binding partner for several MAP kinases involved in a stress-activated signaling cascade[23,24]. Studies in mouse suggested that this protein may interact with, and regulate the activity of numerous protein kinases of the JNK signaling pathway in neuronal cells. Once delivered to the synapse, SYD may function as a membrane-associated scaffolding protein for the JNK signaling cascade and may regulate synaptic vesicle transport possibly by integrating JNK signaling and kinesin-1 transport[11,12,22].

Taking into account that APP can interact with JIP1b/2[25] it is, thereby, of interest that SYD may also has functions outside of its role in axonal transport, because recently, two groups independently cloned mSYD2, calling it JSAP1[26] and JIP3[23], as a neural-enriched scaffolding protein for the JNK MAPK cascade. Particularly, they observed a preferential binding of SYD2 to the JNK3 group of MAPKs[23,26] and, since the JNK3 pathway has been shown to be required for stress-induced neuronal apoptosis[27-29] SYD2 seems to be directly involved in

regulating apoptotic signaling[28]. Given the binding of SYD2 to the proteins of the JNK MAPK cascade, it is possible that SYD is also required to transport this group of proteins down neuronal processes. This finding hints at a potential link between intracellular (apoptotic-) signaling pathways and microtubule-dependent transport. However, further work is needed to determine whether kinesin-I and APP have a major role in the JNK signaling pathway, and the extent of cross-talk between APP, SYD, kinesin-I and JNK proteins. These findings also suggest that the cargo receptors for microtubule motors, such as SYD or APP, may themselves be the cargo of the motors, an evolutionarily efficient mechanism.

The connection between APP's SYD- and kinesin-binding activity and its other JNK-mediated signaling and membrane functions will be an interesting topic for future investigations.

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Does Cerebrovascular Disease Underpin Depression in Patients with Neurodegenerative Disease?

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Abstract

Background: **Although a strong body of evidence points to the "vascular depression" hypothesis, little corroborative neuropathological evidence exists. Our hypothesis was that subjects with neurodegenerative disease and depressive symptomatology will have more microvascular pathology in the frontal white matter and/or basal ganglia than subjects with neurodegenerative disease without depression, and subjects with neither neurodegenerative disease nor depression.**

Method: **Frontal white matter and basal ganglia microvascular pathology were compared in three groups: (i) 17 elderly subjects with neurodegenerative disease (Parkinson's disease, Lewy body disease, Alzheimer's disease, and vascular dementia) and depression; (ii) 16 age-, sex- and disease-matched controls without depression; and (iii) 17 age- and sex-matched controls without neurological disease or depression. Anatomical location and severity of several indices of vascular pathology (e.g. perivascular dilatation (*etat crible*), white matter rarefaction, infarcts) was evaluated.**

Results: **The groups did not differ significantly in either ratings of individual vascular pathologies or our measure of global white matter changes. There was an overall association between white matter disease and vascular risk factors such as cardiac disease alone (significantly more common in controls) and combined hypertension and cardiac disease.**

Conclusions: **This study did not find evidence for microvascular pathology in late life depression. However, there were a number of methodological limitations, principally concerning our retrospectively obtained sample and our examination of persons with neurodegenerative illnesses. Definitive testing of the frontal-basal vascular model of depression is needed in prospectively studied patient groups with neuroimaging correlation.**

Keywords: cerebrovascular, depression, pathology

Introduction

Among depression in patients in later life, there appears to be a subgroup characterised by late onset, cognitive impairment and risk factors for vascular disease. There is converging evidence that disruptions to frontal-subcortical-basal functional circuits account for many of the clinical features and longitudinal course in older patients with depression[1-4].

Neuroimaging studies have demonstrated preferential volume loss of the frontal lobe and the basal ganglia on MRI[1,5-7].Two regions within the frontal lobe have been associated with depressive states: the orbitofrontal region, one of five functionally integrated circuits linking cortex with basal ganglia,[8] and the prefrontal region, involved in dopaminergic projections from the ventral tegmental area[9]. The importance of these regions in depression is supported by functional imaging studies of both primary depressives[10-13] and secondary depressives with Parkinson's disease[9,14].

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While there appears to be a certain specificity to the neuroanatomical distribution of lesions associated with depression, the underlying pathophysiology is less well known. Vascular disease may play an important role. Firstly, depression occurs more commonly in vascular dementia (particularly Binswanger's disease) than other dementias, leading some to suggest that it is an integral part of the disease[15-17]. Secondly, an increased prevalence (30-80%) of hyperintensities in the subcortical white matter (particularly frontotemporally) and basal ganglia is seen in elderly depressives compared with age matched controls on MRI[8-21]. Associated with cerebrovascular risk factors[22] and in the long term with cognitive decline[23], the underlying histopathology of these lesions may range from *etat crible* (expansion of perivascular or Virchow-Robin Spaces) and gliosis to focal non-cavitated and cavitated infarcts, depending on their type and site[22,24-25]. There is little agreement as to their underlying pathophysiology apart from the role of hypertension and blood pressure regulation[26-27].

There have been few neuropathological studies of late-life depression. An earlier study noted sulcal widening, ventricular dilatation, white matter loss and microscopic vascular changes of a slight degree in four of seven subjects with depression[28]. A more recent study found marked reduction in glial number in the subgenual prefrontal cortex in subjects with major depressive disorder (particularly familial forms) and bipolar disorder[29]. One group demonstrated a significant association between depression and microinfarction (i.e.<15ml) in subjects with dementia[30], while another group found a significant increase in atheromatous disease in aortic and cerebral vessels, but not in microvascular disease, in subjects with late life depression[31].

Thus, although a strong body of evidence points to a role for cerebrovascular disease and disruption of frontal-basal circuits in both primary and secondary depression, little corroborative neuropathological evidence exists at the current time. The aim of our study was to explore further the neuropathological correlates of depression in later life. Our hypothesis was that elderly subjects with neurodegenerative disease and significant depressive symptomatology will have more severe microvascular pathology in the frontal white matter and/or basal ganglia than: (i) subjects with neurodegenerative disease without depression; and (ii) subjects with neither neurodegenerative disease nor depression.

Methods

Sampling

Cases were identified initially on the basis of clinical features recorded on the database from the Centre for Education and Research on Ageing (CERA) and the Prince of Wales Medical Research Institute brain bank. At the time of sampling, the brain bank, established for neurodegenerative disorders and including both diseased cases and controls recruited from geriatric and neurological clinics and community samples, had 317 cases. Material from the database was elaborated by reference to original clinical case notes (see below). Cases with a neurodegenerative disorder and depressive symptomatology within five years of the onset of disease or during the course of illness were thus identified. Cases were excluded if there was evidence of a familial degenerative brain disorder or rare primary degenerative brain disorders such as progressive supranuclear palsy, multi-system atrophy or corticobasal degeneration.

This search yielded a group of 17 subjects with both a degenerative disorder (i.e. 14 with either Parkinson's disease or Dementia with Lewy bodies, two with Alzheimer's disease, and one with vascular dementia) and depression (DEP-DISEASE). Two control groups were selected: (i) a group of age-matched controls without neurological disease or depression (n=17; NORMCON); and (ii) a group of age- and disease-matched controls without depression (n=16; NONDEP-DISEASE) (table 1). The latter comprised subjects with the same neurodegenerative disorders in similar distribution to the depressed group (i.e. 11 with either Parkinson's disease or dementia with Lewy bodies, four with Alzheimer's disease, and one with vascular dementia). Diagnoses for degenerative disorders were made clinicopathologically i.e. based on clinical histories and neuropathological findings using standardised protocols[32-33]. Ethical approval was obtained from the Central Sydney and South Eastern Area Health Services and the Universities of Sydney and New South Wales.

Psychiatric ratings

Medical and nursing case notes and medical correspondence were examined carefully for documentation of signs and symptoms of depression, treatment with antidepressants and referral to psychiatrists. Although the case notes of the subjects with neurodegenerative disorders were more detailed than those of the non-diseased controls (whose case notes often consisted of a single admission), they mainly comprised outpatient notes from neurologists. The identification of depression had not been the subject of systematic enquiry. Of those with any symptoms of depression documented in their notes, most only had one or two symptoms recorded, making the use of relevant psychiatric instruments (e.g. Diagnostic Evaluation After Death)[34] inappropriate for this group of medically ill subjects. The 17 subjects with neurodegenerative disease and depression were consensus rated by CP and IH and assigned diagnoses of probable or definite depression according to the following criteria:

Definite depression

Either:

1. fulfilled criteria for DSM-IV major depression
2. patient was seen by a psychiatrist who diagnosed depression and initiated antidepressant treatment, or
3. patient had a prolonged depressive episode (i.e. longer than six months with major symptoms such as suicidal ideation) and was treated with antidepressants.

Probable depression

Either:

1. fulfilled some criteria for major depression (e.g. mood disturbance or diminished interest/pleasure in activities plus >1 but <5 of : weight loss, insomnia/hypersomnia, psychomotor agitation or retardation, anergia, worthlessness /guilt, decreased concentration, thoughts of death or suicide), or
2. patient was seen by a general practitioner, neuropsychologist or neurologist who diagnosed depression and initiated antidepressant treatment.

While general psychiatric comorbidity was common in the subjects with neurodegenerative disease, subjects were excluded from the neurodegenerative control group if they had evidence of depressive symptoms or associated treatment.

Basic demographic details and cerebrovascular risk factors such as smoking, hypertension, cardiac disease and diabetes were recorded for all subjects. The temporal relationship between depression and disease, and depression and death also was recorded for depressed subjects.

Pathological sampling and ratings

Brains were sectioned at 3mm intervals in the coronal plane and samples were taken from comparable levels in each case. All potential candidate frontal lobe regions (vis: dorsolateral prefrontal association cortex - Brodmann area 9; orbitofrontal region – Brodmann areas 11 and 12; cingulate gyrus – Brodmann area 24) were examined along with the basal ganglia. Samples were taken predominantly from the left hemisphere (187 of the 195 sections examined). Sections from frontal motor strip (Brodmann area 4) were taken for internal comparison of small vessel pathology. Tissue blocks were embedded in paraffin, sectioned at 10m and stained with haematoxylin and eosin. Sections were coded and ratings of small vessel pathology performed by CP blind to subject group.

With no consensual rating scheme for quantifying vascular pathology, both ratings of individual white matter changes (e.g. degree of *etat crible*, degree of myelin loss)[35] and composite gradings of overall changes[35-37] have been used previously. In this study individual ratings were made in the white matter of rarefaction (rated 0-3), increased glial density (present or absent), perivascular dilatation (rated 0-3), thickening of vessel walls (present or absent), perivascular infiltrate of macrophages (present or absent), myelin pallor (present or absent), spongiosis (present or absent) and infarcts (size and location).

In addition to the rating of individual features in each of the regions examined, the composite rating system described by Esiri et al[36] was used to give an overall grade of white matter change from 0-3 for each region. A *global composite score* for each case was derived by dividing the sum of the composite scores of the regions sampled by the maximal score for the number of regions sampled to account for any missing data. In addition, *white matter distribution scores* were derived by calculating the sum of white matter changes in frontal lobe and basal ganglia regions relative to the white matter changes in the motor strip, as follows:

Dorsal frontal: sum of the composite scores of the dorsolateral frontal, cingulate and basal ganglia sections divided by the composite score of the motor strip,

Orbital frontal: sum of the composite scores of the orbitofrontal and basal ganglia divided by the composite score of the motor strip,

All frontal: sum of the composite scores of the dorsolateral frontal, cingulate, orbitofrontal and basal ganglia divided by the composite score of the motor strip,

Only frontal: sum of the composite scores of dorsolateral frontal, cingulate, and orbitofrontal divided by the composite score of the motor strip.

Reliability and validity

The vascular ratings were repeated on pilot cases until consistency was achieved. Rater drift over time was assessed using test-retest reliability over an extended period of time. Test-retest reliability was examined on ten sections, randomly selected but inclusive of each of the regions and clinical categories sampled. Ratings for the eight individual white matter changes described above were made for each of the ten sections. There was 91.3% agreement (weighted Kappa = 0.83) between the baseline white matter ratings and the subsequent ratings, up to 12 months later. There was no consistent pattern to the non-agreement which was spread over the various features rated. Inter-rater agreement was tested on a separate sample of ten sections, again randomly selected but inclusive of each of the regions and clinical categories sampled. There was 88.8% agreement (weighted Kappa = 0.78) between Rater 1 (CP) and Rater 2 (JK). Non-agreement was spread over the various features rated.

Statistical analyses

Statistical analyses were performed using Statview 5[38]. Analysis of variance was used to examine group differences (e.g. subject groups; presence/absence of risk factors) on white matter distribution and global composite scores. Chi

square analyses were conducted to examine group differences on categorical data such as gender and risk factor presence.

Results

The demographics and frequencies of the risk factors for the three groups are provided in Table 1. There was no significant difference between the groups in either the frequency of the individual pathological changes or the mean *global composite scores* (which summed all the pathologies). Global composite scores (±SD) for the three groups were; (i) DEP-DISEASE 38.73 ± 21.1; (ii) NONDEP DISEASE 39.17 ± 24.1; and (iii) NORMCON 43.13 ± 25.6 (df = 2; F = 0 17; P = 0.84).

The frequency of each of the pathological changes was low in the entire population (Table 2). In particular the frequency of moderate or severe grades of rarefaction and dilatation was low indicating a general paucity of white matter vascular pathology in this population. Cellular infiltrates and vessel wall thickening were seen more commonly, but these were not specific to either the diseased groups or the group with depression. Infarction was only found in a total of nine of the 195 slides available for examination and occurred in the same frequency in depressed and control individuals (Table 2).

The specificity of vascular pathology to particular neuroanatomical circuits was examined and no difference in the severity of vascular pathology was found to distinguish the depressive group. Specifically, the three groups did not differ in *white matter distribution scores* (Table 3).

The association between our overall measure of white matter pathology, the mean *global composite score*, and cerebrovascular risk factors was examined (Table 4). As there were no differences in the frequency of the pathological changes between the three groups, the association between the pathology score and the presence of absence of each of the risk factors was examined in the entire population. Mean global composite scores were higher in the group with cardiac disease alone and in combination with hypertension (Table 4). Importantly, cardiac disease was significantly more common in the NORMCON group.

Table 1. Demographic and risk factor variables

	DEP-DISEASE n=17	NONDEP-DISEASE n=16	NORMCON n=17	Statistical evaluation
Female, No. (%)	7 (41)	4 (25)	9 (53)	-
Mean age at death (yrs)	76.5 ± 7.4	75.8 ± 7.6	73.6 ± 9.8	df=2; F=0.54; P=0.58
Hypertension, No. (%)	5 (29.4)	4 (26.7)[a]	8 (47.1)	df=2; χ^2 = 1.78; P=0.41
Cardiac disease, No. (%)	4 (23.5)	3 (20)[a]	11 (64.7)	df=2; χ^2 = 8.81; P=0.01
Diabetes, No. (%)	1 (5.9)	2 (13.3)[a]	2 (11.8)	df=2; χ^2 = 0.55; P=0.76
Smoking, No. (%)	1 (16.7)[b]	4 (44.4)[c]	5 (55.6)[c]	df=2; χ^2 = 2.29; P=0.32

[a]n=15; [b]n=6; [c]n=9;

Table 2: Frequencies of individual pathologies over all regions (excluding motor strip) by subject group, No (%)

	Rarefaction				Dilatation				Cell infiltrate	Infarcts	Thickening
	Nil	Mild	Mod	Severe	Nil	Mild	Mod	Severe			
DEP-DISEASE (n=67)	41 (61.2)	18 (26.8)	4 (6.0)	4 (6.0)	58 (86.6)	5 (7.5)	3 (4.5)	1 (1.4)	20 (30.3)	4 (6.2)	15 (22.7)
NONDEP-DISEASE (n=63)	42 (66.7)	10 (15.9)	4 (6.3)	7 (11.1)	57 (90.5)	5 (7.9)	1 (1.6)	0	16 (25.4)	1 (1.6)	11 (17.5)
NORMCON (n=65)	35 (53.9)	19 (29.2)	3 (4.6)	8 (12.3)	57 (87.7)	6 (9.2)	2 (3.1)	0	21 (32.3)	4 (6.0)	14 (21.5)

Table 3: White matter distribution scores by group

	DEP-DISEASE n=16	NONDEP-DISEASE n=15	NORMCON n=14	df	*F*	P*
All frontal	4.61 ± 2.35	4.14 ± 1.54	5.35 ± 2.33	2	1.20	.31
Superior frontal	3.56 ± 1.91	3.12 ± 1.27	4.08 ± 1.94	2	1.13	.33
Orbital frontal	2.4 ± 1.38	2.06 ± 0.87	2.7 ± 1.67	2	0.93	.40
Only frontal	3.23 ± 1.60	3.10 ± 1.21	3.87 ± 1.58	2	1.12	.34

* by ANOVA

Table 4: Mean global composite scores for those with and without cerebrovascular risk factors

	Mean composite score		df	*F*	P*
Risk factor	*Present*	*absent*			
Hypertension	47.75 ± 23.3	36.24 ± 22.9	1	2.74	0.10
Cardiac disease	50.59 ± 26.0	34.68 ± 20.2	1	5.54	0.023
Diabetes	42.67 ± 23.9	40.04 ± 23.7	1	0.06	0.82
Hypertension/cardiac	58.00 ± 24.6	35.66 ± 21.1	1	8.30	0.006
Hypertension/diabetes	57.78 ± 15.4	39.15 ± 23.5	1	1.81	0.19
Diabetes/cardiac	50.0 ± 20.0	38.2 ± 22.5	1	1.02	0.32

* by ANOVA

Conclusions

This investigation failed to demonstrate any pattern of neuropathological change that was associated with clinical symptoms of depression in patients with neurodegenerative disease. This finding may be a true null result or may be a reflection of the methodological limitations of this study, many of which were related to our retrospectively obtained subject population. Firstly, the pathological vascular changes under investigation occurred at such low frequency that is was difficult to demonstrate differences between groups. By using a depressive group that comprised mainly subjects with movement disorders, and matching for disease, we may have inadvertently biased our diseased groups against the presence of vascular disease. Importantly, cardiac disease, which was most strongly associated with white matter disease, was found significantly more often in the control group. This might be addressed in future studies by employing high-risk methodology (e.g. examining subjects with known vascular risk factors or pre-existing lesions such as hyperintensities on neuroimaging).

Secondly, the value of data obtained from postmortem investigations of diseases depends heavily on the adequacy of medical records. The difficulties associated with retrospective assignation of psychiatric diagnoses after death[39-40] are compounded in a medically ill population because medical case notes are often biased in symptom recording towards perceived treatable symptoms or the focus of the specialist physician's expertise. Consequently, low frequency of recording of depressive signs and symptoms in medical case notes is a consistent finding in Australia[41-42] and overseas[43-45]. Depression is often undetected and under-diagnosed in people with neurodegenerative disease. This, together with the lack of prospective study, might have inadvertently contaminated the neurological control group with subjects who might have had a depressive illness.

Further, given the hypothesised involvement of frontal-basal ganglia circuits it is more likely that the predicted pattern of neuropathological changes is associated with definite depressive syndromes associated with psychomotor change or melancholia[46]. Yet, as has been observed, retrospectively identifying full depressive syndromes from medical records in medically ill subjects gone to autopsy is difficult. Ideally, the vascular depression hypothesis would be tested in a group of subjects with antemortem diagnoses of endogenous or melancholic depression. However, in the absence of specific brain tissue collection program for mood disorders, exploration of this model is limited to opportunistic studies of subjects involved in brain donation programs. Although established for schizophrenia, such programs have not been set up in Australia for affective disorders and most brain tissue programs either involve subjects with neurodegenerative disorders or normal, community-based elderly.

Not withstanding the methodological limitations outlined above, the lack of association between late life depression and microvascular pathology in this population was recently supported by Thomas *et al*[31] in a non-neurodegnerative population. Thomas *et al*[31] found no difference for microvascular disease either in the brain generally or locally in the frontal lobe in subjects with a history of major depression compared with controls.

Considerable evidence points to a strong contributory role for cerebrovascular disease and disruption of frontal-basal circuits in the pathogenesis of depression in later life[2, 4, 47-48]. Yet, there has been little investigation of this at a histopathological level. In this study our ability to test this model was limited by our sample; however we have developed methodology for evaluating microvascular pathology at postmortem. We have investigated the broadest possible range of microvascular pathology including quantification of both infarcts and white matter changes often associated with the leukoencephalopathy observed on neuroimaging. We have examined the degree of vascular pathology with respect to the presence or absence of known risk factors for cerebrovascular disease[22, 49] and found an association between these risk factors and our measure of small vessel disease.

This methodology will enable future testing of the frontal-basal vascular model of depression in prospectively followed older patients with depression, preferably with MRI correlation. While neither our study nor that of Thomas *et al*[31] found evidence of microvascular disease in retrospectively studied subjects with depression, we still do not know whether there is a disjunction between suggestive MRI evidence of cerebrovascular disease and direct pathological examination, as Breitner[50] commented recently in an editorial. Further, is there a subgroup of depressed patients, such as those with psychomotor changes, in whom microvascular disease is more relevant as an aetiological factor? Ultimately advancing the understanding of the pathophysiological processes underlying later life depression may provide potential targets for preventative or treatment strategies akin to those offered for other neurobiological disorders of later life.

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Families' Experiences Permitting Brain Autopsy for the Oregon Brain Bank in the Context of the Neuropathology Report

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Abstract

Twenty-three family members were interviewed about their experiences with deciding to consent to a brain autopsy and their reactions to reading a Neuropathology report analyzing brain tissue from a deceased relative. They were interviewed 5 to 45 months after the death of 20 relatives. Using content analysis, seven main categories of concepts were derived from the data, e.g., Follow-Up Information. Twenty-two of 23 family members found the formal report itself technical and difficult to understand. All family members were glad that they had authorized the brain autopsy, but their reactions varied greatly depending on diagnosis. For some the report provided emotional closure; to others it was disturbing. The needs for follow-up information included clarification of the report's technical language, and genetic counseling.

Keywords: Alzheimer's disease, Brain autopsy, Dementia, Neuropathology report, Family member

Introduction

Alzheimer's disease (AD) is the fourth leading cause of mortality in the United States (U.S.) and an inexorably fatal disease, devastating both to its victims and their families. In 1997, the number of Americans affected by AD was estimated to be between 2.32 and 4 million[1]. Because of the projected increased life expectancy of the elder population, especially the fastest growing segment surpassing age 85[2], this number is anticipated nearly to quadruple to 9 million in the next 5 decades[1]. The increased number of Americans who will be diagnosed with the disease, coupled with the current concern over its increasing health care costs (already exceeding $90 billion annually), underscores the urgency in optimizing the planning of this population's health care needs.

Included in this planning are both behavioral and biological approaches to interventions, and treatments to help persons with AD and their family caregivers. Behavioral approaches have focused primarily on individuals who suffer from AD and their caregivers before the death of the individual, whereas less attention has been given to caregivers after the death of the individual. Among these are family members who either consented to donating the brain of a deceased relative, or who were family members of a cognitively normal person who himself or herself had consented to donate his or her brain upon death. Biological approaches have focused on understanding the biochemical and molecular abnormalities underlying the neuropathological lesions of AD, which are unique for the human brain. To accomplish this, autopsy study of the human brain is critical. It remains the only definitive means by which to confirm the clinical diagnosis.

Despite the overwhelming need for postmortem study of human brains both from people with AD and especially from cognitively normal aged people, U. S. autopsy rates generally continue to be low[3]. One way to better understand human brain autopsy rates, and to develop interventions to increase such yield, is to examine the experiences and impact brain autopsy results have on family members who either consented to donating the brain of a deceased relative, or who were family members of a person who had himself or herself assented to donate his or her brain upon death.

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Much of the literature on full autopsy has dealt primarily with three areas: (a) reasons to permit autopsy[4,5,6], (b) attitudes towards autopsy[7,8,9,10], and (c) results of autopsy[11,12,13]. Limited research has studied family members' experiences related to full autopsy and the autopsy report[14,15,16] .

Oppewal and Meyboom-De Jong[14] interviewed 12 family members of 9 autopsied patients, 6 months to 1 year after the autopsies had been performed. In all 9 cases, the death was determined to be the result of natural causes, such as myocardial infarction. According to most family members, hearing the autopsy result was a positive experience, as it provided them clarity, and reassured them that they had not misinterpreted important symptoms experienced by the patients. This reassurance helped family members in their grieving process. In the study by McPhee et al.[16], only 28 of 62 family members of patients who had undergone autopsy received a copy of the autopsy report, lack of which was frustrating to other family members. Most family members who received the report complained about prolonged delays (3 months or more) in receiving it, and about the complexity of the terminology used. Kotch and Cohen[21] examined the autopsy report as a counseling tool for parents of children who were presumed to have died of sudden infant death syndrome (SIDS). Forty-seven of 53 SIDS counselors returned a questionnaire covering the following categories of information: 1) counselor profile; 2) administrative concerns; and 3) autopsy visit. The counselors reported that sharing the autopsy report with the bereaved parents was a valuable part of the counseling process. The majority of the counselors also reported that parents always wanted them to explain the autopsy report as well as the cause of death.

Research has been lacking on family members' experiences related to brain autopsy and to receiving and reading the Neuropathology report. Therefore, our study at the Oregon Brain Bank in Portland, Oregon, U.S. has explored family members' experiences from and reactions to receiving and reading a Neuropathology report describing brain tissue changes found in a deceased relative; and has identified reasons for, benefits of, and concerns resulting from their decision to consent to a brain donation for autopsy.

At the Oregon Brain Bank, since its inception in 1990, more than 1300 brains have been donated for autopsy. These donated brains come from two sources: (a) spontaneous "family interest" donations and (b) individuals enrolled in certain federally funded research studies. The former are families of patients who had some neurodegenerative illness, and who are looking for answers and specific diagnosis by autopsy. The scientific need for age-matched control brains means that family members of such patients also may volunteer to donate their own brain. Therefore, this source includes brains of cognitively normal individuals. For individuals enrolled in the federally funded research studies whether they were cognitively intact or in the early stage of dementia, they and their next-of-kin agreed in advance to brain autopsy permission. In the state of Oregon, the consent for an autopsy is legally binding when it is signed or authorized by the legal next-of-kin. The following persons, in order of priority may provide such consent: (a) spouse, (b) an adult son or daughter, (c) either parent, (d) an adult brother or sister, (e) a guardian of the decedent at the time of death, and (f) any other person authorized or under obligation to dispose of the body.

At the Oregon Brain Bank, to some extent, every donated brain is utilized for some level of research. The ones donated from individuals enrolled in federally funded research studies fulfill more extensive research protocols for targeting cellular studies than the ones from the spontaneous "family interest" group. None of research studies is intentionally designed to be a population-based study.

Spontaneous "family interest" donors learn about the brain donation and autopsy option from various sources such as chronic care facilities and primary health care providers. Solicitation of brains for the Oregon Brain Bank is also done by academic clinicians in the Alzheimer Clinic, through informational advertisement, and at public presentations in the community. During the past decade, about 30 – 35 percent of the donated brains have come from families where their relative has been followed during life in a research study. The other 65 – 70 percent derived from "family interest" donations.

At the Oregon Brain Bank, an extremely thorough and rigorous neuropathology examination is performed on all donated brains, involving sampling 14 standard anatomical regions of the central nervous system, and utilizing the histochemical battery of stains approved by the Neuropathology Core Leaders Group of the 30 Alzheimer's Research Centers funded by the National Institute of Aging (NIH). The diagnostic criteria employed are those published both by the NIH panel[17] and the Consortium to Establish Registry of Alzheimer's Disease (CERAD). The complete neuropathology examination is documented in all Neuropathology reports sent to family members as it is for the medical record. The reports average 6 to 8 single-spaced type-written pages.

Materials and Methods

Sample

Twenty family members were recruited through the Oregon Brain Bank with which their elderly relative had been affiliated. The family members had received and read the Oregon Brain Bank's Neuropathology report describing presence or absence of brain tissue abnormalities from 20 deceased relatives. Three additional family members participated in one interview, increasing the total sample size to 23. Nineteen family members were women and four were men. They were all Caucasian and had a mean

education of 16.3 years (SD = 2.44). Family members ranged in age from 34 to 88 years (M = 61.7, SD = 13.6). Eight spouses (wives = 5, husbands = 3) and 15 children (daughters = 14, sons = 1) had made the decision to donate the brain of their deceased relative for autopsy. They were randomly selected from four groups, categorizing the autopsy result of their deceased relative (AD, non-AD dementias, Parkinson Disease (PD), or normal brains). By using this approach, we could be assured that the sample would include family members who had experienced different results from the various Neuropathology reports.

Deceased relatives ranged in age from 70 to 104 years (M = 82.0, SD = 8.1). Nine were women and 11 were men. They were all Caucasian. Ten of them had the clinical diagnosis AD, three had non-AD dementias, three had PD, and one had AD and PD. Three of the deceased relatives had had normal brains. The brains of 14 were from spontaneous "family interest" donations, unsolicited by the Brain Bank. Six were from individuals enrolled in longitudinal federally funded research studies where the patient and the family were asked to assent to brain donation.

Procedures

A semi-structured interview guide was developed and reviewed by five experts in dementing illnesses, gerontological and family care nursing, and organ donation. Prior to approval of the study, the Oregon Health & Science University (OHSU) Institutional Review Board requested that we pilot test the interview guide using 20 adult volunteers. After they had read one of four anonymized and modified Neuropathology reports, they assessed the questions for clarity, ambiguity, comprehensibility, and sensitivity, and established the approximate length of time it would take to conduct the interviews. Based on the pilot testing, no modifications were made to the interview guide.

Family members of relatives who have had a brain autopsy performed at the Oregon Brain Bank have filled out a Letter of Intent. They have been asked specifically if they would be willing to be contacted at a later date to supply additional information. A letter briefly describing the study was sent to potential study participants alerting them of an upcoming telephone call. The first author (LH) contacted family members by phone after they had received the letter. She explained the study and invited them to participate. If the family members agreed to participate, the principal investigator then scheduled an interview. To obtain a sample of 20, initially 21 family members were contacted. One family member had died. The 20 contacted family members were invited to participate in the study. They and the 3 additional family members who participated in the same interview, all consented to participate in the study.

Using the interview-guide, family members were interviewed once about their decision to consent to a brain donation and their reaction to reading the Neuropathology report. The interviews were conducted 5 to 45 months (M = 16.8, SD =12.6) after the death of the relatives and at places chosen by the family members. With the exception of family members of those deceased relatives who had had normal brains, one inclusion criterion was to conduct the interviews within 24 months after the death of the relatives. Because of the small number of family members who had donated normal brains available to be interviewed, they were selected from the entire group, causing those interviews to be conducted at 45 months after the death of the relative. The interviews lasted for 22 to 120 minutes (M = 55.3, SD = 30.8). Interviews conducted with family members who experienced family conflicts in relation to the death of their relative, or who had not expected their deceased relative to have suffered from AD, tended to last longer than interviews conducted with family members where no family conflicts existed or their deceased relative had been cognitively intact. All interviews were audiotaped, transcribed, and analyzed using content analysis[18,19]. Nine categories of concepts were defined before the analysis, such as Reasons for Autopsy. The remaining categories were derived from the data. After the transcripts had been verified, each interview was then read and coded, line by line, to achieve a complete and thorough coverage of the data. The unit of analysis was words and phrases spoken by study participants which minimized the loss of meaning[19]. As the investigator worked through each interview, codes from each of them were compared with others from the same study participant and other participants to clarify relationships.

Initially, 18 main categories of concepts and 63 subcategories were created. Categories were reconfigured by deleting and condensing, under fewer, more broadly defined categories. For example, since some of the codes were unrelated to family members' experiences they were deleted (e.g., caregiving activities). Other codes had only one non-important coded data bit defining them, and were therefore deleted. Others were moved under other subcategories. Additional subcategories were combined into one concept; such as, "other family members being supportive of autopsy decision" and "other family members not being supportive of autopsy decision" were combined to "family support of autopsy decision." This resulted in seven main categories of concepts, each with two to four subcategories. The main categories include: Decision Related Issues, Donation Process, Autopsy Related Issues, The Report, Effects, Follow-Up Information, and Preparing for the Future.

Validity and Reliability

To enhance the validity of the data, the principal investigator observed participants' non-verbal behavior such as hesitation to answer certain questions or whether they seemed anxious or disturbed by certain questions[19]. Participant behaviors and inconsistencies in responses were noted by the investigator and carefully called to their attention. After the interviews, notes about participants' responses were written.

Reliability of the data was enhanced by having the investigator serve as the sole interviewer[20]. Threats to reliability of the content analysis were addressed using the recommendations of Garvin et al.[21], who emphasize that both unitizing and interpretive reliability are important. Unitizing reliability, defined as "consistency in assessing what is to be coded"[19,21 p.52], was enhanced by using interviews that were tape-recorded and transcribed. The transcriptions were coded line-by-line and the identified units (words and phrases) were examined repeatedly. Interpretive reliability is defined as "consistency in assigning units to categories" [19 p.307,20]. To enhance inter-rater reliability, two other researchers reviewed the initial coding scheme to ensure that they agreed with the investigator's original analysis. Using the coding scheme, the two researchers coded one long and two shorter interviews (approximately 18% of the data). Before discussing the coding with the researchers, inter-rater agreement on the codes was calculated to be 85.7%. Coding decisions were then compared and full agreement on the coding was reached for the remaining 14.3% between the researchers and the investigator. Data were coded using the software program, Non-numerical Unstructured Data Indexing Searching and Theorizing (QRS NUD*IST).

Results

Results are discussed under the seven main categories of concepts: Decision Related Issues, Donation Process, Autopsy Related Issues, The Report, Effects, Follow-Up Information, and Preparing (Table 1).

Decision Related Issues

Family members were asked if other family members had participated in the brain donation/ autopsy decision as well as how they had learned about the brain autopsy option, e.g. from advertisement, the primary health care provider, etc. The main reasons family members described to consent to a brain donation for autopsy were to (a) receive a definitive diagnosis, (b) fulfill familial objectives, (c) advance medical knowledge, and (d) carry out moral obligations. For some family members the decision whether to consent to the brain autopsy was far harder than knowing that the actual autopsy was performed, once they had so decided. After the decision had been made, the autopsy performed, and the Neuropathology report received and read, all family members were glad that they had authorized the brain autopsy. The most frequently described benefit of the autopsy by family members was the fact that they now knew what they had been dealing with, and what they might be dealing with in the future. Ten family members talked about other family members being supportive or not supportive of the decision to consent to the brain donation/autopsy. Two family members elected not to tell other family members that they had authorized the autopsy, in order to prevent judgmental statements from them as well as not to increase existing family conflicts.

Donation Process

Most family members felt that the brain donation process went smoothly. They found health care providers as well as the funeral directors to be helpful and kind. The length of time it took from the death of their relatives until the autopsy had taken place was distressing to two of the family members. This may reflect, e.g. the work load of funeral home personnel who transport the deceased relative between the place of death, (the Brain Bank), and the funeral home. The Brain Bank personnel make an effort to keep the post-mortem interval as short as possible. Approximately 30 percent of all autopsies performed have a post-mortem interval of less than 6 hours. Another source of distress for two family members was when additional organs from the deceased relative had been "donated," but were not harvested due to some bureaucratic inflexibility of the state's health care system.

Autopsy Related Issues

During the study period, due to reduced public funding for the service provided at the Oregon Brain Bank, the Brain Bank personnel discretely requested a charitable donation when Neuropathology reports were sent to spontaneous "family interest" donors, indicating that a voluntary donation would help defray the technical cost of the autopsy (previously $800). However, the monetary donation was never a mandatory requirement and the Brain Bank continued to provide the service at no cost to many of these families. Several family members gave modest donations to cover the technical fee associated with the brain autopsy, whereas still others did not give donations. Six of the deceased relatives had been enrolled in federally funded research studies, and therefore, the cost of those brain autopsies had been covered by the research studies.

An area of concern for four of the family members was the question of disfigurement of the face after the autopsy. Three of them were assured by health care providers that their relative would not look any different after the autopsy, which they then found to be true.

Four of the family members found there to be too little publicity about the brain autopsy option. They spoke about how easily they could have missed the opportunity and the distress they would have felt had they not had it performed. They were thankful for the opportunity for having it done, as it provided them with an option to do something that might be of help to future generations.

The Report

With the exception of one family member, they all found the Neuropathology report detailed, technical, and difficult to understand. In addition, all family members talked about the timing of the report. Most had received it approximately 4 weeks after the death of their relative and found that to be

Table 1.

Main Categories	Subcategories	Quotes Illustrating a Few Categories
Decision Related Issues	1. Who decided on the donation 2. How learned about the autopsy 3. Reasons for autopsy: a. Advance medical knowledge (n = 16) b. Receive a definitive diagnosis (n = 10) c. Fulfill familial objectives (n = 7) d. Carry out moral obligations (n = 3) 4. Consenting a. Support (support, n = 5, no support, n = 5)	"But I didn't know how it [brain donation] happened. I didn't know anything really about it until I talked to Hospice or Visiting Nurses, and they were really good about hooking us up with that [Brain Bank]." "This [brain donation] was top secret in our family, because my mother's family, her brothers and sisters, as soon as my Dad was diagnosed, they wanted him committed."
Donation Process	1. "Smoothness" (smooth, n = 21; not smooth, n = 2) 2. Harvesting (n = 2)	The whole process went "smoothly, real slick or real well."
Autopsy Related Issues	1. Cost (n = 14) 2. Appearance (n = 4) 3. Publicity (n = 4)	Responses to the cost of the technical part of a brain autopsy ranged from believing the cost to be "incredible reasonable" and "recognizing that the work can't be done for free," to not having thought about financial aspects, or to feeling "horrified that it would cost that kind of money to do that kind of research to help others."
The Report	1. Format a. Language (n = 23) b. Timing (n = 23) c. Length (n = 3) 2. Suggestions to improve report a. Lay terms (n = 14) b. Correlate with symptoms (n = 6)	"Well you know they're talking about it [diagnosis] and they use words that are miles two long and you don't know what they're talking about."
Effects	1. Varied based on diagnosis a. Disturbing (n = 6) b. Closure (n = 4) c. Grieving (n = 3) d. Pleased (n = 3)	"I sort of had a mixed reaction to the report [Neuropathology report]. Um, relief that OK, this was what happened. This was what was going on. It wasn't senile dementia, it wasn't alcohol related. It wasn't stroke related. It was truly Alzheimer's. OK, it was helpful to have a definitive answer as a daughter. It was disturbing. Especially with the family history. And the question is, is this a genetic thing?" "She [sister] is now worried that we now are in a data base and going to be denied insurance because we are going to be genetically linked."
Follow-Up Information	1. Would have liked (n = 11) 2. Received (n = 4) 3. Recommendations a. Phone call	"As far as what we got, in my opinion, we got the report but nobody explained anything to it. I mean, I've gone over the report. There's not one part of it that I can glean any information that says anything. We want to know if it's hereditary. We want to know how it could effect us."
Preparing for the Future	1. Keeping informed 2. Sharing information	"I certainly am a candidate as much as anybody else. But I will certainly be watching as far as new research that comes out. New information about vitamins or lifestyle or whatever, I am talking about things with my children right now. That my mom and I never had discussions about because we didn't know. I don't want my children to feel the pressure and fear that I sometimes felt.

n = number of family members who addressed the subcategory

appropriate. Only one family member believed that the report had arrived rather too early, whereas a couple felt that the report had arrived rather late (> 8 weeks) which caused some anxiety. The Oregon Brain Bank personnel make a special effort for family members to receive prompt as well as accurate feedback about the diagnosis of their deceased relative in as few weeks as possible. Three family members found the report to be "too lengthy."

Although an explanatory letter (which includes a non-technical description of the deceased relatives' diagnoses) from the Consultant Neuropathologist accompanies all Neuropathology reports, more than half of the family members wished that the report could have been explained in more extensive lay terms, either by the Neuropathologist or (less desirable) by the deceased relative's primary care provider. Several of them expressed a need to have the findings of the report correlate with symptoms their deceased relative had experienced, in order to be able to make sense of the report and its medical terminology.

Effects

The effects family members experienced after receiving and reading a Neuropathology report describing brain tissue from a deceased relative varied greatly based on diagnosis. For some the Neuropathology report provided closure; for a few it intensified their grieving; for still others it was disturbing. It was particularly concerning to family members of relatives diagnosed with AD who had a strong family history of AD, to family members who had been treated for AD but who were found to have suffered from a considerably different disease, and to family members who had not expected their relative to have had AD. Family members of the cognitively normal deceased relatives were pleased to have that information confirmed by autopsy.

Follow-Up Information

Both the family member who authorized the brain autopsy and the deceased relative's primary care provider receive a complete copy of the Neuropathology report. Eleven of the 23 family members felt a need for follow-up information, after having received and read the Neuropathology report. Eight of the family members did not feel that they had a need for follow-up information. Four of the family members had in fact been contacted by their deceased relatives' primary care provider or research investigator, and had discussed the autopsy report and result with him or her, about which they were pleased. Two of those four family members were among the ones who felt that they did not need follow-up information.

The need for follow-up information varied from clarification of the report's technical language to genetic counseling. In particular, family members with a family history of AD expressed a desire to know about genetics, and worried (unnecessarily) about potential ramifications of genetic testing. Due to Oregon state law, genetic information that could potentially be derived from brain autopsy tissue may never be communicated to family members or other parties such as insurance companies. However, if family members make inquiry about genetic pre-disposition they are always referred to genetic counselors in the community.

Although in the explanatory letter from the Consultant Neuropathologist that accompanied the Neuropathology report family members were encouraged to contact their deceased relatives' primary care provider to interpret and discuss further the findings of the report, only three of the family members had actually followed this advice. Two of the three family members found the discussion with the care providers helpful. Others for various reasons were uncomfortable contacting their deceased relative's primary care provider. A few felt that they did not know their deceased relative's primary care provider very well, and that he or she would make them feel as they were wasting his or her time or uncomfortable because of their lack of understanding of the report. A couple did not believe the primary care provider to be knowledgeable in regard to the Neuropathology report, and wanted to speak directly with the Consultant Neuropathologist as they thought him or her better equipped to answer questions that they had. A follow-up phone call was the most frequent recommendation by the family members.

Preparing for the Future

Family members, especially of relatives diagnosed with AD, described how they were trying to prepare and plan for their and other family members' uncertain future. They did this by keeping up on the latest published literature and research on AD. Several of the family members were interested in participating in research studies so as to be part of an environment that could provide them with information such as the newest pharmaceutical and therapeutic developments. During the interviews, several family members expressed an interest in learning more about genes as a cause of AD. If more specific questions were asked in relation to their family, the family members were encouraged to contact their primary care provider for further information.

Discussion

This study explores family members' experiences from and reactions to receiving and reading a Neuropathology report describing brain tissue changes from a deceased relative and identifies reasons for, benefits of, and concerns resulting from their decision to consent to a brain donation for autopsy. As expected, family members of cognitively normal relatives (n = 3) and family members of relatives who died from different dementing illnesses (n = 17) had differing experiences and reactions to the autopsy result. Family members of the deceased who had had normal brains were pleased to have that information confirmed, and lacked the level of concern for developing AD in the future expressed by family members of relatives who had been diagnosed/confirmed with AD.

Reasons for wanting or not wanting autopsy have been documented in the literature[16]. Stress of allowing autopsy has been described as one reason for not having an autopsy performed. This supports the experience of some family members in our study, who found that the decision to consent to the brain autopsy was far harder than knowing that the actual autopsy was performed, once they had so decided.

Disfigurement of the body after an autopsy has been described in the literature as a concern and reason for family members not wanting to consent to an autopsy[22,23]. In our study, disfigurement of the face was of concern to family members, although written information provided to family members from the Pathology department clearly states that the autopsy procedure does not cause disfigurement. Because this concern continues to be an issue, and perhaps a barrier to obtaining brain autopsies, it should be reiterated in particular detail by health care providers who interact with family members or patients who may be thinking about

consenting to a brain donation. Another reason why family members, patients or volunteers may not want to consent to a brain donation for autopsy is related to the cost potentially associated with the procedure. Unless people are enrolled in special research studies and have the cost for the brain autopsy covered by such studies, money to support autopsy programs is less available as health care systems are cutting costs. This is unfortunate, as the genetic aspects of AD and other dementing diseases are becoming better understood and the autopsy is a way for family members to know if they may have an increased risk of contracting the same disease as their deceased relative.

To our knowledge, this is the first study that has explored family members' experiences from and reactions to receiving and reading a Neuropathology report describing brain tissue changes from a deceased relative. Limited research has examined family members' experiences to autopsy[14,15,16]. In the study by McPhee et al.[16], family members described each day they had to wait for the autopsy results as agonizing. Therefore, the timing of the arrival of the Neuropathology report may be of importance in order to help family members have closure. In our study, family members received the Neuropathology report within 4 weeks after the death of their relative, and found that to be appropriate.

Not surprisingly in our study, 22 of 23 family members found the formal Neuropathology report difficult to understand, and more than half of the family members wanted the report to have been explained in more lay terms, even though an explanatory letter from the Consultant Neuropathologist accompanied all reports. Furthermore, several of the family members expressed a wish to have the findings of the report correlate more precisely with symptoms their deceased relative had experienced, in order to be able to make sense of the report and its medical terminology. Some suggested this might be addressed in some cases by adding a diagram of the brain to the Neuropathology report. The diagram of the brain could show the major areas of the brain, their general function, and the main symptoms associated with disease in such areas. These major areas could have different numbers that could even be referred to in the report description.

At our institution, a copy of the Neuropathology report is always sent to the deceased relative's primary care provider, who is encouraged to consult with family members in the interpretation of the report, unless the deceased relative was part of a federally funded research study. At the same time, a copy of the Neuropathology report is sent to the family member who authorized the brain donation and autopsy. In a letter accompanying the copy of the report sent to the family member, the Consultant Neuropathologist specifically recommends that the family member contact the deceased relative's primary health care provider for any clarification or amplification he or she may desire in regard to the report. If the deceased relative was enrolled in a research study, the family member is scheduled to receive a Neurologist's telephone follow-up review of the neuropathology results. We believe that this semi-structured interview with the family member is an effective means of follow-up and allows him or her an opportunity to address concerns or questions about the result. It was nevertheless not surprising that 11 of the 23 family members felt a need for some sort of follow-up information after having received and read the Neuropathology report, as the report is very technical and often beyond the expertise of most primary care providers. This raises some questions: (a) Who should be responsible for follow-up information to family members of the spontaneous "family interest" group, in particular: the deceased relative's primary care provider, the Consultant Neuropathologist, or the family member who authorized the brain autopsy?; (b) what is the best mechanism to provide follow-up information?; and (c) what is the ideal amount and type of follow-up information needed? For various reasons family members were uncomfortable contacting their deceased relative's primary care provider, even though Webster et al.[24] have indicated such feedback and closure are properly the job of the clinician. Webster et al.[24] suggest that timely follow-up information by clinicians would provide an opportunity for them to support families, help them with unresolved issues, and assist them with their grieving process. As family members were uncomfortable contacting their deceased relative's primary care provider, the provider might be uncomfortable contacting family members[5,25]. This might be due to his or her own discomfort with organ donation and autopsy, or a fear of upsetting family members. Family members suggested a phone call as the most frequent recommendation for follow-up information. Can such a phone call be made by a Brain Bank coordinator? Should it be done by a Consultant Neuropathologist? Should it be done, preferably, by the deceased relative's primary care or terminal provider? Currently about one third of the donations come from families where their relative has been followed during life in a research study. In this particular setting, an academic Neurologist is responsible for follow-up discussion of the autopsy results.

Our study has several limitations. The first is the small sample size (n = 23) which limits the generalizability of the study findings. A subgroup of the deceased relatives (n = 6) were part of formal research studies and their family members may have had different attitudes about autopsy. Further, the findings were derived from family members' recall of their experiences from and reactions to receiving and reading a Neuropathology report describing brain tissue changes from a deceased relative. Nevertheless, the information on experiences recalled by family members is valuable because these experiences were real to them. None of the family members seemed to have any difficulty recalling and describing their experiences, although it might have been likely that family members who read the report more than a year before they were interviewed would have less clear recollection of the experience than those who read the report within the past year. The second is the family members' mean education of 16.3 years. Burroughs et al.[26] found that the

more formal education an individual had, the more likely he or she was to donate organs. In keeping with the general observation in populations that women are somewhat more frequently afflicted by AD, we also found that more women (n = 19) than men (n = 4) were included in our study. The educational level between the two genders did not differ. The third limitation is that the study-sample lacked ethnic variation, which is not surprising because research has shown that whites have a higher brain autopsy rate than minorities[9,27]. To increase brain donation in different ethnic communities including that from persons who are expected to have normal brains, addressing factors such as lack of knowledge about brain donation, lack of communication between lay people and health care providers, cost, spirituality, and culture are necessary[4,9]. Age may also be a factor necessary to address, as Kaye et al.[8] found that older persons (>85 years) were more likely to consent to donate their own brains than those younger (65-84 years), and that educational level was not a predictor of willingness to donate.

This study provides a picture of family members' reasons for deciding to consent to a brain autopsy, their experiences related to the brain donation process, and their reactions to brain autopsy results. Such reactions, experiences, and reasons have clinical relevance. This includes, but is not limited to, a need for interventions to improve the presentation of the Neuropathology report, follow-up information, and the need for later interventions such as genetic counseling. Based on these family members' experiences a questionnaire is now being developed to survey a larger sample of family members who either consented to donating the brain of a deceased relative, or who were family members of a person who had (himself or herself) pre-consented to donate his or her brain upon death.

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Relation between Vascular Risk Factors and Carotid Plaque Cell Composition and Viability in Elderly Patients

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Abstract

Objectives: **Carotid stenosis is a crucial cause of ischemic stroke. Recent studies suggest that one of the most important effects of lipid-lowering statins is to stabilize vulnerable plaques. However, it remains to be determined if this effect is secondary to the lowering of plasma cholesterol levels or due to a direct effect of statins on plaques stability.**

Design and main outcome measures: **In this study we have analyzed if plaque cell composition and the frequency of apoptotic DNA fragmentation are related to cholesterol levels or any of the major risk factors for vascular disease. The study group consisted of 49 patients undergoing carotid endarterectomy. The plaques were stained by immunohistochemical and TUNEL techniques and scored semi quantitatively by a blinded observer.**

Results: **Rupture sites contained significantly more TUNEL-positive cells and T-cells, but less smooth muscle cells than intact areas of the fibrous cap. Plaques from hypercholesterolemic patients were found to have less TUNEL-positive cells, but otherwise hypercholesterolemia, low HDL cholesterol, hypertension, diabetes and smoking did not influence plaque cell composition or the frequency of TUNEL-positive cells.**

Conclusions: **Our observations suggest that there are no associations between major vascular risk factors and plaque cell composition. Accordingly, they favor the notion that the effects of statins are due to a direct effect on plaque structure rather than solely secondary to lowering of plasma cholesterol.**

Key words: atherosclerosis, plaque rupture, hypercholesterolemia, inflammation, hypertension, elderly patients

Introduction

A consistent finding of lipid-lowering intervention trials is that the reduction in cardiovascular events is greater than the effect on plaque size. This suggests that the major effect of lipid lowering is on plaque stability rather than on plaque regression[1-3]. Unstable and ruptured atherosclerotic plaques are characterized by increased macrophage infiltration, expression of matrix degrading enzymes and sparse smooth muscle cells (SMC) in the fibrous cap region[4-6]. Studies using TUNEL technique to label cells with DNA fragmentation have suggested presence of cell death by apoptosis in human plaque cells[7-10]. The factors responsible for induction of DNA damage and inflammatory activity in atherosclerotic lesions remain poorly understood. In cell culture, SMC exposed to INF-γ, TNF-α and IL-1β undergo cell death by apoptosis[11]. Oxidized low density lipoproteins (LDL) and products generated during oxidative modification of LDL, such as oxysterols, have also been implicated in SMC apoptosis [12-14].

Induction of hypercholesterolemia in animals results in activation of endothelial adhesion molecule expression and intimal leukocyte infiltration[15]. If lipids influence the inflammatory activity also in more advanced lesions, and in

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particular in the inflammatory degradation of the fibrous cap preceding plaque rupture, remains to be established. Experimental evidence suggests that LDL particles undergoing oxidative modification in the vascular extracellular matrix may be involved in lipid-induced vascular inflammation[16-18]. Oxidized LDL is cytotoxic for cultured endothelial and SMC[19], increase endothelial-leukocyte interactions[20,21] and influence cytokine production[21].

We have recently shown that treatment with pravastatin decrease the inflammatory activity and increases cell viability and collagen content in human atherosclerotic plaques[22]. The aim of the present study was to determine whether there is an association between vascular risk factors such as high LDL cholesterol, low HDL cholesterol, smoking, diabetes and hypertension and markers of plaque cell damage and inflammation. The study included risk factor screening as well as immunohistochemical and TUNEL analysis of atherosclerotic plaques from 49 patients undergoing surgery because of symptomatic ipsilateral high-grade carotid stenosis.

Methods

Subjects

Forty-nine patients with symptomatic (hemispheric TIAs, amaurosis fugax or minor stroke within the past 6 months) ipsilateral carotid artery stenosis ≥70% diameter reduction according to carotid angiography measurements (NASCET criteria)[23] undergoing carotid endarterectomy (CEA) within 3-6 month after the acute event were included in the study. Risk factor analysis included hypertension (current or previous use of antihypertensive drugs and/or blood pressure ≥ 160/95 mm Hg), diabetes mellitus (taking oral antidiabetic drugs and/or insulin or being on antidiabetic diet) and smoking habits (nonsmoker, former smoker and current smoker).

Tissue preparation

One part of the endatherectomy specimens was immediately snap frozen in liquid nitrogen and stored at -70°C. The residual part of the tissue was fixed in 4% paraformaldehyde/1µM EDTA. Tissues were cut in 14µm thick section on a cryostat and stored at -20°C. Fresh frozen tissue sections were fixed in acetone for 20 minutes prior to storage.

Blood sampling and laboratory measurements

Blood samples were taken after 12 h of fasting. Venous blood was drawn into vacutainer tubes, left for 30 min at room temperature and centrifuged at 1400 x g for 20 min at room temperature. Fasting cholesterol and triglyceride concentrations in serum were assayed by enzymatic techniques (the Fully enzymatic method; Hitachi 917, Naka Japan). High-density lipoprotein cholesterol was also analyzed by a direct enzymatic technique (Hitachi 917, Naka Japan). Low-density lipoprotein (LDL) cholesterol was calculated using Friedewald's formula.

TUNEL

TUNEL was performed using the Apoptag in situ detection kit (Oncor) on both paraformaldehyde-fixed and frozen specimens. Sections were pretreated in 20 µg/ml of proteinase K for 20 minutes, rinsed in 1µM EDTA in PBS for 5 minutes and then covered with the TdT-enzyme solution for 2 hours at 37°C. The reaction was detected by peroxidase-conjugated anti-digoxigenin (room temperature, 20 minutes) and visualized by DAB (diaminobenzidine) followed by counterstaining with hematoxylin-eosin. Control slides were incubated in PBS alone.

Immunohistochemistry

Fresh frozen sections and/or formaldehyde fixed were rinsed for 2x5 minutes in PBS. Endogenous peroxidase was quenched with 0.3% H_2O_2 in methanol for 30 minutes and rinsed in PBS for 20 minutes. Slides were then covered with normal horse serum for 20 minutes and washed with PBS for 10 minutes. Subsequently the sections were covered with the following primary antibodies; CD6 and CD3 for T-cells (Dako, 1:50), CD68 for macrophages (Dako, 1:50) and HHF-35 for SMC (Dako, 1:50-1:100). Control slides were incubated in an irrelevant primary antibody or PBS alone. The sections were then incubated with biotinylated anti-mouse IgG as the secondary antibody for 30 minutes, washed in PBS for 10 min and stained with avidin-biotin for 30 minutes. The slides were exposed to DAB for 2 minutes and counterstained in methyl green and/or hematoxylin-eosin.

The frequency of cells showing positive TUNEL or T-cell, macrophage and SMC immunoreactivity was graded by a blinded observer according to a modified version of the technique described by Galis et al.[24]. Consistent positive staining involving more than 50% of the plaque cells was recorded as (4), positive staining of 20-50% of the cells as (3), positive staining of 5-20% of the cells as (2), positive staining of 1-5% of the cells as (1) and staining of less than 1% of the cells as (0). One section from every patient was used for each analysis.

Statistical analysis

Statistical tests were performed using the JMP software program (SAS Institute Inc.). Values are expressed as mean and standard deviation. Student's t-test or ANOVA were used to study differences in continuous variables between groups.

Results

Carotid plaques from 49 patients undergoing endarterectomy due to symptomatic ipsilateral high grade stenosis were analyzed for the presence of DNA fragmentation as assessed by TUNEL and plaque content of macrophages, T cells and SMC as assessed by immunohistochemistry. Before surgery serum triglycerides, LDL and HDL cholesterol were determined. The mean age of the patients was 70.9±7.1 years.

Mean serum cholesterol, LDL cholesterol, HDL cholesterol and serum triglycerides were 6.39±0.97 mmol/L, 4.25±0.96 mmol/L, 1.27±0.41 and 1.96±0.70, respectively.

Morphological and immunohistochemical characteristics of the plaques

Organized thrombus could be observed macroscopically in all endarterectomy specimens. The surface of the plaques was disrupted and highly irregular. The plaques contained areas of lipid deposits, calcification and necrotic debris. Histological examination of the plaques revealed that calcium deposits, intra-plaque hemorrhage, necrosis and plaque rupture was a prominent feature of the majority of the plaques.

Cells containing DNA fragmentation as assessed by TUNEL staining were identified in 45 of the 49 specimens. The mean TUNEL score for all lesions was 1.85±1.1. TUNEL positive cells were found throughout the plaque, but were particularly abundant in the shoulder region of the fibrous cap and in the vicinity of necrotic areas. At similar locations signs of plaque rupture with organized thrombus was often observed. All plaques contained SMC α-actin (HHF 35) positive cells with a mean score of 2.35±0.93. Parallel sections stained for SMC α-actin and TUNEL suggested that the majority of cells with DNA damage were SMC. However, presence of TUNEL positivity was also observed in macrophages and T-cell rich areas. Lymphocytes present in organized thrombus generally showed positive TUNEL staining. Mean scores for T-cell and macrophage stainings were 1.94±0.97 and 1.94±0.92, respectively. As previously reported normal arterial tissue did not contain TUNEL-positive cells[9].

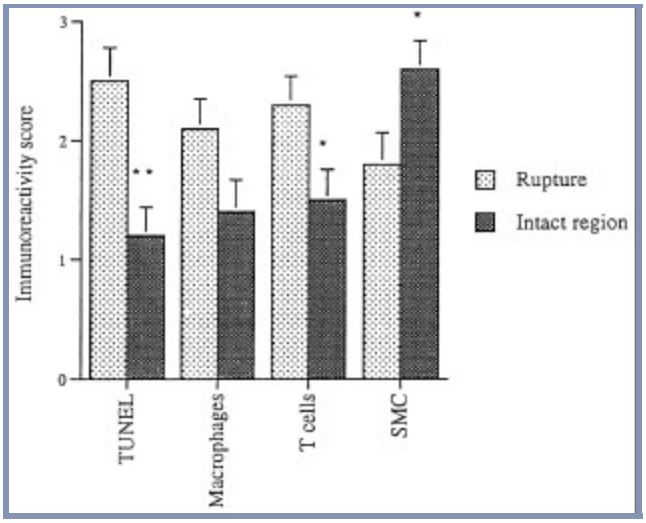

Figure 1. Comparison of TUNEL, macrophage, T cell and SMC staining in rupured and intact regions of the fibrous cap. The frequency of cells showing positive staining was graded according to a modified version of the technique described by Galis et al[22]. Consistent positive staining involving more than 50% of the plaque cells was recorded as (4), positive staining of 20-50% of the cells as (3), positive staining of 5-20% of the cells as (2), positive staining of 1-5% of the cells as (1) and staining of less than 1% of the cells as (0). *p<0.05, **p<0.0001.

Difference in DNA fragmentation and cell composition between ruptured and intact regions of the fibrous cap

In 26 of the 49 lesions distinct acute rupture sites could be identified. A ruptured or a very thin fibrous cap covering thrombotic material characterized these lesions. Scoring of TUNEL and cell-specific immunoreactivity in ruptured versus morphologically intact regions of the fibrous cap demonstrated that TUNEL positive cells, as well as T cells, were more frequent at rupture sites than in intact tissue (fig. 1). In contrast, there were fewer α-actin-positive cells at rupture sites than in intact tissue (fig.1), suggesting that rupture is associated with loss of SMC. Several previous studies have shown an increased rate of macrophage infiltration at rupture sites [5,25]. A similar trend was observed also in the present study, but did not reach significance using this scoring system.

Relations between plaque cell DNA fragmentation, cell composition, serum lipoprotein lipid levels and other cardiovascular risk factors

Plaques obtained from patients with a total plasma cholesterol above 6.5 mmol/L or a LDL cholesterol above 4.5 mmol/L were found to contain less TUNEL-positive cells than patients with lower cholesterol levels, whereas no association was found between HDL cholesterol and the number of TUNEL-positive cells in the plaques (table 1). Total plasma cholesterol, LDL cholesterol and HDL cholesterol levels did not influence macrophage, T-cell or smooth muscle cell content in plaques as assessed by immunohistochemistry. Moreover, plaque cell composition and cell viability was not affected by the presence of hypertension, diabetes or smoking (table 2).

Out of the 49 subjects included in the present study 6 were on treatment with simvastatin. It has previously been shown that treatment with pravastatin increases plaque cell viability and collagen content and decrease the inflammatory activity in plaques.[22] Similar trends were observed also in this study with a lower staining for TUNEL-positive cells (1.33±0.45 versus 1.93±0.17), for macrophages (1.50±0.37 versus 2.00±0.14) and T-cells (1.67±0.40 versus 1.98±0.15) in plaques from statin-treated subjects.

Discussion

The cellular composition of atherosclerotic lesions in different stages of development has been carefully characterized. These studies have also demonstrated that the destabilization of the fibrotic cap that precedes rupture of coronary plaques is associated with an increased cell death and infiltration of inflammatory cells [4,25]. The results of the present studies on human carotid plaques are in good agreement with these studies. However, there is little information on how the presence of different risk factors affects the cellular composition, inflammatory activity and cell viability of human atherosclerotic plaques in elderly

Table 1. Effect of total plasma, LDL and HDL cholesterol levels on plaque cell composition and DNA fragmentation (TUNEL)

	TUNEL	SMC	T-cells	Macrophages
Total plasma cholesterol				
=6.5 mmol/L (n=20)	1.35±0.18	2.25±0.16	2.10±0.23	1.95±0.20
<6.5 mmol/L (n=29)	2.21±0.21*	2.42±0.20	1.83±0.17	1.93±0.18
LDL cholesterol				
=4.5 mmol/L (n=17)	1.29±0.21	2.29±0.19	2.06±0.28	2.18±0.17
<4.5 mmol/L (n=25)	2.22±0.23*	2.57±0.22	2.00±0.15	1.74±0.21
HDL cholesterol				
=1.3 mmol/L (n=18)	1.78±0.29	2.28±0.21	2.17±0.20	1.78±0.21
< 1.3 mmol/L (n=24)	1.92±0.22	2.50±0.20	1.83±0.21	1.92±0.19

The frequency of cells showing positive TUNEL or T-cell, macrophage and SMC immunoreactivity was graded by a blinded observer according to a modified version of the technique described by Galis et al[22]. Consistent positive staining involving more than 50% of the plaque cells was recorded as (4), positive staining of 20-50% of the cells as (3), positive staining 5-20% of the cells as (2), positive staining of 1-5% of the cells as (1) and staining of less than 1% of the cells as (0). LDL and HDL cholesterol values were only available for 42 of the patients. Results are presented as the average score±standard error. *p<0.01.

Table 2. Effect of hypertension, diabetes and smoking on plaque cell composition and DNA fragmentation (TUNEL)

	TUNEL	SMC	T-cells	Macrophages
Hypertension				
no (n=27)	1.85±0.21	2.51±0.17	2.00±0.19	1,96±0.18
yes (n=22)	1.86±0.23	2.13±0.20	1.86±0.21	1.91±0.20
Diabetes				
no (n=37)	1.86±0.18	2.37±0.15	1.89±0.16	1.97±0.15
yes (n=12)	1.83±0.32	2.25±0.27	2.08±0.28	1.83±0.27
Smoking				
non-smokers (n=19)	1.89±0.26	2.21±0.20	1.89±0.22	1.84±0.21
former smokers (n=12)	1.75±0.32	2.08±0.26	1.75±0.28	2.25±0.26
current smokers (n=18)	1.89±0.26	2.67±0.21	2.11±0.23	1.83±0.22

The frequency of cells showing positive TUNEL or T-cell, macrophage and SMC immunoreactivity was graded as described in table 1. Results are presented as the average score±standard error.

patients. The present observations demonstrate that ruptured atherosclerotic plaques from subjects with different risk factors have very similar general cellular composition and structure. The presence of diabetes, hypertension and smoking did not influence plaque inflammatory activity or cell viability as assessed by the TUNEL technique.

Plaques from subjects with hypercholesterolemia show equal signs of inflammatory activity as plaques from subjects with low or only moderately increased total or LDL cholesterol levels. Neither did HDL levels influence plaque inflammatory activity. The lack of association between plasma LDL cholesterol levels and plaque inflammatory activity is of interest in view of recent studies demonstrating that treatment with lipid-lowering statins results in a reduced plaque content of inflammatory cells and increased expression of matrix proteinase inhibitors[22].

Several lines of evidence suggest that the beneficial effect of statins in cardiovascular disease is due to plaque stabilization rather than plaque regression[26]. However, it remains to be fully elucidated whether the effect on plaque stability is due to a direct effect on plaques or secondary to the lowering of plasma cholesterol levels. Experimental studies demonstrating that statins suppress T cell responses[27], reduce expression of HLA-DR receptors on antigen-presenting cells[28] and reduce chemokine synthesis in peripheral blood mononuclear cells[29] suggest that statins also have a direct anti-inflammatory effect. The anti-inflammatory effects of statins are primarily mediated by an inhibition of mevalonate synthesis[27-29], but recent studies have also identified a statin-binding integrin site that mediates inhibition of leukocyte function antigen–1[30]. Increased plasma levels of inflammatory markers, such as C-reactive protein (CRP), are independent predictors of risk for development of cardiovascular disease[31]. Statin therapy has been shown to reduce CRP levels and this reduction was found to be independent of the effect on LDL cholesterol levels[32]. Moreover, statins reduce risk for development of cardiovascular events primarily in subjects with signs of increased inflammatory activity[33]. Taken together these data suggest that statins have an anti-inflammatory effect on atherosclerotic plaques that is independent of the effect on LDL cholesterol levels. The present data also provide some indirect support for this notion. If the decrease in plaque inflammatory activity is secondary to a decrease in plasma cholesterol levels one would also expect to find an association

between LDL cholesterol and the amount of inflammatory cells in plaques. Our studies provide no support for the existence of such an association. In contrast, signs of decreased inflammatory activity were found in the small sub-group of patients treated with statins also in the present study.

Signs of increased cell death, as assessed by TUNEL staining, in patients with low cholesterol levels was the only significant association between vascular risk factors and plaque characteristics observed in the present study. This finding was unexpected since several experimental studies suggest that lipid accumulation is an important cause of cell death in the atherosclerotic plaque [13,19]. Only two patients in the low cholesterol group were on statin treatment, suggesting that the low rate of plaque cell death in this group was not a statin effect.

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The Use of Informant Data in the Diagnosis of Early Dementia

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Abstract

Retrospective data on functioning, behaviour and cognition, scored by carers, from 484 persons seen at a memory clinic were related to the outcome variable „Cognitive Impairment“ in subjects with a MMSE sum-score of 20 and above. Eleven of the 78 examined items, all scored binary, related to the out-come „Cognitive Impairment“ had sensitivity and specificity >0.5 and an accuracy of 75 % or higher. Factor analysis of these items demonstrated a single principal construct. Restricting the number of items to six, the set that best predicted the outcome was: „Remembers shopping lists?“, „Manages own possessions?“, „Appears confused?“, „Initiates a conversation?“, „Activates him/her-self?“ and „Knows the time?“. ROC-curves showed that this set was a better indicator of „Cognitive impairment“ than the MMSE. A cut-off of 4/5 on their sum-score gave a diagnostic sensitivity of 0.83, a specificity of 0.81 and a LR + of 4.4. Data from carers on activities and behaviour thus provide valid diagnostic information about an elderly person's cognitive functioning and may be of use when the issue is to identify those who could possibly benefit from a diagnostic work-up regarding dementia.

Keywords: cognitive impairment, dementia, elderly, informant-based data, case-finding

Introduction

Dementia is a common disorder in the elderly, the prevalence in the age group 70-74 years being about 5%, 15% among those over 75 years, and more than 40 % among those aged over 85[1,2]. Dementia tends to be overlooked by doctors and is frequently diagnosed at a later stage of the disease[3,4]. Many of the elderly are unaware that they are developing symptoms of dementia, or if they are aware, they try to conceal it. In such cases, the responsibility for initiating a diagnostic procedure will often be with a carer.

Screening for dementia does not yet seem warranted[5], however early detection is important for people suffering from cognitive impairment that is potentially reversible, e.g. caused by vitamin deficiencies, side effects of drugs, space-occupying cerebral lesions, depression or hypothyroidism[6]. An assessment at an early stage is also paramount to exploit properly non-pharmacological and pharmacological treatment[7]. Carers seem to have an important role in trigging evaluation of elderly suspected of developing dementia. One strategy for early diagnosis would therefore be to develop and assess instruments intended to capture information from carers that can be used to select elderly who could be offered a diagnostic work-up regarding dementia, e.g. with a GP or a memory clinic.

A prerequisite for a diagnosis of dementia is the definite loss of cognitive skills and reduced functioning with respect to the activities of daily living (ADL)[8-12]. An informant may easily observe such skills. Several comprehensive instruments for measuring ADL functioning, reported by a proxy, are being used in dementia diagnostics[13,14] and in one study[15] this type of information proved to give a high likelihood ratio for a positive test (LR + = 4.5). We hypothesise that family carers, relatives, friends, home-helps, nurses and (or) unskilled health service personnel, provided with a simple check-list for activities that are typically hampered by cognitive impairment, would be able to provide data which could contribute to the detection of the elderly in need of assessment, e.g. at a memory clinic.

In the present study, we have used informant-based data collected at a memory clinic, to identify markers of ADL, behaviour and memory that strongly relate to the specialists' diagnosis of dementia, and explore their properties regarding the future development of an instrument for carer-based case-finding of elderly with cognitive impairment.

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Materials and methods

The data were retrospective covering referrals seen at the Memory Clinic, Ullevaal University Hospital during the first six years of practice. The diagnostic work-up here is standardized and to a large extent based upon validated methods. All subjects seen are supposed to bring a carer. The physician performs the cognitive testing (in younger patients, a neuropsychologist) and the physical examination, and also takes a history from both the patient and the proxy who, assisted by a nurse, fills in standardized evaluation scales regarding ADL, behaviour and cognition, and also carer's burden. Information from this examination, together with the results from blood-tests and CT scans, makes the basis for the final diagnosis of dementia that is reached in consensus between the staff at the memory clinic. This procedure has been described in detail elsewhere [16,17].

In all, 946 data records were available. However, 94 of the subjects had not been accompanied by a carer, and in 66, there had been ambiguity regarding the final diagnosis of cognitive impairment. We furthermore excluded data from subjects with a Mini-Mental State Examination [18] (MMSE) sum-score of 19 or less. This left data records from 484 subjects (66% women, mean age 74 years, range 43-96) for the analyses. Their length of education was: 7 years or less, 39%; 8-12 years, 45%; and 13 years or more, 15%. The informant was a spouse in 44%; a daughter in 22%; a son in 13%; a sibling in 5% or another carer in 16% (a more distant family-member, a friend or a representative of the municipal health care system). Dementia was diagnosed according to the DSM III-R criteria for dementia with the addition that a cognitive decline should have been present for at least six months. A diagnosis of cognitive impairment, no dementia (CIND)[19], was made if the subject had evidence of impairment in short-term and long-term memory, but otherwise did not meet the DSM III-R criteria, or if the cognitive problems had been present for less than 6 months. The diagnoses were: 262 (54.1%) with dementia (mean MMSE = 23.5); 69 (14.3%) with CIND (mean MMSE =27.2); and 153 (31.6%) without definite cognitive impairment (mean MMSE = 28.5). Using a cut-point of 6 / 7[20] on the Montgomery & Åsberg Depression Rating Scale (MADRS) [21], depressive symptomatology was found in 44% among the subjects diagnosed with dementia, 56% in those diagnosed with CIND and in 51% in those without cognitive impairment. The term „Cognitive Impairment" was used as our principal outcome variable, and this encompassed those diagnosed with dementia or CIND.

Carer data on ADL were scored using the „Rapid Disability Rating Scale-2" (RDRS-2, 18 items)[22], behaviour and mood on the „Behaviour and Mood Depression Scale" (BMD, 33 items)[23], and general mental functioning on 20 items on the „Informant Interview" of the „Cambridge examination for mental disorders of the elderly" (CAMDEX)[24]. Subjective memory complaints were scored by means of the seven questions of the memory section of the „Interview with the patients" from the CAMDEX [24].

Results

Two-by-two tables were used to study the association between cognitive impairment and all the items from the RDRS-2, BMD and CAMDEX - scales, 78 in all. Many of these items are on an ordinal scale (0 - 4 or 1 - 4), and categories representing any degree of a problem whatsoever were merged, thereby creating binary items. Items with a sensitivity and specificity over 0.5 and a diagnostic accuracy over 0.75, 11 in all, were pursued in the subsequent analyses (Table 1).

Table 1. Items from RDRS-2, BMD, CAMDEX selected for further analyses

Items	Source	Sensitivity	Specificity	Accuracy
Manages possessions	RDRS - 2	0.84	0.70	0.81
Appears confused	RDRS - 2	0.82	0.68	0.79
Participates in conversation	BMD	0.81	0.61	0.76
Reads newspapers	BMD	0.77	0.67	0.75
Initiates a conversation	BMD	0.85	0.61	0.80
Activates him-/herself	BMD	0.99	0.57	0.81
Knows the time (day/date/month/year)	BMD	0.81	0.80	0.81
Keeps to the subject in conversations	BMD	0.80	0.63	0.76
Remembers shopping lists	CAMDEX	0.83	0.63	0.81
Does not concentrate	CAMDEX	0.92	0.53	0.87
Remembers happenings	CAMDEX	0.92	0.52	0.89

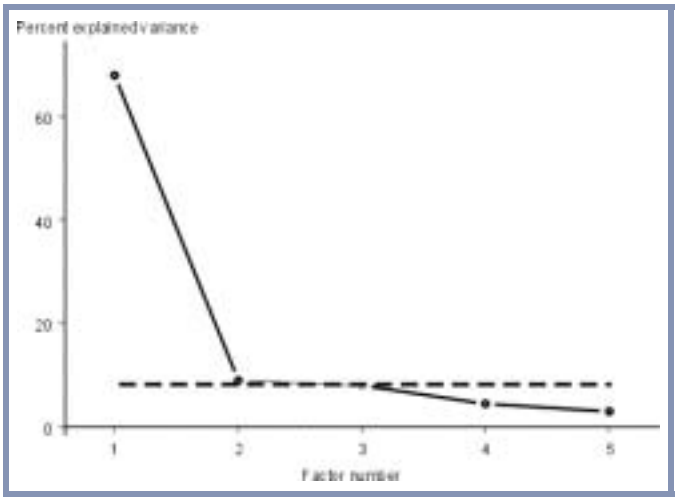

Figure 1. Scree plot of eigenvalues by construct number. Horizontal line corresponds to an eigenvalue of 1.0

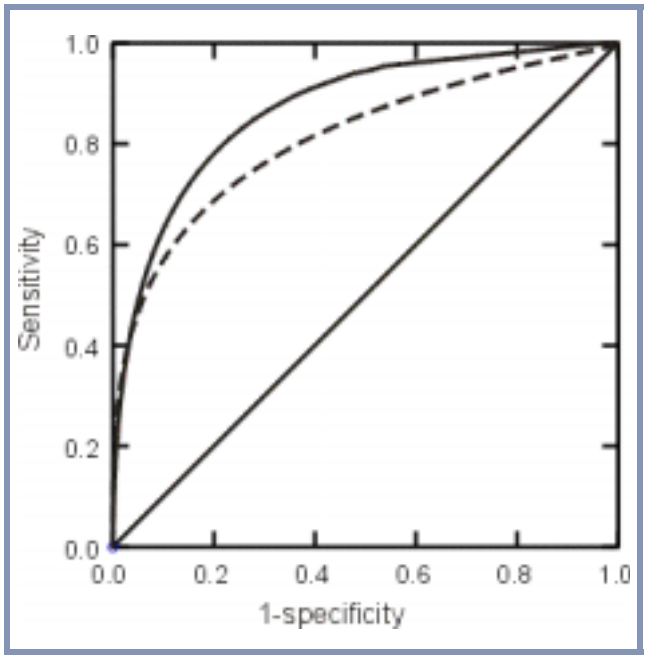

Figure 2. ROC-curves for the sum-score on the six-item set versus the MMSE sum-score (broken line)

Factor analysis was used to explore the dimensionality of these items (all scored 0 / 1). Binary factor analysis should be based upon tetrachoric correlations[25] which are the Pearson correlation one would obtain if the variables were measured continuously. There were many high correlations (>0.5), and factor analysis could therefore be applied. This revealed one principal construct. A screen plot of the eigenvalues (explained variance) supported the assumption of unidimensionality among the items by showing that the first factor, which explained 68% of the variance, dominated the other factors in this dataset. This allows the construction of an index[26] (Fig. 1).

For the possible development of a new instrument for detecting milder cognitive impairment in the elderly living at home, in this particular case to be scored by relatives, laypersons or district nurses, we decided to limit the number of items to six to make it more applicable. All the selected 11 items had proven to measure the same construct, and limiting the number of items to six would not weaken the reliability much but reduce problems owing to missing values, e.g. owing to item inapplicability. Best subset multiple logistic regression analysis, using the distance measure Mallow Cp[27] was applied to identify the six-item set that predicted the outcome variable „Cognitive Impairment“ most precisely. Table 2 presents the resulting six items and their correlations. These data were very well described by one factor (Chi-square = 2.33, DF = 9, p = 0.99) that explained 71% of the total variance (Chronbach's alpha = 0.78)

The diagnostic properties of the sum-score on these six items (range 0-6) versus the outcome variable „Cognitive Impairment“ was studied by means of a ROC curve and compared with the ROC-curve for the MMSE sum-score (Fig. 2). The six-item subset was a somewhat better indicator of „Cognitive Impairment“ than the MMSE sum-score and also superior to the complete informant-based instruments RDRS-2, BMD and CAMDEX (results not shown). The ROC-curve showed that a threshold value of 4/5 provided satisfactory diagnostic sensitivity (0.83), specificity (0.81) and LR + (4.4).

When checking for a health problem with low prevalence, false positives have a significant impact on expenditure and may cause unnecessary concern for the screenees and their families. Table 3 shows the percentage of false positives using the sum-score of these six-items, all scored 0 / 1, at cut-point 4/5 for various prevalence rates of cognitive impairment, e.g. at a hypothetical prevalence rate of 40 %, only every fourth case or less would be a false positive.

Discussion

Matthews et al. reported that most of the elderly with dementia in a large population-based survey live in their own homes and are admitted to institutional care first when their disability had reached a certain level[28]. The Oslo study on the prevalence of dementia by Engedal and Haugen showed that 60% of those with dementia lived at home[29]. Neuropsychological test batteries are known to distinguish well between cases with and without dementia with high LR + figures, e.g. 8 and 13[30,31]. In the well-designed study by Tierny et al.[31], the patients were followed by GPs looking out for the development of dementia, whereas in the study by Incalzi et al.[30] the sample was not at all natural, since the participants were hospitalised patients. A major problem with the neuropsychological test batteries is that they require specially trained personnel and are time-consuming. The MMSE, which is much shorter, was shown by Grut et al.[32] to have good diagnostic properties among unselected

Table 2. Correlation matrix of the six-item set

Items	Remembers shopping lists	Manages possessions	Appears confused	Initiates a conversation	Activates him-/herself
Manages possessions	0.60				
Appears confused	0.52	0.80			
Initiates a conversation	0.44	0.66	0.70		
Activates him-/herself	0.63	0.85	0.80	0.67	
Knows the time (day/date/month/year)	0.46	0.73	0.62	0.55	0.73

Table 3. Percentage of false positives on the sum-score of the six-item set cut at 4/5 at various prevalence rates

	Prevalence rate					
	5%	10%	15%	20%	40%	60%
False positives	81%	67%	56%	48%	26%	13%

subjects aged 75 years and over, when cut at 23 / 24, where it had similar diagnostic properties as our six-item set having a LR+ of 4.4. Ritchie et al.[33] found a higher LR+ for the MMSE at the cut-off of 23/24 (8.4), but this figure seems biased for the diagnostic situation as they collected their cases in institutions, where the prevalence of moderate to severe dementia was high, and compared them with persons without dementia living in the community. Furthermore, the MMSE is test-based with a significant learning effect[34], and the testing may offend the subject. It should only be used by trained persons and is therefore unsuitable for carers who want to assess cognition in the elderly.

The present study identify six ADL, behaviour and cognitive-related items, all derived from validated instruments, that have the potential of being developed into an instrument intended to identify elderly who have a significantly higher probability of being diagnosed with cognitive impairment at a memory clinic. Unlike the Informant Questionnaire on Cognitive Decline in the Elderly (IQ-CODE)[35], which also measures proxies' evaluations of change in cognition over 10 years but necessitates long-term knowledge of the person, these six items can be scored by any carer who has observed the person in various ADL-situations. We used data from a memory clinic where the diagnosis of cognitive impairment is based upon clinical consensus and established criteria. However, those making the final diagnosis were not fully blind to the information provided by carers on scales measuring ADL and behaviour, so there is a possibility for a diagnostic tautology with biased values for sensitivity and specificity. However, the information on the ADL - and behaviour scales provided by carers is only used to a smaller extent when the diagnosis is being discussed at the memory clinic, and we believe that this bias is small. On the other hand, we did not include data from patients with a MMSE sumscore of 19 or less, thereby to a large extent excluding those with moderate and severe impairment where a diagnosis of dementia usually is unambiguous. If these cases were included, the values for sensitivity, specificity and LR+ would have appeared better, but the figures would have been unrealistic regarding mild cognitive problems, which is how early dementia cases present themselves to family and other carers.

The properties of a diagnostic test depend strongly on the population to which it is applied. Persons referred to a memory clinic are clearly not fully representative of the general elderly population, e.g. concerning psychiatric and physical co-morbidity. Physical impairments, e.g. chronic heart failure, chronic obstructive pulmonary disease, cancer, diabetes mellitus and osteoporosis have been found to occur more frequently among those with normal mental status, who also receive more medication than the cognitively impaired, but physically unimpaired elderly[36]. By contrast, in the cross-sectional study by Doraiswamy et al.[37], the prevalence of co-

morbid medical illness in 679 Alzheimer's patients with moderate and advanced dementia (mean MMSE 11.8) was high (61%), and they conclude that co-morbidity and cognition in Alzheimer patients are strongly associated. The set of six simple items identified in this study can only be developed to a diagnostic tool after cross validation on a more realistic sample, i.e. the elderly living at home, and a follow-up study of reliability and sensitivity to change.

Acknowledgement

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Resource Utilization in Dementia: "RUD Lite©"

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Abstract

The Resource Utilization in Dementia (RUD) instrument was developed for clinical trials to capture demented patients´ use of resources, which in a further step could be calculated into costs. RUD has been used in several studies and proven to be a powerful and comprehensive instrument. However, in large trials with several kinds of scales, RUD may be too extensive for practical reasons. Therefore, RUD Lite© has been developed. It covers resource use corresponding to 95% in trials where the complete RUD has been used. Without losing strength, RUD Lite© can therefore be regarded as a good alternative in trials where the complete assessment battery is large.

Introduction

Alzheimer's Disease and other dementias are devastating disorders that influence the life situation for the patients and their families for several years, often decades. These disorders also have an enormous impact on resource utilization and costs. There is also a situation in many countries that is characterized by an economical crisis and a great need for priorities in the health care and social security sectors. Demographic changes also show that the number of demented persons will increase considerably during the forthcoming years [1]. This scenario that puts pressure on all parts of the care system for demented persons also highlights the need for health economical studies as a support for decisions and priorities.

In a complete health economical analysis both costs and outcome are measured. The costing process consist of two phases; in the first phase the use of resources is quantified and in the second phase, costs are calculated by the multiplication of the resource use by a per item/per diem cost. Today there are discussions about what outcomes that are clinically relevant[2].

Any health economical analysis must define its perspective. A societal perspective is recommended in most cases[3]. The most obvious consequence regarding dementia care is that informal care must be given a cost, a process that includes several complex issues[4 5]. The amount of informal care has been approached with different methods using various instruments [6-8].

So far, economical models have been used rather frequently, particularly to catch the long-term effects of treatment of dementia. Even if models are necessary, since it is difficult to accomplish long-term clinical trials [9], the use of models has been criticized as they do not rely on empirical data [2]. Prospectively collected resource utilization data in randomized controlled studies are essential from that perspective. It is necessary to use instruments that make it possible the comprehensively collect and quantify the use of resources in dementia care.

The Resource Utilization in Dementia Instrument (RUD)

The Resource Utlilization in Dementia (RUD) instrument was developed as a comprehensive tool to assess the amount of resource use among demented patients [8]. It has been used in pharmacoeconomical studies [10 11] and it has proven to be a useful tool in the evaluation of dementia care [12 13].

RUD assesses both formal and informal resource use (table 1) of patients and the primary caregiver, making it possible to calculate costs from a societal perspective. It consists of two parts with a similar content, but the wording is adapted to a baseline situation (part A) or follow-up situation(s) (part B). RUD can be administrated as an interview with the primary caregiver or other persons with knowledge about the patient's situation. Regarding the caregiver time and social services the primary caregiver is the

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best informant in most cases, while information about hospital care and home nursing care can be collected from staff at clinics etc. If register data is available and acceptable to use for ethical reasons (regarding e.g. days of hospital care) such sources can also be used.

In addition, lists of use of medications are added to RUD. Lists concerning the study patient's drug use are in most cases already included in the CRFs (Case Report Forms). Caregivers´ drug use is, however, included in RUD.

"RUD Lite©"

RUD is a comprehensive instrument and it may be difficult to collect all data. This may be a problem in large RCTs where RUD is just one of several instruments. An RCT of a drug for the treatment of e.g. Alzheimer´s Disease may consist of several scales assessing severity of dementia/staging, cognition, personal ADL (activities of daily living), Instrumental ADL, Quality of life (of patients and/or caregivers), BPSD (behavioural and psychiatric symptoms in dementia), physical status and medical history, caregiver burden, drug use, side effects/adverse events, and resource utilization. Such comprehensive tools provide a lot of valuable information but may also be stressing for patients, caregivers, and staff. From that perspective it is of great value to use instruments that are simplified without losing too much validity and reliability as well as clinical relevance. Based on experiences from trials with RUD, we have therefore developed a new and shorter version of the instrument: RUD Lite©. Based on the results from two prospective RCTs of drug treatment in dementia where RUD was used [10 11], it is obvious that the two major cost drivers are costs of long-term institutional care and the societal costs of unpaid informal care. These two components as well as other components of resource utilization are included in RUD Lite©. As compared with the complete RUD, work status of both patients and caregivers are excluded as well as drug use (except from costs of studying drugs in drug trials) and outpatient visits. These components demand a lot of work to collect, but they constitute a very low proportion of the total costs. For caregivers, only the assessment of caregiver time is maintained as compared with the complete RUD. With this new version of RUD, the RUD Lite©, 95% of the costs are still captured as compared with the complete RUD. The time frame for informal care is 1 month in RUD Lite©, irrespective of whether it is a baseline or a follow-up assessment.

However, in studies where there is a specific research question based on cost-effectiveness, there should only be costing and one specific outcome (except from basic patient data and, if not collected earlier, safety data). For such studies, the complete RUD is recommended.

Table 1. Components of the resource utilization battery in RUD[8].

Patient	Caregiver
Accommodation/ long-term care	Informal care time (for patient)
Work status	Work status
Respite care	Respite care
Hospital care	Hospital care
Outpatient visits	Outpatient visits
Social services	Social services
Home nursing care	Home nursing care
Day care	Day care
Drug use (study medication)	Drug use (study medication)

Table 2. Components of RUD Lite©

Patient
Accommodation/long-term care
Respite care
Hospital care
Outpatient visits
Social services
Home nursing care
Day care
Drug use (study medication)
Caregiver
Informal care time (for patient)

Appendix 1

THE RESOURCE UTILIZATION IN DEMENTIA (RUD LITE©) GUIDELINES/MANUAL

A. Wimo, B. Winblad

1. BASELINE QUESTIONNAIRES

1.1 Caregiver

1.1.1 Description of primary caregiver
1.1.2 Caregiver time

1.2 Patient

1.2.1 Accommodation
1.2.2 Health care resource utilization

2. FOLLOW-UP QUESTIONNAIRES

2.1 Caregiver

2.1.1 Caregiver time

2.2 Patient

2.2.1 Accommodation
2.2.2 Health care resource utilization

GUIDELINES FOR THE RUD LITE©

SECTION 1: BASELINE

1.1.1.4 Staff are not accepted as informal caregivers (but are, of course, are accepted to care for the patient and as informants). If the first caregiver for some reason during the study period (disease, death, moving etc) no longer fulfils the criteria, a new caregiver can be included according to the defined criteria. The date and the type of new caregiver must be noted.

1.1.2 Caregiver time

The first question refers to how much time that is spent on care during the typical care episode while the b-question refers to the number of days that support was given during the period. Example: if a daughter makes 16 visits during the period (here one month) to her demented mother and supports her in ADL, and the daughter spends 2 hours there at every visit, then the first answer is ”2” and the second is ”16”. If part of the total time is Instrumental ADL and supervision activities, then the total time should be divided in its parts (ADL, IADL, and supervision). The number of days/month for the activities may also vary (example: there may be support in ADL on 4 occasions/week, supervision every day but Instrumental ADL-support only once a week. The time frame is one month, since some caregiver activities do not occur every day or every week. One decimal is allowed for half hours (e.g. 03.5 hours).

1.1.2.1 This question covers personal ADL (Activities of Daily Life) such as toilet visits, eating, dressing, grooming, walking, and bathing. If two persons support the patient during eg 1 hour, then multiply it by 2, resulting in a figure of 2 hours.

1.1.2.2 This question covers Instrumental ADL, such as shopping, food preparation, housekeeping, laundry, transportation, taking medication, and managing financial matters. Such activities are often done for more than one person. Example: a spouse prepares food during 3 hours for two persons. If so, divide this figure by two, resulting in a figure of 1.5 hours. If support in shopping takes place once a week for 2 hours each time, then the answer is 2 hours on the 2a question and 4 on the 2b question.

1.1.2.3 Supervision (or surveillance) is related to the risk for dangerous events, such as risks of fire, walking into a road alone, walking outside without sufficient clothes during cold weather etc. Help questions: can the patient be left alone for part of the day? If so, for how many hours? Can the patient be left alone at night?

Responsibilities: All kinds of caregiving of the patient; that is not just because of the patient's dementia.

1.1.2.4 Caregivers sick leave <= 1 month (1 month=30 days) are regarded as working for pay (option 1) while on sick leave > 1 month = option 2).

1.1.2.5 Early retirement: pension insurance, part pension, contract pension that is not related to disease.

Own health problems: Early pension due to disease, sick leave > 1 month.

To care for subject: includes both if the caregiver has stopped work and is paid for the care or is not paid for it.

1.2.1 Patient Living Accommodation

Care organisation and types of accommodation for the elderly vary between countries and the following "definitions" are supposed to serve as a guide for the classification of the accommodation. Even if there are grey areas, the accommodation of the subject should be classified.

Own home

"Usual living", is a flat (apartment) or a house or similar which was not originally designed for care or social support. However, in an own home, some adjustments for care may be done due to the needs of a patient. If the subject lives in the same house as for instance his/her son, and the house is owned by the son, the living of the subject is classified as "own home".

Intermediate forms of accommodation (not dementia-specific)

Service house: A permanent habitation where the residents live in their own apartment in a particular building. The apartment fulfils quality criteria for private living in physical terms. Home service is available from staff when needed. Day Center activities (see below) are mostly available in the building. Staff density is lower than in a home for the aged.

Home for the aged/old people's home: A permanent habitation where the residents live in their own room in a particular building where home service is regularly available from staff in the home. Few or no medical-technical equipments are available. There is usually staff there also during the night, even if the number is limited.

Dementia-specific residential accommodation

Group living (Group homes, group dwellings, collective living, and similar): A permanent habitation for 4-10 demented persons where the residents live in their own room or a small apartment and where facilities/rooms for mutual activities such as meals and other sorts of social life are available. Staff are trained in dementia care and are available around the clock. Staff provide supervision, community, and care for the demented persons according to specified goals for dementia care. The specified goals may vary but are mostly based on a nursing theory of dementia care (such as managing behavioural disturbances, apraxia, agnosia, memory impairment). Staff may be nurses, licensed practical nurses (assistant nurses), home aids, or special dementia carers.

Long-term institutional care

A care unit with care wards where nursing care is provided around the clock to those who are chronically ill and unable to perform daily activities. Staff are nurses, licensed practical nurses (assistant nurses), and orderlies. Staff are available around the clock. Medical-technical equipments are available. This care option is often referred to as a "nursing home" or similar.

Nursing home care may sometimes not be allowed at baseline in some studies (depending on inclusion/exclusion criteria).

1.2.2 Patient Health Care Resource Utilization

1.2.2.5

District nurse option: all kinds of visits by registered nurses even if the entitlement is different.

Home health aid/orderly option: also register visits by licensed practical nurses/assistant nurses here.

Food delivery (meals on wheels): register just publicly paid delivered meals.

Day care

Day care units are usually defined as "day care" (the names may vary) in administrative terms. All the following types of day care should be noted as "day care".

Day care for demented: Staff provide supervision, community, and care to demented persons according to specified goals for dementia care at a particular place, mostly during 5-7 hours/day. The specified goals may vary but are mostly based on a nursing theory of dementia care (such as managing behavioural disturbances, apraxia, agnosia, memory impairment) and the staff are trained in dementia care. Staff may be nurses, licensed practical nurses (assistant nurses), home aids, or special dementia carers. The patients mostly come from their own accommodation but may also come from service houses and homes for the aged. Mostly 2-3 staff serve 7-12 demented persons.

Day Center. Elderly persons, not necessarily ill, receive activation, occupational therapy, and social support during part of the day at a particular place. Staff are often occupational therapists with assistants. If no registered staff (such as occupational therapists or physiotherapists) are available, do not register. The patients mostly come from own living, service houses, or homes for the aged.

Somatic Day Care. Patients receive care at a particular place, mostly during 5-7 hours/day according to specified goals, focused mainly on rehabilitation and physical training. Staff may be nurses, physiotherapists, occupational therapists, or psychologists. Assistants to these staff categories are often available. Somatic day care may be located at hospitals ("day hospitals") or as independent units. The patients mostly come from own living but may also come from service houses, homes for the aged, or nursing homes.

Transportation: just register publicly paid transport, not transport provided by spouses - children etc.

Other: If volunteer organisations work is registered here, note that it is this type of support.

SECTION 2: FOLLOW UP VISIT

It is recommended that RUD Lite© is administrated at least every 3rd month (or in multi-year trials at least every 6 month).

2.2.1 Patient Living Accommodation.

2.2.1.4 The first four options deals with deterioration while options 5-8 describe improvement. There is a risk for overlap between these alternatives, but try to choose the most relevant and just one alternative.

2.2.1.5 This question deals with short time accommodation/respite care (not emergency hospital care). If there are difficulties differing between for instance nursing home care and short time care at nursing homes organized by geriatric clinics, just register at one place, preferably at **2.2.2.4** (see next section) but make a note in the margin about these problems.

2.2.2.5. If it is not possible to catch information for the whole period since the last visit, then the last month approach can be used, but make a note of it!

Appendix 2

THE RESOURCE UTILIZATION IN DEMENTIA (RUD LITE©) QUESTIONNAIRE/CASE REPORT FORM

A. Wimo, B. Winblad

1. BASELINE QUESTIONNAIRES

1.1 Caregiver

1.1.1 Description of primary caregiver
1.1.2 Caregiver time

1.2 Patient

1.2.1 Accommodation
1.2.2 Health care resource utilization

2. FOLLOW-UP QUESTIONNAIRES

2.1 Caregiver

2.1.1 Caregiver time

2.2 Patient

2.2.1 Accommodation
2.2.2 Health care resource utilization

THE RESOURCE UTILIZATION IN DEMENTIA (RUD Lite©) QUESTIONNAIRE

1. BASELINE QUESTIONNAIRE

1.1 CAREGIVER

1.1.1 Description of Primary Caregiver

1. Date of birth ___ / ___ / ___
(dd/mm/yy)

2. Age _____ years

3. Sex:
1. Male ☐
2. Female ☐

4. Relationship to patient

1. Husband ☐
2. Wife ☐
3. Child ☐
4. Friend ☐
5. Other ☐

(Staff not allowed)

5. Marital status

1. Married/Cohabiting ☐
2. Never married ☐
3. Divorced/Separated ☐
4. Widowed ☐

6. Number of children currently living with you

_____ child(ren)

7. Do you live with the patient?

1. Yes ☐
2. No ☐

1.1.2 Caregiver Time

1a). On a typical care day during the last month (when you provided support to the patient), how much time per day did you assist the patient with personal tasks such as toilet visits, eating, dressing, grooming, walking, and bathing (also called personal ADL (ADL=activities in daily life))?

Total time ☐☐.☐ hours per day

1b). During the last month, how many days did you spend providing these services to the patient?

_____ days

2a). On a typical care day during the last month (when you provided support to the patient), how much time per day did you assist the patient with tasks such as shopping, food preparation, housekeeping, laundry, transportation, taking medication, and managing financial matters (also called instrumental ADL (activities in daily life))?

Total time ☐☐.☐ hours per day

2b). During the last month, how many days did you spend providing these services to the patient?

_____ days

3a). On a typical care day during the last month (when you provided support to the patient), how much time per day did you spend supervising the patient (i.e. preventing dangerous events)?

Total time ☐☐.☐ hours per day

3b). During the last month, how many days did you spend providing these services to the patient?

_____ days

4. Do you currently work for pay?

1. Yes ☐ If **yes**, go to next section
2. No ☐ If **no**, answer question 5

5. Why did you stop working?

1. Never worked ☐
2. Reached retirement age ☐
3. Early retirement (not disease-related) ☐
4. Was made unemployed ☐
5. Own health problems ☐
6. To care for patient ☐
7. Other ☐

1.2 PATIENT

1.2.1 Patient Living Accommodation

1a). Please specify the patient's current living accommodation.

1. Own home ☐
2. Intermediate forms of accommodation (not dementia-specific) ☐
3. Dementia-specific residential accommodation ☐
4. Long-term institutional care ☐
5. Other: ☐

1b). If the patient lives in her/his own home, who does the patient live together with?

1. Alone ☐
2. Spouse ☐
3. Other ☐
4. Not applicable ☐

1.2.2 Patient Health Care Resource Utilization

1. During the last month, was the patient admitted in a hospital (for more than 24 hours)?

1. Yes ☐ If **yes**, go to question 2
2. No ☐ If **no**, go to question 5

2. During the last month, how many times was the patient hospitalized?

☐☐ times

3. For each hospitalization (during the last month), please provide the diagnosis or reason for hospitalization.

Hospitalization number	Major diagnosis or reason for hospitalization	DRG code (if possible)
1		
2		
3		
4		

4. Please specify the **total** number of nights spent in each type of ward (for all hospitalizations during the last month).

Ward	Number of nights
Geriatric	
Psychiatric	
Internal medicine	
Surgery	
Other (please specify)	

5. For each service listed below, please specify the number of times the service was received during the last month, and the average number of hours per visit.

Service	Number of visits during last month	Number of hours per visit
District nurse		
Home aid/orderly		
Food delivery		
Day care		
Transportation (publicly paid)		
Other (e.g. please specify)		

2. FOLLOW-UP QUESTIONNAIRES

2.1 CAREGIVER

2.1.1 Caregiver Time

1a). On a typical care day during the last month (when you provided support to the patient), how much time per day did you assist the patient with personal tasks such as toilet visits, eating, dressing, grooming, walking, and bathing (also called personal ADL (ADL=activities in daily life))?

Total time ☐☐.☐ hours per day

1b). During the last month, how many days did you spend providing these services to the patient?

_____ days

2a). On a typical care day during the last month (when you provided support to the patient), how much time per day did you assist the patient with tasks such as shopping, food preparation, housekeeping, laundry, transportation, taking medication, and managing financial matters (also called instrumental ADL (activities in daily life))?

Total time ☐☐.☐ hours per day

2b). During the last month, how many days did you spend providing these services to the patient?

_____ days

3a). On a typical care day during the last month (when you provided support to the patient), how much time per day did you spend supervising the patient (i.e. preventing dangerous events)?

Total time ☐☐.☐ hours per day

3b). During the last month, how many days did you spend providing these services to the patient?

_____ days

2.2.1 Patient Living Accommodation

1. Long term care and similar: Since the last visit in the study, did the patient **permanently** change his/her living accommodation (i.e. moved to another location and is currently living in this new location)?

1. Yes ☐ If **yes**, answer questions 2 to 4
2. No ☐ If **no**, answer question 5

2. Please specify the patient's current living accommodation.

1. Own home ☐
2. Intermediate forms of accommodation (not dementia-specific) ☐
3. Dementia-specific residential accommodation ☐
4. Long-term institutional care ☐
5. Other ☐

3. Please specify the date at which the change occurred.

___ / ___ / ___
(dd/mm/yy)

4. Please specify the principal reason for this change in living accommodation.

1. Worsening of patient's cognitive function ☐
2. Worsening of patient's ability to perform daily tasks (e.g., feeding, dressing, housekeeping, etc.) ☐
3. Increase in patient's behavioral problems ☐
4. Poor caregiver health ☐
5. Improvement of patient's cognitive function ☐

6. Improvement of patient's ability to perform daily tasks (e.g., feeding, dressing, housekeeping, etc.) ☐
7. Improvement of patient's behavior ☐
8. Improved caregiver health ☐
9. Other ☐

5a). Respite care and similar: Since the last visit in the study, did the patient temporarily change living accommodation (i.e. moved to a new location for more than 24 hours and then back to the original location)?

1. Yes ☐
2. No ☐

5b). If **yes**, please specify where the subject temporarily moved.

1. Own home ☐
2. Intermediate forms of accommodation (not dementia-specific) ☐
3. Dementia-specific residential accommodation ☐
4. Long-term institutional care ☐
5. Other ☐

5c). Please specify the number of nights spent in this temporary living accommodation.

	Number of nights
1. Own home	___
2. Intermediate forms of accommodation (not dementia-specific)	___
3. Dementia-specific residential accommodation	___
4. Long-term institutional care	___
5. Other	___

2.2.2 Patient Health Care Resource Utilization

1. Since the last visit in the study, was the patient admitted to a hospital (for more than 24 hours)?

1. Yes ☐ If **yes**, go to question 2
2. No ☐ If **no**, go to question 5

2. Since the last visit in the study, how many times was the patient hospitalized?

☐☐ times

3. For each hospitalization (since the last visit in the study), please provide the diagnosis or reason for hospitalization.

Hospitalization number	Major diagnosis or reason for hospitalization	DRG code (if possible)
1		
2		
3		
4		

4. Please specify the **total** number of nights spent in each type of ward (for all hospitalizations since the last visit in the study).

Ward	Number of nights
Geriatric	
Psychiatric	
Internal medicine	
Surgery	
Other (please specify)	

5. For each service listed below, please specify the number of times the service was received since the last visit in the study and the average number of hours per visit.

Service	Number of visits	Number of hours per visit
District nurse		
Home aid/orderly		
Food delivery		
Day care		
Transportation (publicly paid)		
Other (e.g. please specify)		

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Spontaneous Intracerebellar Hemorrhage-Case Report

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Spontaneous hemorrhage in the cerebellum represents 6-18% of the brain spontaneous parenchymal hemorrhages and 70% of the cases occurred in elderly patients, most commonly due to systemic arterial hypertension.
The surgical treatment is indicated for patients presenting progressive neurological deterioration, with a hemorrhage image bigger than 3 cm, obstructing the cerebrospinal fluid drainage.

Case report

A 68 years old patient, female, was presented in emergency room with altered clinical condition, systemic arterial hypertension (240/120 mmHg) and GCS=6. The onset was sudden with headache, nausea and vomiting followed by neurological deterioration and coma.

CT scanning revealed left cerebellar hemorrhage with secondary hydrocephalus due to IV intraventricular hemorrhage (fig. 1).

Surgical intervention, considered the most appropriate, consisted of external ventricular drainage and then surgical treatment of the left cerebellar hemorrhage. CT postoperative images are showed in fig. 2. Postoperative evolution was good with complete neurological recovery after one month.

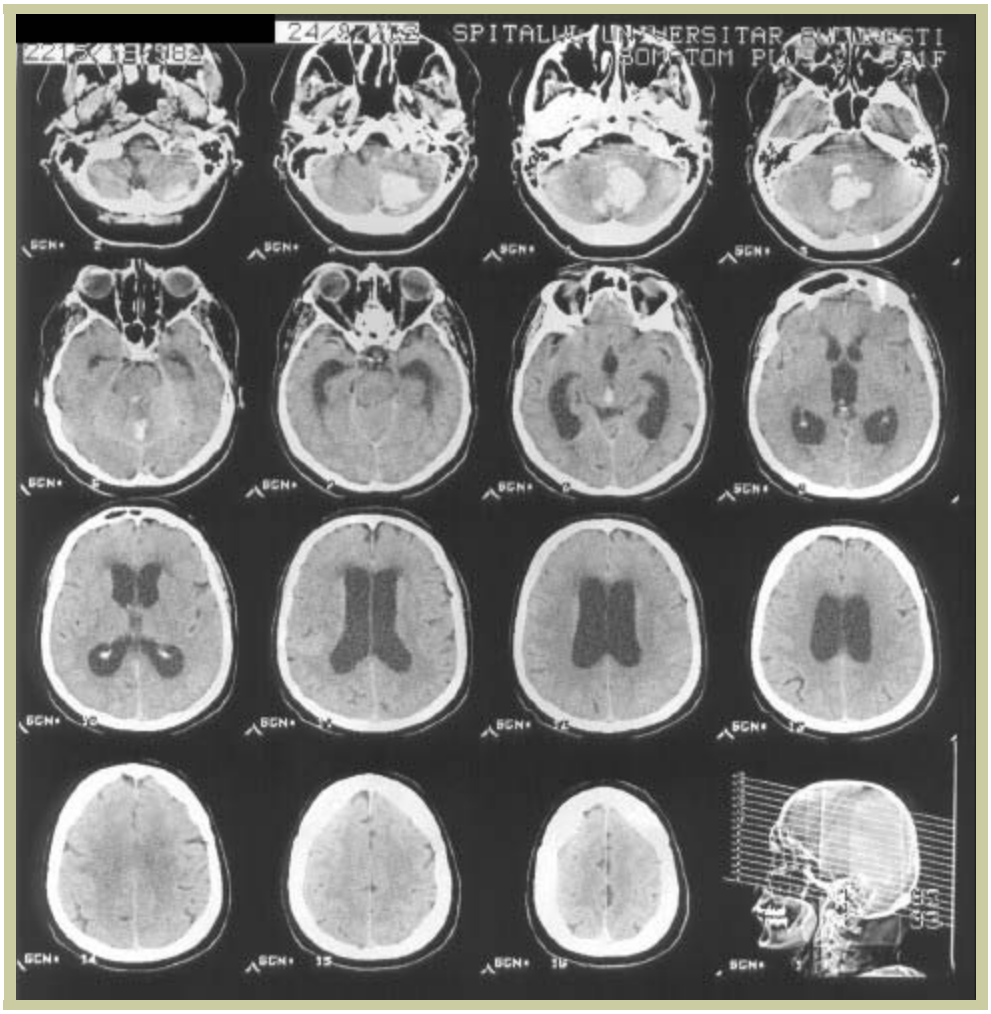

Figure 1. CT scanning images of intracerebellar hemorrhage before surgical intervention.

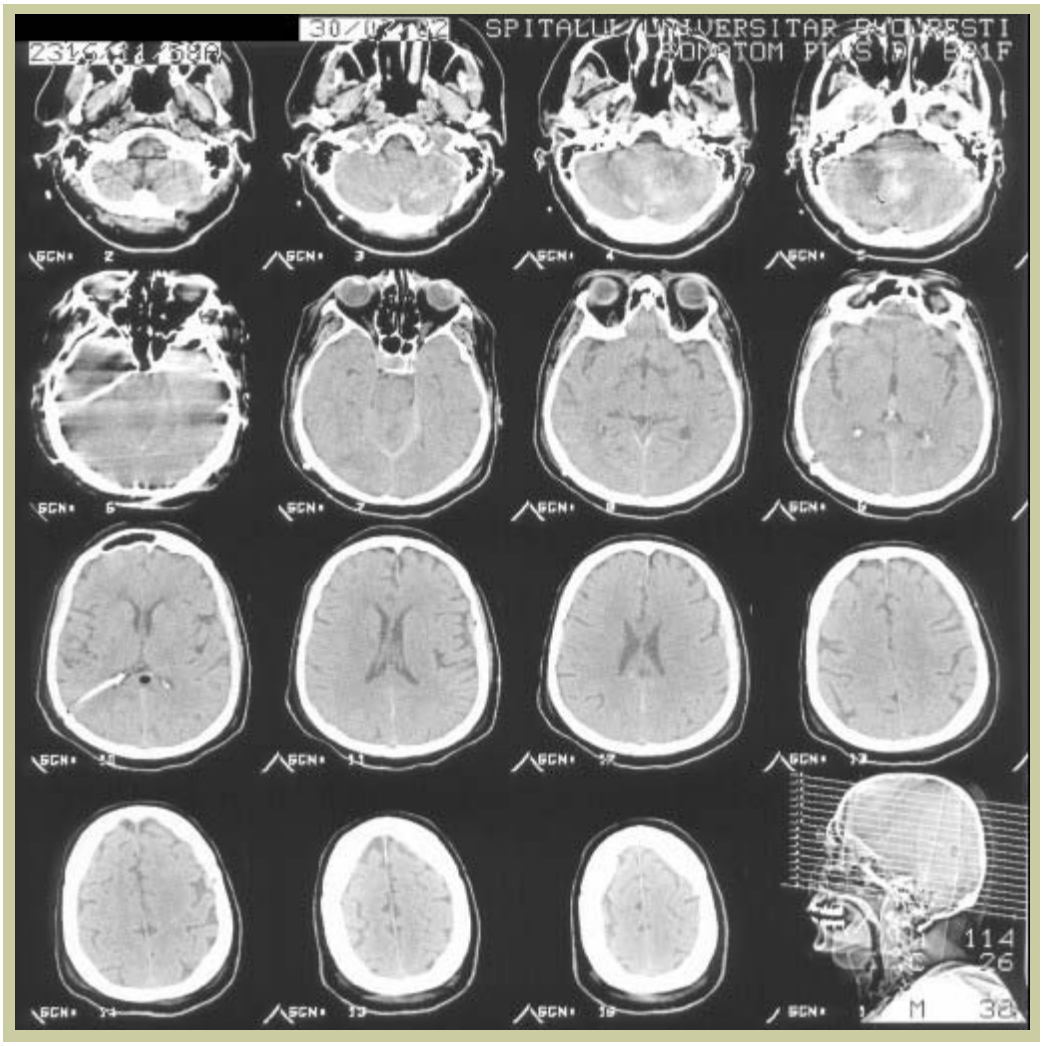

Figure 2. CT scanning images of intracerebellar hemorrhage after surgical intervention.

Discussion

In case of patient with intracerebellar hemorrhage and secondary hydrocephalus, the surgical intervention in two steps is the best choice: first, external ventricular drainage and second surgical procedure of the hemorrhage in the posterior cerebral fossa. The external ventricular drainage carried out in emergency allows the improvement of patient's condition and reduces the vegetative accompanying phenomena, temporizing the surgery hemorrhage treatment.

Medical Examination of the Elderly Patient

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Medical examination is the most important part of the Geriatrician's task. On this depends the proper medical management of the patient and often, as at all ages, the quality of his future life. The Specialist in Geriatric Medicine must be first of all trained in General Internal Medicine, familiar and well acquainted with the medical examination of the younger patient. What follows will focus on the main differences which apply when examining the older patient.

Medical Examination is geared to the detection of physical ***signs*** of disease. It must be preceded by patient and comprehensive Medical History or Anamnesis taking, to elicit any relevant ***symptoms*** of disease.

Anamnesis

There are many pitfalls when taking the medical history from an ageing patient. This is often made more difficult by loss of short term memory, confusion, apathy, or even fear of admission to hospital. Also deafness and blindness can make it difficult for the patient to collaborate.

Try not to hurry your older patient, give him/her lots of time. Place yourself in a well lit situation in front of him/her so she can see your face and lips clearly. It is surprising how many deaf old people depend on lip reading to understand the spoken word. When you finish taking your history try to corroborate it, if in doubt, with a witness such as a relative or friend, or carer.

Clinical Examination

It is advisable to have a nurse to help you and act as a chaperon, especially with female patients. Give your patients plenty of time to undress and lie comfortably on the couch. Make a record of any aids he/she may be using: spectacles, hearing aids, dentures, walking sticks, walking frames. Make yourself comfortable as well so that your body will be relaxed during the whole examination. Put him and yourself at ease. Always follow the same scheme or routine so it will be less likely that you will overlook any essential part of the clinical examination of your patients. Insists your patients undress properly (Fig. 1 & 2) and only remain in their underwear, but under a blanket so they will not shiver in the cold. Remember that the capacity to elicit clinical signs depends on the interaction between you and your patient: make sure you maximise this by gaining the patient's trust.

Make sure you record the patient's height and weight, and measure the blood pressure at the beginning of your examination. Always check it as well while the patient is standing. Generally proceed from the top of the head to the tip of the toes. You can examine by region or by organ systems. I use a combination of the two.

Sit the patient with his trunk at an angle of 45 degrees on the horizontal. Try to inspect the greatest part of his/her body. Always check all pressure areas if the patient has mobility problems. Look for bruising, the patient may have forgotten to tell you he/she fell. Observe the general status: whether he/she is neglected and unkempt (Fig. 3), or properly groomed. Use all senses of your body; including your nose: this will help you assess incontinence of urine and faeces, or detect necrotic pressure sores. It may also help you to smell diabetic keto-acidosis, liver and renal failure. All are quite common in the elderly.

Head and neck (Fig 4 & 5).

Look for skin malignancies, particularly common in the face (Fig 6 & 7). Examine the scallop carefully (Fig 8). Observe the conjunctival and oral mucosae (Fig 9 & 10). Look inside the mouth as far back into the fauces as possible, with the aid of a spatula. Check the state of dentition and possible gingival disease, which is common in the elderly. Look for the presence of neck scars, thyroid nodules or goitres with the head in extension (Fig 11); palpate the jugular chain lymphnodes. Always observe and measure the jugular venous pressure (Fig 12), and remember not to confuse it with a carotid pulsation: the carotid arteries are often tortuous and very prominent in the elderly. Listen for neck bruits especially in stroke patients.

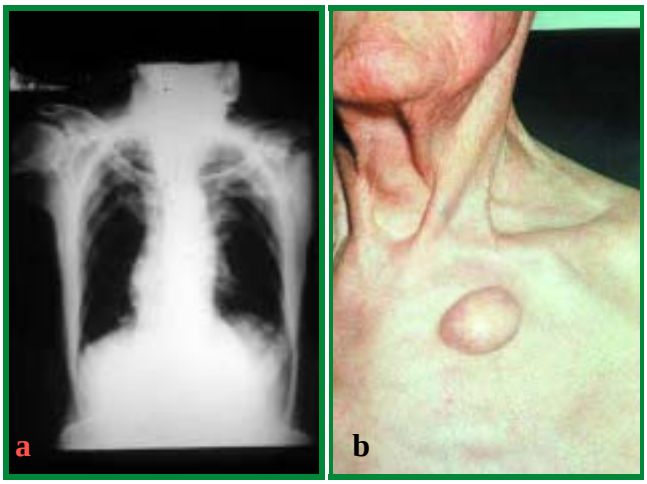


Figure 1

a: Chest X-ray of a 76 years old man, showing a left upper lung field rounded opacity. This was investigated as malignancy, all tests proved negative

b: On re-examining the patient with his chest fully undressed, the cause for the opacity on the chest x-ray was revealed: a long standing lipoma.

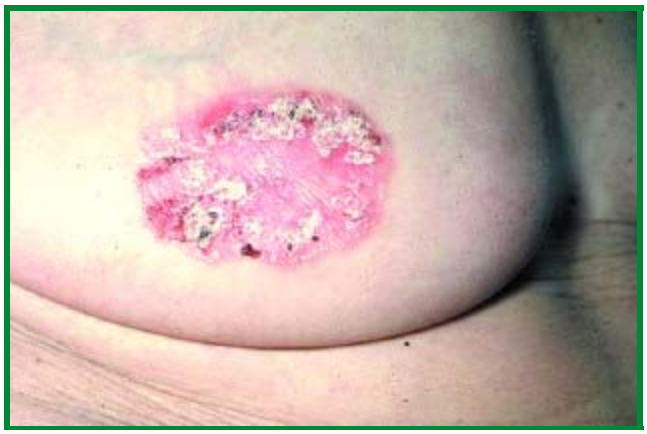


Figure 2.

Paget's disease of nipple in an 82 years old woman who had been reticent to undress fully.

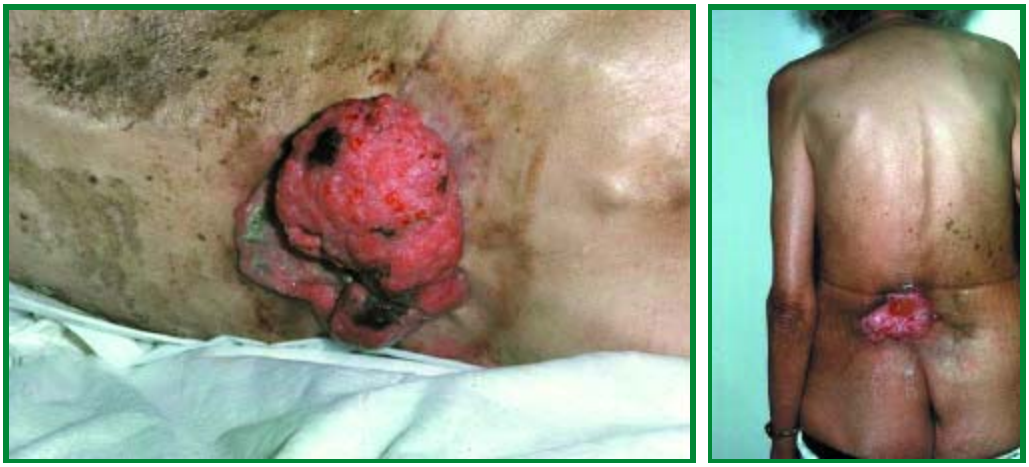


Figure 3.

a: Large (30 x 10 x 5 cm) squamous cell carcinoma of the skin of the lumbar region. 79 years old unkempt woman with a 12 months' history of weight loss and anaemia. Even her husband was unaware of the existence of this lesion;

b: same patient after three month's radiotherapy.

Figure 4.

Gross deformity of skull due to Paget's disease of bone in an 89 years old woman.

Figure 5.

Osteosarcoma complicating Paget's disease of bone in a 78 years old woman.

Figure 6.
Large basal cell carcinoma (rodent ulcer) in an 85 years old woman

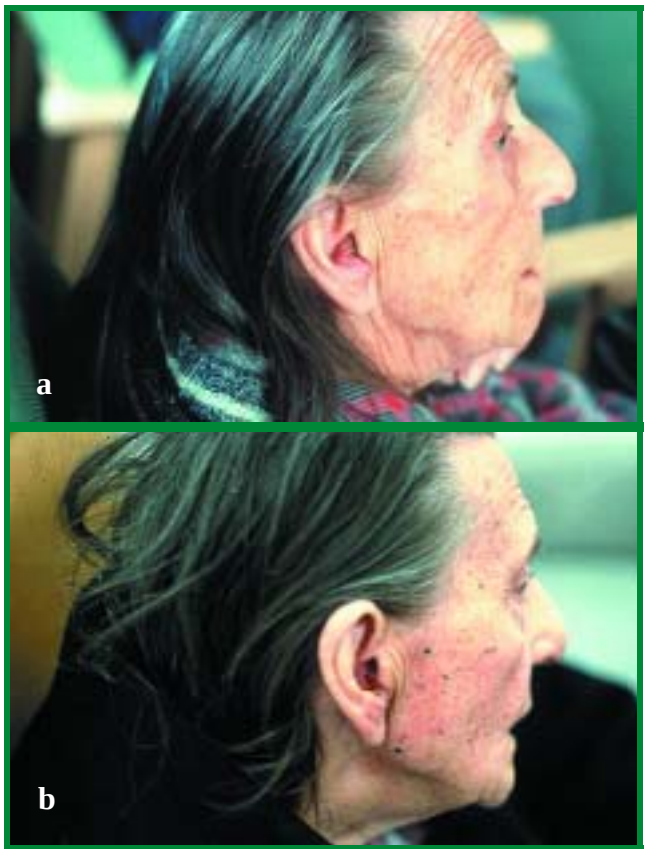


Figure 7.
a: Squamous cell carcinoma in a 90 years old woman with a history of unilateral deafness for one month.
b: Same patient after one month of radiotherapy.

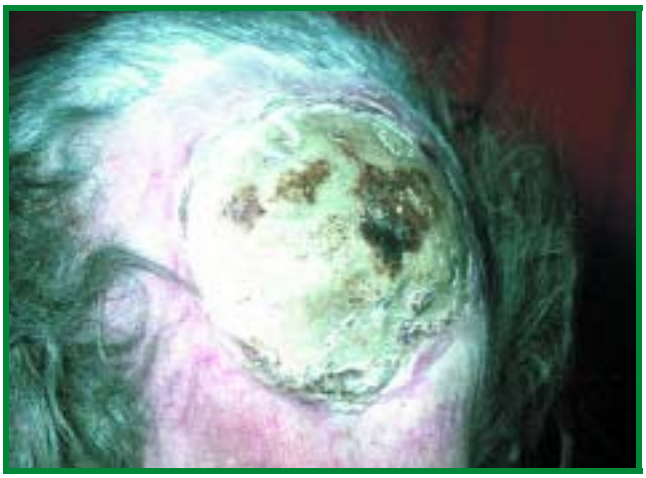


Figure 8.
82 years old woman with radionecrosis of scalp. She had a six months' history of confusion, falls and incontinence. She had undergone radiotherapy for squamous cells carcinoma of scalp, but had failed to attend follow up one year before admission. She was found to have multiple brain abscesses. Bare bone is visible in the centre of this picture.

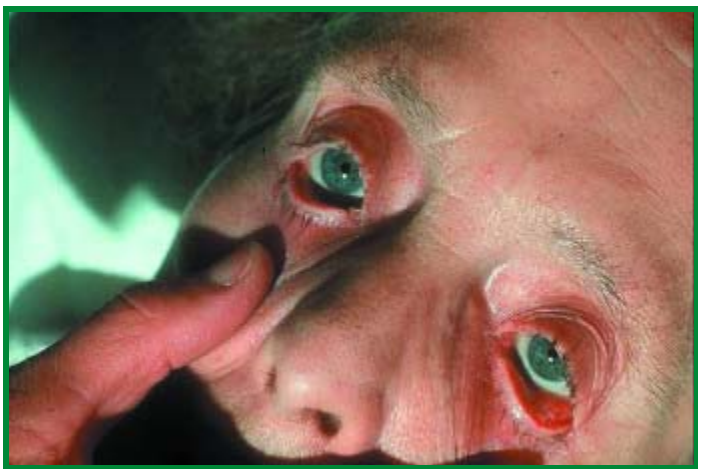


Figure 9.
Sunconjunctival petecchiae in a 76 years old woman with infective endocarditis

Figure 10.
Macroglossia in an 87 years old woman with myxoedema.

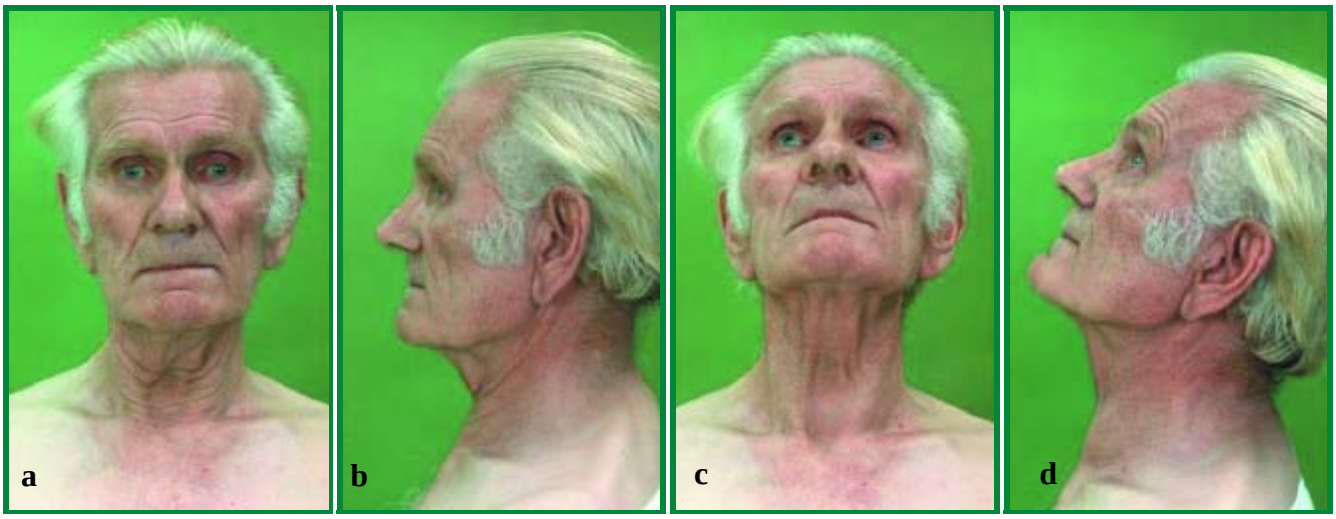


Figure 11.

a: Frontal view of an 82 years old man;
b: lateral view of same patient.
c: same patient with neck in extension reveal a left thyroid nodule, which did not manifest in the previous position;
d: lateral view of same patient showing the now prominent thyroid lump.

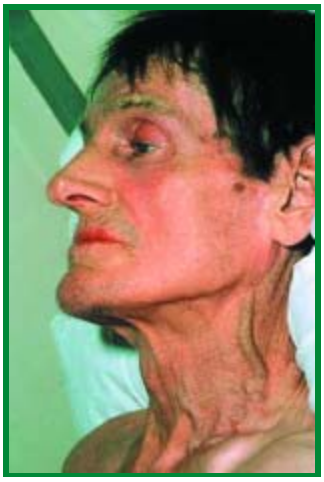


Figure 12.

Distended external jugular vein indicating increased jugular venous pressure in a 73 years old man with biventricular cardiac failure.

Figure 13.

84 years old woman with severe dosarl kyphosis due to senile osteoporosis.

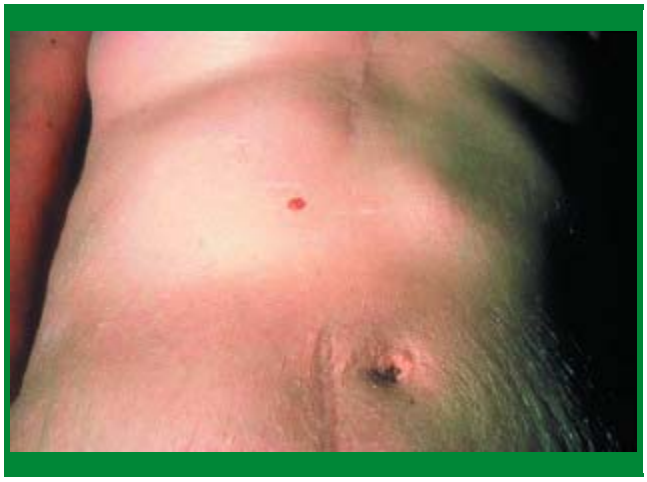


Figure 14.

Obvious liver enlargement in a patient suffering from biventricular cardiac failure. Haepatomegaly is easily detectable if the patient lies flat.

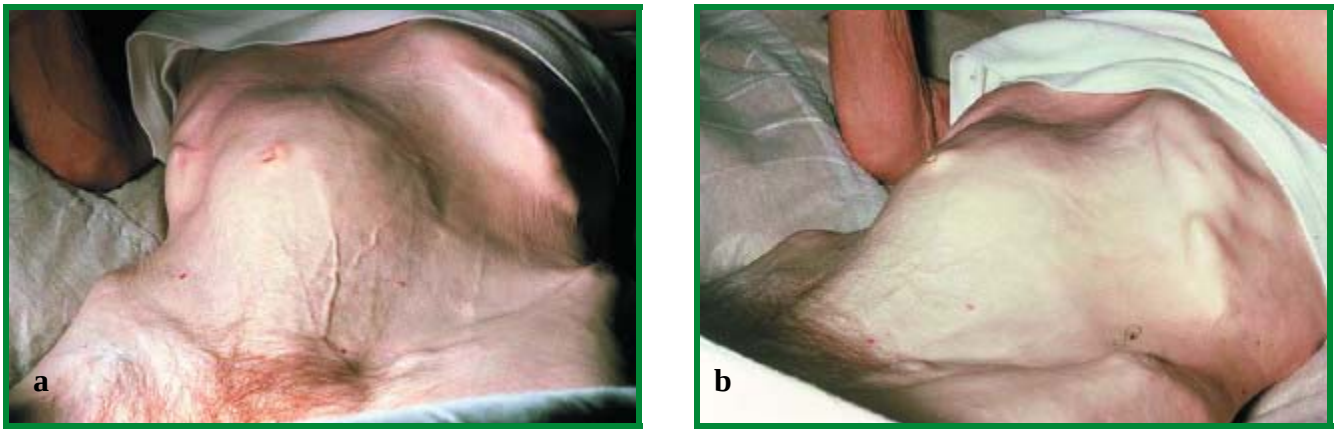


Figure 15. **a:** Large abdominal aortic aneurysm in an 87 years old man; **b:** lateral view of the same patient.

Chest and heart

Measure the respiratory rate: tachypnea is the commonest sign of bronchopneumonia in the elderly. Dorsal kyphosis and pidgeon chest are common in the elderly as a result of osteoporosis (Fig 13). Sit the patient symmetrically and observe whether the two hemithoraces are symmetrically moving during respiration. If asymmetrical you must suspect loss of lung volume on the side which is moving less, that is lung malignancy. When listening to the lungs, remember basal crackles are common when old people lie in bed: they usually disappear after coughing or deep breathing.

Always check the apex beat, measure its rate, and look for gallop rhythms and thrills. Auscultation will reveal a systolic ejection murmur in two thirds of the oversixtyfives, thought to be due to "aortic sclerosis", not stenosis. Remember valvular heart disease is more common in the elderly population nowadays. Cardiac failure is the commonest acute disease seen in the hospitalised elderly patient in the UK.

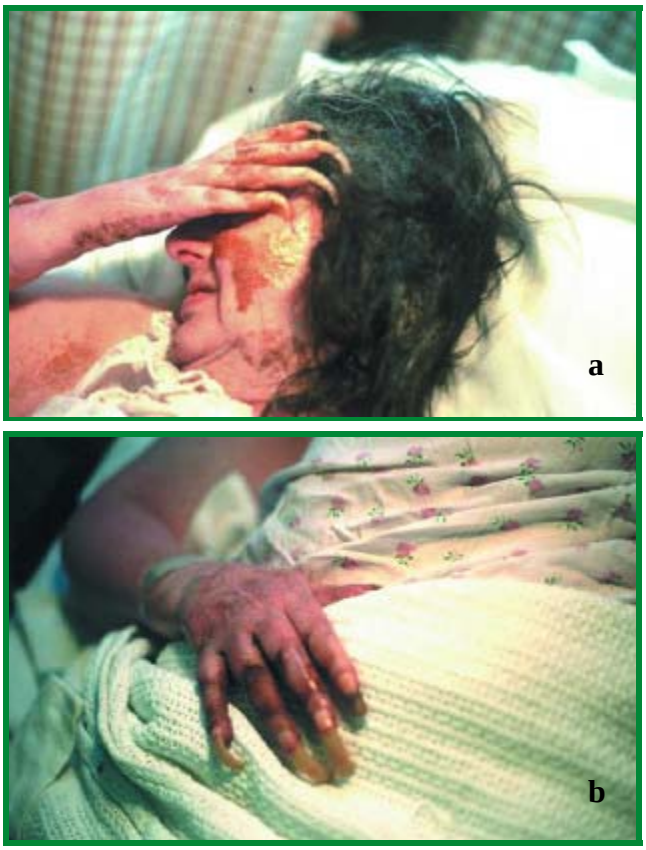


Figure 16.

a: 85 years old woman, gross self-neglect, matted hair and uncut finger nails;
b: detail of grossly neglected hands and nails in the same patient.

Abdomen

Always examine this when the patient is lying flat: this will allow enlarged organs or pathological masses to stand out (Fig 14). Look for surgical scars, herniae, and then proceed to palpation. Look for tenderness, search for masses (Fig 15), and pay particular attention to faecal masses, as constipation is frequent in the elderly when their mobility is impaired. Also for a distended bladder: chronic retention of urine in the elderly is not infrequently painless. You may need to do a rectal and/or vaginal examination. Always make sure you explain this procedure well before you carry it out and you are chaperoned, or this manoeuvre will be, in law, a physical assault on your patient and you may be prosecuted.

Central Nervous System

Start by assessing mood and mentation (Fig 16). Assess the presence of confusion at least with a short Mental Test Score questionnaire. Examine chranial nerves properly: look inside the fundi for cataracts, macular degeneration, glaucoma; test eye pressure by palpation and look for cupping of the disc. Remember that one third of the over 65s are partially sighted and two thirds are deaf or hard of hearing. Look for nistagmus.

Always assess muscle tone and power, and remember that paratonia (an increase in muscle tone in the limbs when they are passively moved) is ubiquitous in the elderly and may interfere with eliciting the deep or tendon reflexes. Use augmentation (Jendrassik manoeuvre) if necessary. Always look for abnormal movement, such as tremor and tardive diskynesia. Myoclonic jerks and flapping tremor occur in acute metabolic encephalopathies, particularly common in the acutely ill elderly patient. Always observe the patient's gait. Test the sensory side of the nervous system, but remember the patient's answers may be not dependable if the patient is confused.

Locomotor system

Look for deformity of the fingers: degenerative arthritis with Heberden's nodes is common (Fig 17), and frequently accompanied by atrophy of the small muscles of the hands. Rheumatoid arthritis nearly always causes specific hand and fingers deformity (Fig 18). Gouty tophi of the fingers are beconing increasingly common, as the numbers of elderly people with chronic heart failure nowadays survive for years on diuretics (Fig 19). Finger clubbing will help you diagnose lung disease (Fig 20). Always look out for nicotine staining of the finger nails. Always observe the length of both legs, remember that shortening and external rotation is the commonest sign of fractured neck of femur (Fig 21), but may also be due to advanced degenerative arthritis of the hip. When looking at the legs, remember to check the status of the toe-nails (Fig 22), and always note the presence or absence of oedema (Fig 22). Always palpate the peripheral pulses.

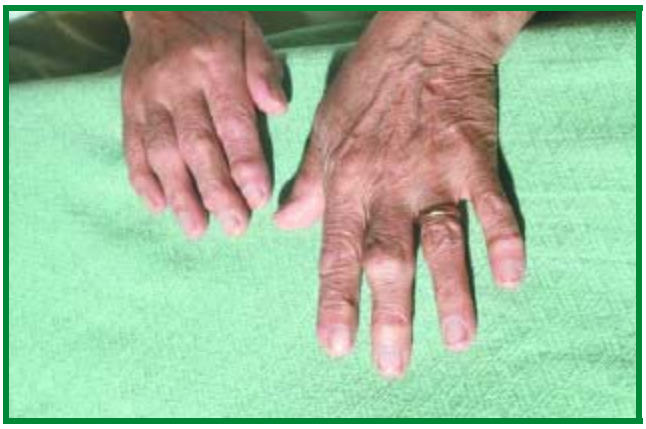


Figure 17.
Bouchard's and Heberden's nodes (proximal inter-phalangeal and distal inter-phalangeal joints osteophytes): osteo-arthritis of the hands.

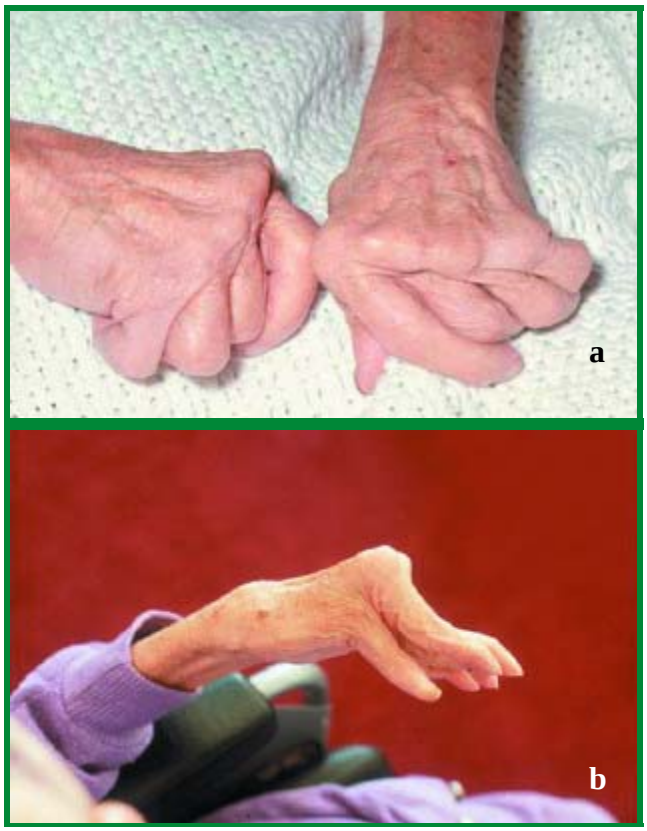


Figure 18.
a: Rheumatoid arthritis of hands in a 83 years old woman.
b: Rheumatoid arthritis of hands with swan neck deformity.

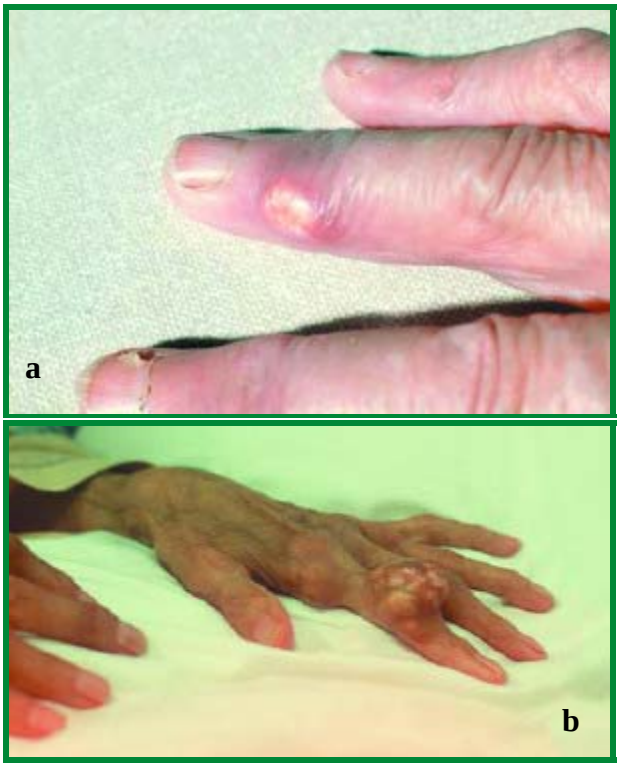


Figure 19.
a: Uric acid crystals deposition (tophus) in an elderly patient on long term frusemide for cardiac failure.
b: Very large tophus in another patient on long term diuretics treatment.

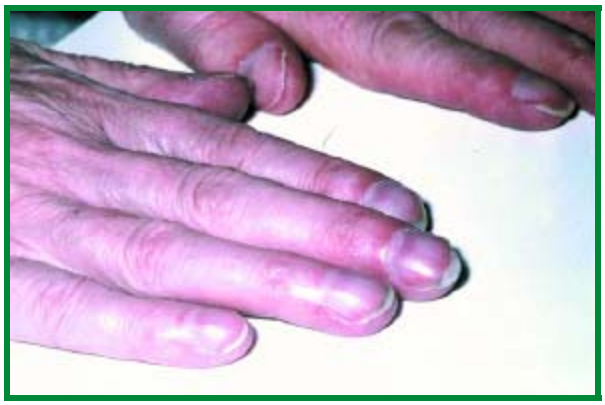


Figure 20. Finger nails clubbing in a 73 years old woman with bird fancier's lung disease.

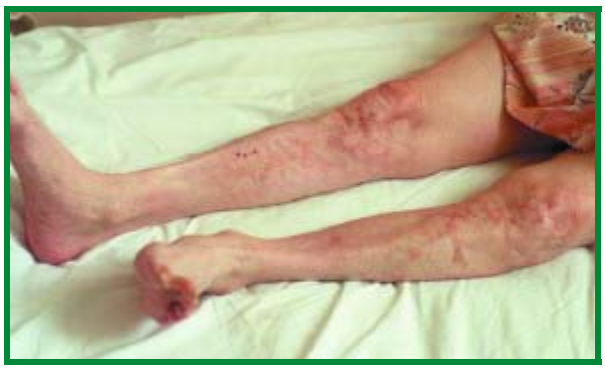
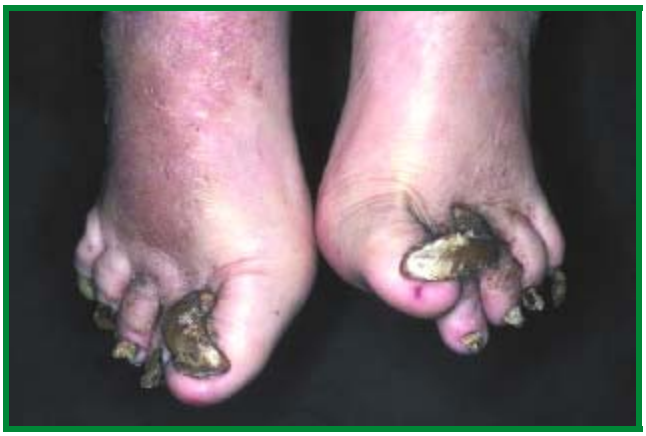
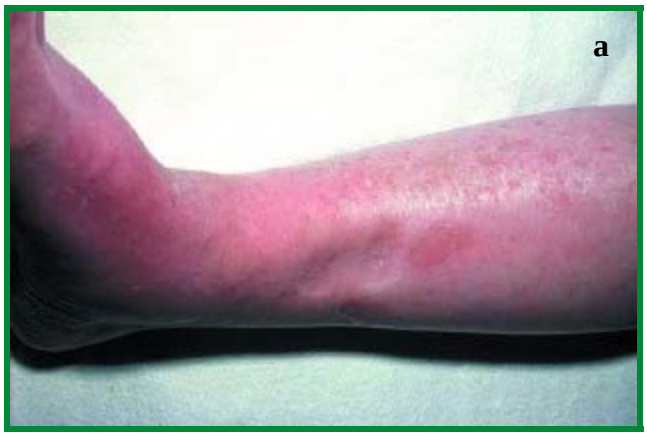


Figure 21. Shortening and external rotation of the left leg in a 92 years old woman with fractured neck of left femur.





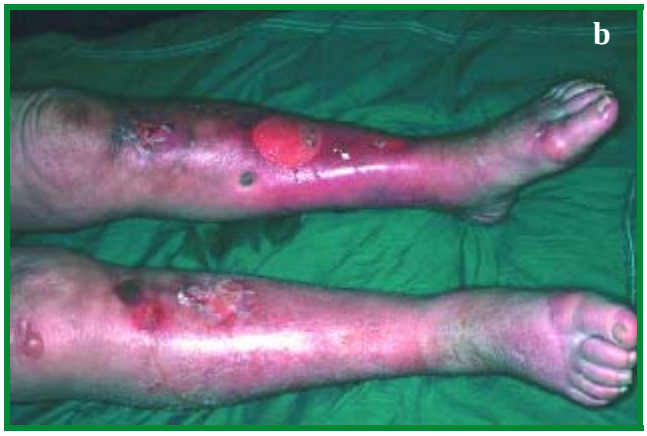


Figure 22.

Onycho-griphosis. This patient did not have his toe-nails cut for two years. This condition can cause gross discomfort and may impair the patient's mobility.

a: Pitting oedema of legs in a patient suffering from cardiac failure.

b: Ruptured blisters due to severe oedema in another patient suffering from cardiac failure.

This brief and practical review of clinical examination of the elderly is entirely based on my clinical experience, and is meant to be only a guideline for you to develop your own technique. Make sure whatever method you use that it is as comprehensive and stereotyped as possible, to make it more difficult for you to omit detecting important signs. Without an adequate examination the patient's management is likely to go astray.

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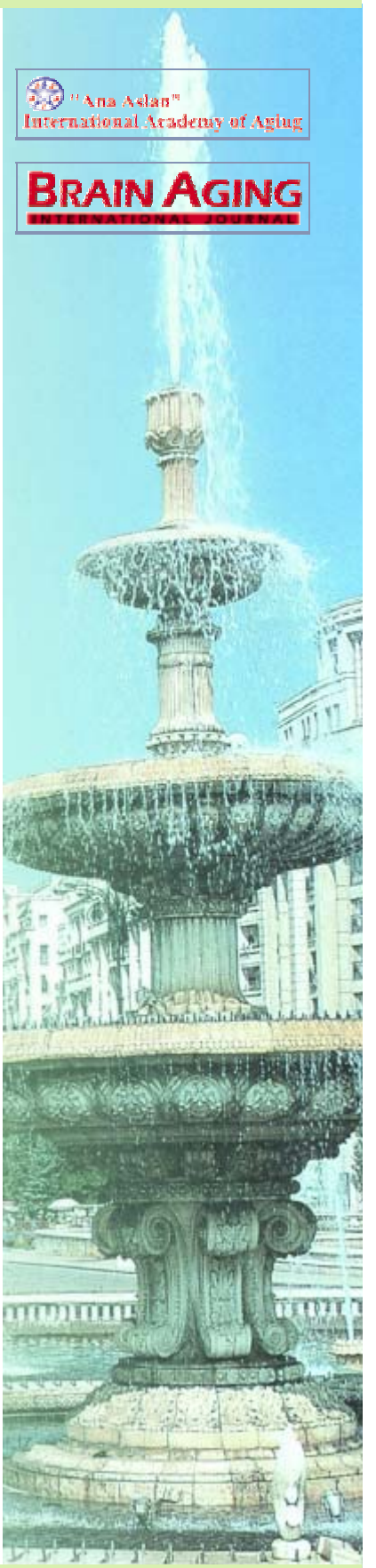

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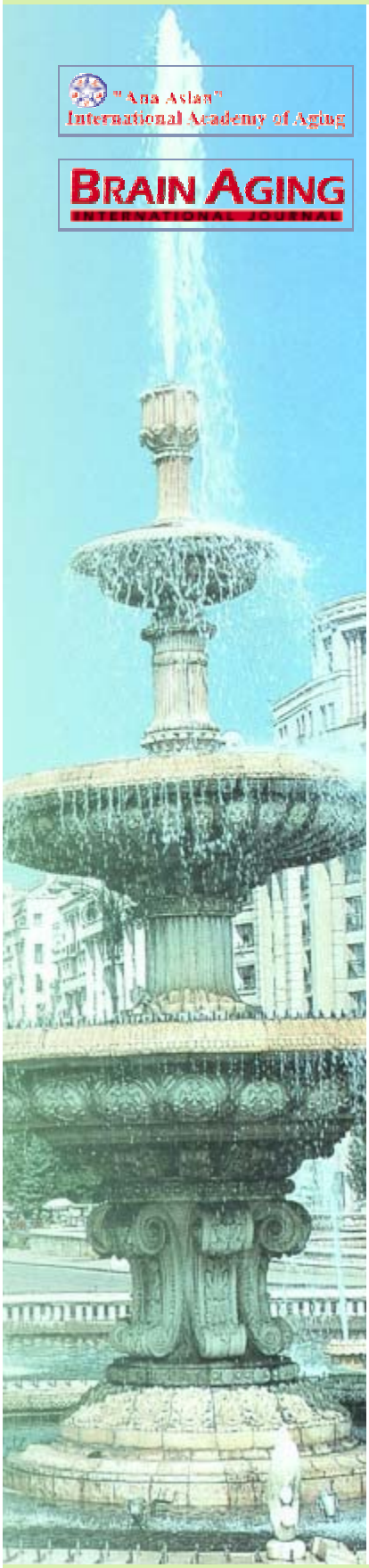

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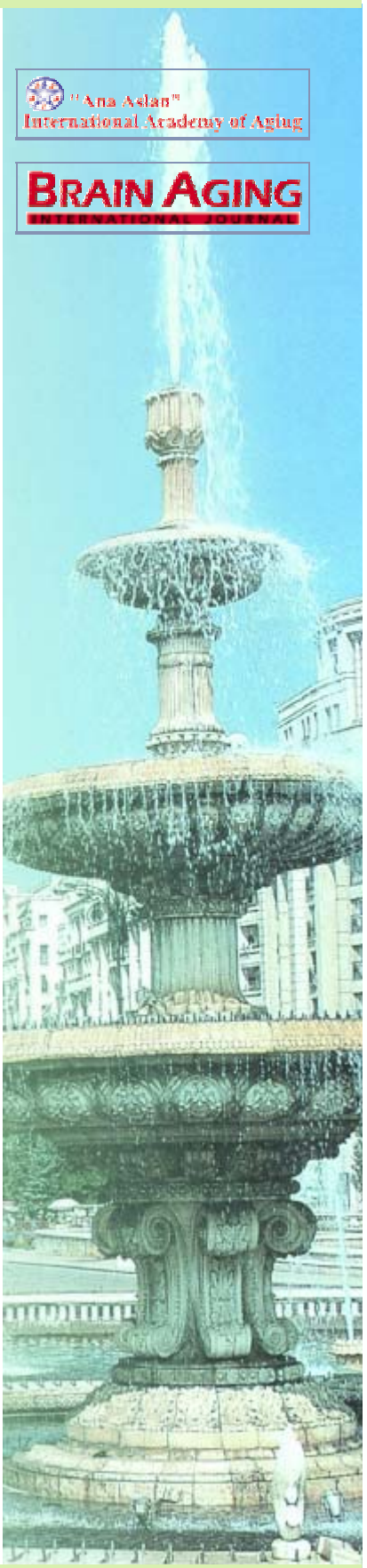

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