# Alzheimer's disease:

# studies of diagnosis and therapy

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# Alzheimer's disease:

Studies of diagnosis and therapy

De ziekte van Alzheimer: diagnostische en therapeutische studies

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# Contents

# Publications and manuscripts based on studies in this thesis

**Chapter 3.1**

Claus JJ, van Harskamp FH, Breteler MMB, Krenning EP, Hofman A, Hasan D. Comparison of different methods for rCBF measurements with SPECT in normal controls and in patients with Alzheimer's disease. Submitted.

**Chapter 3.2**

Claus JJ, van Harskamp FH, Breteler MMB, Krenning EP, de Koning I, van der Cammen TJM, Hofman A, Hasan D. The diagnostic value of SPECT with $^{99m}$Tc HM-PAO in Alzheimer's disease: a population-based study. Submitted.

**Chapter 3.3**

Claus JJ, Breteler MMB, Krenning EP, Bots ML, Grobbee DE, de Jong PTVM, de Bruijn AM, Hofman A. Vascular risk factors and regional cerebral blood flow in a population-based study: The Rotterdam Elderly Study. Submitted.

**Chapter 4**

Claus JJ, Hofman A, Jolles J. Farmacologische therapie voor de ziekte van Alzheimer. Nederlands Tijdschrift voor Geneeskunde 1992;136:2463-2466.

**Chapter 5.1**

Claus JJ, Mohr E, Chase TN. Clinical trials in dementia: learning effects with repeated testing. Journal of Psychiatry and Neuroscience 1991;16:1-4.

**Chapter 5.2**

Claus JJ, Ludwig C, Mohr E, Giuffra M, Blin J, Chase TN. Nootropic drugs in Alzheimer's disease: symptomatic treatment with pramiracetam.
Neurology 1991;41:570-574.

**Chapter 5.3**

Claus JJ, van Harskamp FH, Verschoor CJ, de Koning I, Breteler MMB, van der Cammen TJM, Hofman A. Serotonergic mechanisms in Alzheimer's disease: preliminary results of a controlled clinical trial with lisuride. Submitted.

# Chapter 1

# Introduction

*Chapter 1*

# Introduction

Despite tremendous recent advances in the clinical neurology, neurobiology and epidemiology of Alzheimer's disease, the cause as well as its treatment remains as much a mystery today as when it was first described in 1907 by Alois Alzheimer.[1] Alzheimer's disease, the most common type of dementia in the elderly[2] and already referred to as the disease of the century, is one of the major social and medical challenges for clinical practice as well as for scientific medicine, especially since the number of Alzheimer patients is predicted to rise strongly into the next century.[3]

Studies presented in this thesis deal with issues that confront patients suffering from Alzheimer's disease and their families and caregivers every day: the uncertainty of the clinical diagnosis and the lack of adequate treatment. The focus will be on single photon emission computed tomography (SPECT) as a method to assess regional cerebral blood flow (rCBF), its methods of analysis and the contribution to the clinical diagnosis. In addition, aspects of two therapeutic strategies will be explored: stimulation of neuronal metabolism and modulation of neurotransmitter dependent processes. Much of the work presented is guided by epidemiological principles and methods. This coincides with the clinical neurological point of view that is a leading thread running through this thesis.

**Chapter 2** presents an overview of SPECT in aging and dementia. In **chapter 3.1** different methods of analysis of SPECT and the consequences for research studies of Alzheimer's disease are evaluated. **Chapter 3.2** examines the contribution of SPECT to the clinical diagnosis of Alzheimer's disease with emphasis on dementia severity and decision analysis. In **chapter 3.3**, age, risk factors for atherosclerotic vessel disease as well as indicators for atherosclerosis are studied in relation to rCBF in a sample from a population-based study, the Rotterdam Elderly Study.[4]

A review of current therapeutic strategies for Alzheimer's disease and future possibilities is given in **chapter 4**. One of the methodological aspects in clinical trials, the occurrence of learning effects with repeated neuropsychological testing, receives attention in **chapter 5.1**. Treatment of Alzheimer patients with the nootropic compound pramiracetam, a putative enhancer of neuronal metabolism, is evaluated in

a two-phase trial design with dose-finding over a broad range of doses (**chapter 5.2**).

In **chapter 5.3** the effects of lisuride on cognitive and non-cognitive functions are studied in a preliminary trial with 16 Alzheimer patients. Finally, the limitations of the studies in this thesis are discussed from a methodological point of view, the results are reviewed in the context of the present knowledge of Alzheimer's disease and future possibilities for research are suggested (**chapter 6**).

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Chapter 2

# Brain imaging with SPECT in aging and dementia

*Chapter 2*

# Brain imaging with SPECT in aging and dementia

## Introduction

Brain imaging techniques play an important role in the evaluation of the patient presenting with a dementia syndrome. Structural imaging techniques that visualize the brain, such as computed tomography (CT) or magnetic resonance imaging (MRI), are used to detect lesions that may cause dementia, which may lead to effective treatment in some cases. Their role is limited in the diagnosis of Alzheimer's disease, however, since some of the observed changes, including ventricular enlargement and cortical atrophy, are also found in other dementias and in the aged non-demented population. Recent interest has focused on the contribution of physiological imaging techniques, such as single photon emission computed tomography (SPECT) and positron emission tomography (PET), to the understanding of functional correlates of the biological and structural changes observed in the elderly. Areas of research include their role in the differential diagnosis of dementia and the exploration of disease-specific impairments in various dementing disorders. In this chapter, we describe several techniques for measurement of cerebral blood flow (CBF) and address the methods of data analysis in SPECT studies. Finally, results of SPECT and PET studies in normal aging and dementia will be summarized.

## Measurement of cerebral blood flow

Several methods have been developed for measurement of CBF. The first method for measurement of global cerebral perfusion and metabolism was developed in 1945 by Kety and Schmidt.[1] This technique employed the inert diffusible gas nitrous oxide and measured an average value of blood flow per minute per unit weight for the brain as a whole.[2] Cerebral metabolic oxygen consumption ($CMRO_2$) and cerebral glucose utilization (CMRGlc) could be calculated from arteriovenous oxygen and glucose differences, respectively. In 1963, the $^{133}$Xenon ($^{133}$Xe) clearance technique

with intracarotid injection of $^{133}Xe$ was introduced for measurement of CBF.[3] The principle was similar to the Kety-Schmidt technique: measurement of the uptake and clearance in the brain of an inert diffusible gas. Later, non-invasive administration of $^{133}Xe$ was developed by $^{133}Xe$ inhalation. Inhalation of $^{133}Xe$ or intravenous injection of $^{133}Xe$ was combined with the monitoring of radioactivity with multiple collimated scintillation detectors over each hemisphere permitting assessment of regional CBF (rCBF).[4,5] The $^{133}Xe$ method has the advantage that, due to the rapid wash-out, studies can be repeated after as little as 20 minutes. A problem is that rCBF changes in the deep regions of the brain, such as the medial temporal cortex, can not be reliably detected with this method.[6] In addition, the $^{133}Xe$ inhalation method has a poor resolution,[7] and due to the short half-life, the method is not well suited for tomographic imaging.[8]

The need for radioisotope rCBF tracers suitable for tomographic imaging led to the development of new tracers. These tracers should cross the intact blood-brain-barrier (BBB), distribute proportionally to blood flow and retain a fixed regional distribution for a certain period to permit tomographic acquisition.[9] In 1980, iodine-123 labeled isopropyl-iodoamphetamine ($^{123}I$-IMP) was introduced as a radiopharmaceutical that crossed the BBB and was retained in the brain proportionally to rCBF.[10] Imaging with IMP has several drawbacks, however, including the relatively long half-life, the cost, the unfavorable dosimetry, and the redistribution.[11-13] The better dosimetry characteristics and general availability of technetium-99M initiated the search for an appropriate pharmaceutical to link this radionuclide into a lipophilic complex that meets the criteria above. In 1985, Neirinckx and coworkers synthesized the $^{99m}Tc$ labeled hexamethyl-propylene amine oxime (HM-PAO),[14] a lipid soluble molecule that is readily converted to a secondary hydrophillic form in brain cells and that is retained in the brain for several hours proportionally to rCBF without redistribution.[15] Images with IMP give a poorer quality than those with HM-PAO, but more lesions were seen with IMP than with HM-PAO in Alzheimer patients but not in multi-infarct dementia.[16] Especially when using high resolution cameras, and given the better image quality and availability of HM-PAO, this agent is preferred for imaging rCBF in dementia.[16] Comparable results were obtained in patients with various neurological disorders when HM-PAO imaging was compared to the $^{133}Xe$ clearance method.[7] The introduction of HM-PAO provided the opportunity to use rCBF tomography on a wide clinical scale with the use of a conventional slowly-rotating gamma camera.[17]

In the 1970's Sokoloff and associates developed the $^{14}$C-deoxyglucose technique with PET to measure regional cerebral metabolism and blood flow.[18] Extension of this method was made possible with the synthesis of [$^{18}$F]-fluoro-deoxyglucose (F-18-FDG), fulfilling the requirements of a pharmaceutical for measuring regional cerebral glucose metabolism in humans. PET is now a well established technique to measure physiological parameters in the brain such as rCMRGlc, $rCMRO_2$, and rCBF. The advantage of PET, in contrast to SPECT, is that quantitative estimates of these parameters are provided and that the resolution is relatively high. Extensive research has been performed with PET in several degenerative neurological disorders and PET studies continue to add to the scientific knowledge of these diseases. The high costs of PET and the limitation to centers with on-site cyclotrons led to renewed interest in SPECT imaging and to the development of the tracers IMP and HM-PAO. Cameras for SPECT imaging are widely available and the finding that rCBF is coupled to both rCMRGlc and $rCMRO_2$ in normal subjects and in patients with Alzheimer's disease,[19,20] possibly through both chemical and neuronal mechanisms,[21] suggests that SPECT may be a good clinical alternative for PET.

Comparison of SPECT imaging with PET for the measurement of rCBF has been performed in several studies. HM-PAO images were shown to reflect rCBF as measured by $C^{15}O_2$ PET.[22] In a rCBF comparison study of HM-PAO SPECT with fluoromethane PET in cerebrovascular disease, it was found that HM-PAO images showed less contrast between high and low activity regions than PET.[6] The application of the Lassen correction logarithm did not improve the correspondence of SPECT data with PET rCBF because this procedure increased the variance in the high flow areas.[6] In another study, FDG PET was superior in the detection of mildly affected areas as compared to SPECT, but IMP and HM-PAO SPECT were considered useful in the detection of abnormal regions in dementia including Alzheimer's disease and Pick's disease.[23]

## Methods of analysis in SPECT studies

In general, two methods are used to analyze SPECT images: the semi-quantitative analysis and the qualitative analysis. The data sampling in the semi-quantitative analysis requires the construction of a region of interest (ROI) to measure rCBF in a

particular area of the brain. Since SPECT does not yield quantitative data, the mean activity (average counts per pixel) in this ROI is then divided by that of a defined reference area, for example the cerebellum. In qualitative analysis the data set is rated by visual inspection on a classification scale. Both methods of analysis will be discussed in the following section.

*Semi-quantitative analysis: the ROI definition*

The need for ROI's comes from the necessity to have an anatomical reference area and to reduce the enormous amount of information contained by the 3 dimensional data set comprising thousands of pixels. In most studies of Alzheimer's disease the ROI location is with reference to the normal anatomy of the brain and guided by the underlying assumption that structure and function are related. Defined regions usually include the areas of frontal, temporal, parietal, and occipital cortex.

For ROI definitions, variations occur across studies in shape, size and location, and different reference areas are used. Geometrically shaped ROI's range from rectangular regions of 2x4 pixels (13x25 mm),[24] to square regions of 4x4 pixels (2.5x2.5 cm),[25] or 3x3 pixels (18x18 mm).[26] Regions are also drawn by hand with reference to a standard CT brain atlas to optimally conform to the anatomical configuration of the region.[27,28] Waldemar and colleagues[29] used a computerized method with semi-fixed ROI templates from an anatomical atlas that could be individually adjusted and redrawn only for the outer contour of the brain. In the method described by Gemmell et al, an operator gives an outline of the outer brain contour after which a computer inscribes a second ROI within the first outline at a fixed distance of 7 pixels (22.4 mm).[22]

The number of slices per cortical region included in the analysis shows considerable variation, ranging from one to six.[24-30] The choice of the reference area is the cerebellum in most studies,[26,29,30] but also the occipital cortex is used,[28,31] as well as the activity in the whole tomographic slice.[27] The results of rCBF measurements with SPECT will be dependent on the way an ROI is defined.

Potential sources of bias for the ROI construction are discussed by Fox[32] in relation to PET studies, and the issues raised by the author may well translate to the ROI definition in SPECT studies. The a priori selection for "interest" of a brain structure may be a source of sampling bias, because detection of changes outside the defined region is not possible. The sensitivity for this change depends on the anatomical precision of the ROI placement in the affected brain area, which is referred to as the

"small signal problem".[32] The dimensions of the ROI pose another problem. It is argued by Fox that the more closely the dimensions of the ROI conform to the area of change, the more sensitive is the ROI for the magnitude of this change. Small ROI's may be more sensitive but have less anatomical precision, and geometrically shaped ROI's may not optimally conform to the affected brain area.

*Qualitative analysis*

Qualitative rating of tomographic images is another possibility to analyze SPECT images. Rating procedures by one or more raters blind to the clinical diagnosis include dichotomous ratings of images, e.g. as normal or abnormal, or on an ordinal rating scale. If an absolute scaling method is employed for analysis of SPECT images, the reference area chosen will determine the relative count activity in a certain area and thereby the appearance of the particular regions. Several qualitative rating methods have been used. In a study by Johnson et al,[33] only the parietal cortex was taken into account, and an Alzheimer patient was classified as abnormal when the parietal activity was lower than 60% of the maximum of the slice. Employing the same rating procedure, patterns of hypoperfusion were identified in all cortical areas, including bilateral posterior temporal and/or parietal cortex and multiple small cortical defects.[34] In a study by Neary et al[35] two individuals independently rated cerebral activity as normal or abnormal. Cerebral regions were defined as anterior, posterior, left and right, but no information was given on the scaling method of the image display in this study. With another method employed by Sharp et al,[36] the brain was divided in 5 equal-sized sections, and regions did not follow internal anatomical borders. The decrease in average count density in focal regions was expressed as percentage reduction from visually appearing normal tissue.

## SPECT and PET studies in normal aging

Several studies have been reported in healthy elderly individuals from the 2nd to the 8th decade of life, assessing rCBF, rCMRGlc or $rCMRO_2$, or combinations of these variables. Most studies have been conducted with PET, but recently also a SPECT study addressed this subject. The first studies measuring global CBF and oxygen and glucose utilization in normal aging were conducted by Kety and

coworkers. They found that CBF, $CMRO_2$ and CMRGlc show a gradual decrease with advancing age, possibly secondary to neuronal cell loss.[37] Dastur and colleagues selected healthy elderly control subjects with and without hypertension and arteriosclerosis.[38] In contrast to the findings by Kety et al, CBF and $CMRO_2$ were not significantly different between the young and old healthy groups in this study and appeared to be age invariant, although CMRGlc was significantly decreased in the elderly subjects. In a selected sample of elderly subjects with subclinical vascular disease, CBF showed a significant decline of 10-16% compared to the young group, suggesting a role of vascular factors in CBF decline.[38] The role of cerebrovascular disease in CBF decrements and oxygen and glucose consumption of the brain was also suggested in another study.[39] Other studies with PET in healthy individuals have suggested a decrease with aging of rCBF,[40,41] with the exception of a study by Yamaguchi et al.[42] Martin and associates performed a pixel by pixel analysis in a PET rCBF study of normal aging, because variability in methods and limited precision of ROI placement could have affected results in previous reports.[43] They reported an age related decline in adjusted rCBF in limbic and association cortices and suggested that the decrease with age of rCBF could be due to structural changes, to decreases in the activity of individual neurons independent of structural change, or to a combination of the two.[43]

SPECT studies of normal aging have been sparse. In a single study by Waldemar and colleagues 53 healthy elderly subjects aged 21-83 years were assessed with HM-PAO SPECT and the $^{133}Xe$ inhalation technique.[29] An age related decline in rCBF was observed in the frontal cortex, and an area of subcortical low-flow increased with age. In addition, the side-to-side asymmetry of several regions increased with age.

PET studies investigating $rCMRO_2$ and rCMRGlc have not yielded consistent results. The first studies found reductions in rCMRGlc and $rCMRO_2$,[44-46] with a faster rate for CMRGlc with advancing age than for $rCMRO_2$.[45,47,48] A decrease in $rCMRO_2$ and rCMRGlc was also observed in later reports.[40-42,49] However, no age related changes in parameters of brain metabolism were observed in other studies.[50-55] In a reanalysis of their former study Frackowiak et al could not confirm the decline in $rCMRO_2$ while the effect of age on rCBF was reproduced.[56] Varying selection criteria, in particular those involving inclusion of subjects with cerebrovascular disease, could possibly account for study differences.[41,50]

The effect of cerebral atrophy has been demonstrated in several studies. When corrections for cerebral atrophy are made, global CMRGlc increased in Alzheimer

patients and elderly controls with 16.9% and 9.0%, respectively.[57] Yoshii and coworkers found a significantly lower CMRGlc in the elderly compared to young subjects, that disappeared after correcting for brain volume and atrophy.[58] Brain volume explained 17%, brain atrophy 8%, and the combination of these two factors 21% of the variance in CMRGlc. Cerebrovascular risk factors were not related to CMRGlc measures, leaving almost 80% of the variance in CMRGlc unexplained.[58]

The oxygen extraction ratio (OER) showed no statistically significant age effect, although a small upward trend has been observed, and it is suggested that OER is not determined by $CMRO_2$ but compensates reciprocally for different values of CBF.[41] The trend to increased OER with a decline in CBF could reflect a maintenance of metabolism,[43] compensating for a falling CBF, to maintain $CMRO_2$ at higher levels than would be expected if $CMRO_2$ fell in parallel with CBF, or if the fall in CBF were a consequence of a primary fall in $CMRO_2$.[59]

## SPECT and PET studies in dementia

*Alzheimer's disease*

A large number of studies have been performed with both SPECT and PET imaging to examine rCMRGlc and rCBF changes in Alzheimer's disease. The earliest studies in the 1970s with the $^{133}Xe$ method in patients with presumptive Alzheimer's disease showed reductions in overall CBF.[60] The conclusions of these studies in dementia are limited, mainly because selection criteria for patients were not rigorous. In PET research, a general finding is that metabolic reductions appear in Alzheimer's disease. Association cortices are predominantly involved and most studies show a relation of rCMRGlc and rCBF reductions and dementia severity. In one of the first PET studies using $^{15}O$ to estimate oxygen utilization, defects were observed in parietal and temporal lobes of patients with mild Alzheimer's disease whereas in severe dementia also the frontal lobe was found to be profoundly impaired with relative sparing of the occipital lobe.[61] Other studies also showed relative decreases in cortical glucose metabolism in the temporo-parietal association cortex, as well as in the frontal cortex.[62-64] In mild and moderate Alzheimer's disease reductions in rCMRGlc were reported in parietal cortex whereas severe Alzheimer patients demonstrated both parietal and temporal metabolic decreases,[65] and in another study the number of

regions involved in Alzheimer patients increased with dementia severity.[66] In mildly impaired patients with Alzheimer's disease neocortical abnormalities in rCMRGlc may precede the appearance of non-memory cognitive deficits.[67] Longitudinal study of these patients showed that the heterogeneous nonmemory language and visuospatial impairment was related to and predicted by these early metabolic abnormalities in the neocortex, and that asymmetry indices were directionally stable and became more pronounced over time.[68]

SPECT investigations have largely confirmed results obtained in PET studies. The most consistent finding in these studies is a reduction in rCBF in Alzheimer's disease compared to healthy controls in temporo-parietal and parietal cortex.[26-28,33,36,69] Results of frontal and occipital cortex are not consistent, however. Several studies found a rCBF decrease in occipital cortex[26,33] and frontal cortex,[26,28,33] while other studies reported no changes in frontal cortex,[27,68] in superior frontal cortex,[28] and occipital cortex.[70] In studies using a qualitative analysis, some investigators make a distinction between "anterior" and "posterior" deficits. The heterogeneity of metabolic disturbances in Alzheimer patients reported with PET[71] was confirmed by these studies. The percentage of Alzheimer patients with decreased rCBF in both anterior and posterior areas was 46% in one study[72] and 33% in another study.[35] Reduction in only posterior areas was found in 33%[35] and 29%[75] of Alzheimer patients and in only anterior areas this was 14%.[35] The percentage of Alzheimer patients with no abnormalities was reported to be 19%.[35]

Dementia severity correlates with rCBF values measured with SPECT in Alzheimer's disease, most strongly with rCBF decreases in posterior cortical areas in patients with severe dementia.[73] In a study with the $^{133}Xe$ inhalation method a temporo-parietal rCBF deficit was consistently found in early Alzheimer patients, and it was concluded that rCBF procedures provide an accurate and sensitive marker for early Alzheimer's disease.[74] The diagnostic value of SPECT in early Alzheimer patients before structural abnormalities are present on CT or MRI was demonstrated in a study by Perani et al.[25] They found globally decreased cortical rCBF with largest reductions in frontal and temporo-parietal regions and uptake asymmetries corresponded to lateralized neuropsychologic deficits in selected patients. Temporal as well as parietal reductions of rCBF were found in groups of mild, moderate, and severe Alzheimer's disease.[31,33] In addition, occipital rCBF was decreased in patients with dementia severity ranging from mild to moderate.[33] Regional CBF in dorsolateral frontal cortex in mild and moderate Alzheimer's disease was reported

normal compared to controls,[31] and 33% of mild and 50% of moderate Alzheimer patients in this study had values beyond the 95% confidence interval of the control group means.

*Vascular dementia*

Vascular dementia (VaD) is a broad term that encompasses all patients with dementia associated with ischemic and hemorrhagic cerebrovascular disease, according to recent diagnostic criteria for research studies.[75] Several underlying illnesses include small vessel disease with dementia such as in Binswanger's disease (subcortical atherosclerotic encephalopathy) and multiple lacunar strokes, and multi-infarct dementia (MID) and strategic single-infarct dementia, terms used to describe the dementia resulting from multiple cerebral infarcts or localized ischemic damage. The prevalence of VaD increases with advancing age[76] and VaD is, after Alzheimer's disease, the second most common type of dementia and may account for 15-20% of all dementia cases, in addition to another 15-20% that may suffer from a combination of VaD and Alzheimer's disease.[77]

Several studies have searched for characteristic patterns of rCMRGlc and rCBF deficits in VaD, but results are not consistent. With PET and SPECT hypometabolism and hypoperfusion were found to be multiple, focal and asymmetric in VaD.[78] Some studies found no differences between MID patients and controls.[27,68] Gemmell et al studied 15 patients with MID and found no characteristic pattern, with images ranging from fairly normal to multiple rCBF deficits, while in 13% bilateral temporo-parietal deficits were observed.[36,72]

More extensive areas of hypoperfusion than observed on structural imaging may be present, illustrating the value of SPECT to establish the functional significance of vascular lesions.[79] Deficits in rCBF were seen in MID patients that did not match infarcts on MRI or CT scan.[80] Cortical metabolism is dependent on afferent and efferent pathways as was demonstrated in a patient with multiple cerebral infarcts.[81] The degeneration of fiber tracts may result in disconnection of remote structures that is reflected by distant metabolic defects.[81] In the study by Jagust et al, MID patients showed a propensity for frontal involvement.[27] The patients in this study had multiple lacunar infarcts and deep white matter involvement and it was suggested that frontal rCBF decrease may be characteristic for these patients, representing distant flow effects.[27]

*Other dementias*

SPECT studies have also been conducted in other, less common dementing disorders, that are to be excluded when making the diagnosis of probable Alzheimer's disease. Patients with "frontal lobe type dementia" showed selective anterior hypoperfusion.[82,83] In patients with Parkinson's disease no specific pattern was demonstrated in the basal ganglia but those parkinsonians with dementia showed parietal and frontal cortical uptake abnormalities compared to those without dementia.[26] In progressive supranuclear palsy frontal areas of hypoperfusion were present with SPECT[84] and reduced metabolic values were found with FDG PET in all cortical and subcortical areas investigated.[85] Another SPECT study of progressive supranuclear palsy patients demonstrated significant rCBF reductions in basal ganglia, frontal and parietal regions.[86] In early Huntington's disease PET studies have shown decreased glucose metabolism in the basal ganglia before the appearance of abnormalities on CT and it was shown that patients with Huntington's disease have a diffuse decrease in cortical metabolism with early involvement of the frontal lobe.[87]

*The role of SPECT in the diagnosis of Alzheimer's disease*

SPECT imaging as a valuable aid in the diagnostic evaluation of dementing disorders is suggested by some studies that show distinctive rCBF patterns in different types of dementia. SPECT as a diagnostic tool is only useful when sufficient sensitivity and specificity is demonstrated, in particular for the diagnosis of early Alzheimer's disease. Various studies have investigated sensitivity and specificity of SPECT in Alzheimer's disease. Johnson and associates used a cut-off score of 0.66 below which the parietal to cerebellar activity ratio was defined abnormal. This semi-quantitative method was 76% sensitive and 78% specific in 37 Alzheimer patients and 9 controls.[88] The investigators tried to improve diagnostic accuracy by examining all image data in each subject with a qualitative rating method. A data set was classified as abnormal when activity in the parietal lobe was reduced. In 58 mildly to severely affected Alzheimer patients, this method yielded a sensitivity of 88% and specificity of 87%.[33] Jagust and coworkers were able to differentiate all Alzheimer patients from both controls and MID patients with the activity ratio of bilateral temporo-parietal cortex to the whole slice.[27] With a grading scale from 0 (normal) to 5 (severely abnormal) for the whole data set, Duara and colleagues defined an optimal cut-off as 2 or more in a group of 40 patients with probable or possible Alzheimer's disease and 30 controls. They found sensitivity and specificity 58% and 77% with SPECT, and 75%

and 78% with PET, respectively.[89] Powers et al used a blinded clinical evaluation of bilateral temporo-parietal rCBF reduction in patients with probable Alzheimer's disease in a PET study and reported sensitivity 38% and specificity 88%, while these figures were 92% and 85%, respectively, when diagnostic criteria were liberalized.[90]

The correct identification of Alzheimer patients in an early stage of the disease is clinically important, but poses most difficulties. In almost all studies the temporal, parietal or temporo-parietal cortex is used to differentiate between Alzheimer patients and controls, and sensitivity is reportedly lower in early Alzheimer's disease compared to more severe patients. For example, Johnson et al found that the sensitivity was lowest (80%) in the mild Alzheimer's disease group compared to the severe patients (95%).[33] Gemmell et al found that a bilateral temporo-parietal rCBF deficit was not present in 25% of their least impaired Alzheimer patients.[72] Reed et al defined SPECT abnormalities as values beyond the 95% confidence interval of the control group means. They reported in 9 patients with probable and possible Alzheimer's disease a sensitivity of 56%, whereas this value was 83% in 12 moderate Alzheimer patients.[31] Prohovnik and associates used the $^{133}Xe$ inhalation technique and reported sensitivity and specificity 88% and 75%, respectively, in a subgroup of mild Alzheimer patients.[74] In a recent study of 132 patients evaluated for memory loss it was reported that the probability of Alzheimer's disease is 82% when bilateral temporo-parietal defects are present.[34]

No characteristic patterns of hypoperfusion or hypometabolism in SPECT and PET studies have been found to separate patients with Alzheimer's disease and VaD. The presence of a bilateral temporo-parietal deficit has been described in VaD patients, while focal rCBF reductions were found in Alzheimer patients.[68,72] The co-occurrence of these disorders in some patients provides the diagnostic evaluation with an extra challenge and complicates the interpretation of results in SPECT studies of Alzheimer's disease and VaD.

## Conclusion

SPECT with $^{99m}Tc$ HM-PAO has gained recent interest in research studies of aging and dementia, based on reported distinctive patterns of hypoperfusion in different dementing illnesses. Similar results were reported for rCBF abnormalities with

SPECT and PET, and SPECT may therefore provide a clinical alternative for PET. Encouraging results of sensitivity and specificity of SPECT in Alzheimer's disease and normal controls have been reported and some authors have suggested the use of SPECT as an adjunct for the clinical diagnosis of Alzheimer's disease. Some studies also show that considerable overlap may exist in rCBF values between Alzheimer patients, VaD patients, patients with other dementing illnesses and normal controls. For their methods of analysis, various SPECT studies use different definitions of regions of interest. It is unclear how these differences affect study results. Most studies report a decrease in rCBF with advancing age, possibly related to the presence of vascular risk factors and indicators of atherosclerosis, while inconsistent results were reported for $CMRO_2$ and CMRGlc.

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# Chapter 3

# Studies of diagnosis

*Chapter 3.1*

# Comparison of different methods for rCBF measurements with SPECT in normal controls and in patients with Alzheimer's disease

## Introduction

Regional cerebral blood flow (rCBF) measurements by means of single photon emission computed tomography (SPECT) studies is widely applied in research regarding Alzheimer's disease (AD).[1-4] In contrast to positron emission tomography (PET), it is not possible to obtain quantitative rCBF with SPECT. In SPECT studies, semi-quantitative rCBF values are calculated by dividing the mean activity (counts per pixel) in a given region of interest (ROI) by that of a defined reference area (for example the cerebellum). Consequently, the results of rCBF measurements with SPECT will vary depending on the criteria used to define the ROI's and the reference area.

Various definitions of an ROI have been employed in SPECT studies. ROI's vary between studies in shape, size, and location, and in the number of slices included in the analysis. Some investigators drew ROI's by hand with reference to a CT or anatomical atlas,[3-5] others employed geometrically shaped ROI's, such as rectangular and square regions of varying sizes and localization,[1,2,6,7] or included either one single-slice[1,3,4,7] or multi-slices in their analysis.[2,6,8,9] However, it is not known whether variations in methods of defining the ROI's will have different results. In this study, we compared the discriminative ability of 3 different methods of semi-quantitative rCBF measurement with technetium-99M labeled hexamethyl-propylene amine oxime ($^{99m}$Tc HM-PAO) SPECT in normal controls and AD patients.

## Subjects and methods

### Patients

Forty-eight patients referred from the metropolitan area of Rotterdam to the outpatient clinic of the Departments of Neurology and Geriatric Medicine, University Hospital Rotterdam Dijkzigt, for the evaluation of memory complaints in the period 1989 to 1991, fulfilled NINCDS-ADRDA criteria for probable AD[10] and were included in this study. Mean age was 72.2 years (range 50 to 89) and dementia severity was assessed with the Global Deterioration Scale (GDS).[11] Patients with scores of 3 on the GDS were classified as having mild dementia (n=12), patients with the score 4 as moderate dementia (n=20), and patients with scores 5 or higher as severe dementia (n=16). The mean Mini Mental State Examination (MMSE)[12] score was 19.9 and ranged from 9 to 25 (Table 3.1.1).

*Table 3.1.1 Entry characteristics of 60 controls and 48 Alzheimer patients*

| | Controls (n=60)* | Alzheimer patients (n=48)* |
|---|---|---|
| Sex | | |
| male | 27 | 22 |
| female | 33 | 26 |
| Age | | |
| mean (in years) | 74.1 (0.8) | 72.2 (1.2) |
| range (in years) | 62-86 | 50-89 |
| GDS | - | 4.1 ± 0.1 |
| range | | 3-7 |
| MMSE | | |
| mean | 28.3 (0.2) | 19.9 (0.7)† |
| range | 25-30 | 9-25 |

*SEM in parentheses
†Significantly different from controls by t-test ($p < 0.01$)

### Controls

Controls for the SPECT study were recruited from a random sample of 111 persons from the Rotterdam Elderly Study that participated in an additional magnetic resonance

imaging (MRI) study (response rate for the MRI study: 87%) and were matched for age and sex to the AD patients (Table 3.1.1). The Rotterdam Elderly Study is an ongoing single center prospective follow-up study of the total population aged 55 years and over from the suburb Ommoord in Rotterdam. The total eligible population of this study comprises 11,854 persons and the refusal rate in those aged 65 to 84 years was 18 percent. Rationale and design of the Rotterdam Elderly Study have been described elsewhere.[13] A careful screening procedure for dementia was employed.[14] Written informed consent was obtained from all subjects (controls and patients). Twenty-one of the 111 persons were excluded on the basis of a neurologic disease, such as history of stroke or transient ischemic attacks, Parkinson's disease and AD. The remaining 90 subjects were eligible for the SPECT study. Twenty-four of these 90 refused participation and one died before the SPECT study could be performed. SPECT was performed in the remaining 65 persons, 5 of these scans were not available for analysis because of technical reasons. The remaining 60 persons were included in the control group.

**SPECT**

Regional CBF studies were performed with $^{99m}$Tc HM-PAO. Technetium-99M labeled HM-PAO was prepared by adding 1,110 megabecquerel (MBq) freshly eluted sodium pertechnate to a vial containing freeze dried 0.5 mg *d,l*-HM-PAO, 7.6 microgram stannous chloride dihydrate, and 4.5 mg sodium chloride (Ceretec). From this mixture, 740 MBq was intravenously administered within 30 minutes after preparation. SPECT was carried out 10 to 20 minutes later to avoid possible interference from cerebral uptake of free sodium pertechnate. Data acquisition was performed with either a single-head rotating gamma-camera (model Siemens) with a low energy all purpose collimator or a 3-headed rotating gamma camera (model Picker). Total acquisition angle was 360°, 60 projections of 30 seconds each, with a 64x64 matrix and spatial resolution of approximately 12 mm for the Siemens camera, and 128x128 matrix and approximately 7 mm spatial resolution for the Picker camera. Transversal (parallel to orbito-meatal line) slices were reconstructed with a ramp filter, after correction for attenuation, by calculation of the geometric means.

**Regions of interest**

Analysis of the results of rCBF measurements was performed on a personal computer with GAMMAPC 1.41 (Dr O. Nickel, Department of Nuclear Medicine, University of Mainz, Germany). The matrix of images acquired with the Picker camera was reduced

from 128x128 to 64x64. ROI's were drawn blinded to the clinical diagnosis by the following standardized procedure. The reference slice included one cerebellar hemisphere that contained a pixel with the highest counts of the entire cerebellum. After drawing a region by hand roughly around this hemisphere, the computer defined the reference region by activity higher than 70% of the maximum value in this slice (Figure 3.1.1). The boundary of this reference region corresponds to the anatomical boundary of the cerebellum. The mean activity of this region of interest in counts per pixel was regarded as a reference value. All other slices were displayed in a color scale set relative to this value. Two types of ROI were defined in the cerebral hemispheres: regions corresponding to the normal anatomy of the brain in one single-slice and in multi-slices; four rectangular ROI's consisted of 2x4 pixels in one single-slice, as described by Johnson et al.[1] First, the ROI's corresponding to frontal, temporal, temporo-parietal, and occipital cortex, were drawn in each slice by hand with reference to an anatomical atlas[15] (Figure 3.1.1). The temporo-parietal region was defined in the transition area of the temporal and parietal cortex where no reliable differentiation between these two areas can be made. We placed the outer border of these anatomical ROI's at the first line of pixels with a fast decline in activity, assuming that this reflects the transition from brain tissue to cerebrospinal fluid. The inner border was set to a fixed distance of 2 cm from the outer border to approximate the area of grey matter in the brain. With the exception of the outermost slices, all slices were included in the multi-slice analysis. Second, only 1 slice with reference to the normal brain anatomy was used in the single-slice analysis. Third, the 4 rectangular ROI's were located in the frontal, temporal, temporo-parietal, and occipital cortices and were based on the ROI definition from Johnson et al.[1] (Figure 3.1.1). Semi-quantitative rCBF was defined as the average counts per pixel within a region of interest divided by that of the cerebellar reference slice and multiplied with 100 (percentage).

**Statistical analysis**

Comparison of the mean semi-quantitative rCBF in 3 different ROI methods was performed with analysis of variance for repeated measures with the type of SPECT camera as a covariate, and with the classification normal control or AD as grouping variable. When there was a significant difference between methods, a post-hoc analysis was performed between all 3 methods separately with the same statistical method. To evaluate variability between methods in individual subjects, the difference scores were computed; the standard error of the difference score was used as a measure of variability

between methods. The discriminative ability of the combined left and right semi-quantitative rCBF for the classification control person or AD patient was assessed with multivariate logistic regression, adjusted for age, sex, and type of SPECT camera and is expressed as adjusted odds ratio with 95% confidence interval. All regions significantly predicting the diagnostic classification were simultaneously entered into a second logistic model. Regions of interest that were no longer significant in this model were not used in further analyses. Receiver-operator-characteristic curves were plotted for the mean of right and left temporal semi-quantitative rCBF of the 3 ROI methods in patients with mild, moderate and severe AD separately on the basis of the results of the logistic regression analysis.[16] The logistic model calculated the predicted probability of being a patient with AD for all subjects and subsequently generated the sensitivity and specificity for a series of probability cut-off points. In receiver-operator-curves, the true-positive rate (sensitivity) is plotted against the false-positive rate (1 minus specificity) and the discriminative ability of a test is shown by the position of the full curve: the farther upward and to the left the curve lies in the graph, the better is the test.

## Results

Entry characteristics of control subjects and AD patients are presented in Table 3.1.1. Analysis of variance with repeated measures for patients and controls combined revealed a significant difference between the mean semi-quantitative rCBF values of the 3 methods for all regions ($p < 0.001$ for all ROI's). For these differences, no significant group interactions were observed. Analysis for groups separately showed that semi-quantitative rCBF values measured with the rectangular ROI method were significantly higher than from that of both methods with ROI's corresponding to the normal anatomy of the brain in both controls (Table 3.1.2) and AD patients (Table 3.1.3). No differences between the multi-slice and single-slice method with ROI's corresponding to the normal anatomy of the brain could be found in both controls (Table 3.1.2) and AD patients (Table 3.1.3). Analysis of individual variability between all 3 methods revealed that the standard deviations of the differences of the mean rCBF values between the rectangular ROI and each of the two anatomical ROI's were consistently larger than the standard deviations of that between the two anatomically defined ROI's in both controls (Table 3.1.2) and AD patients (Table 3.1.3).

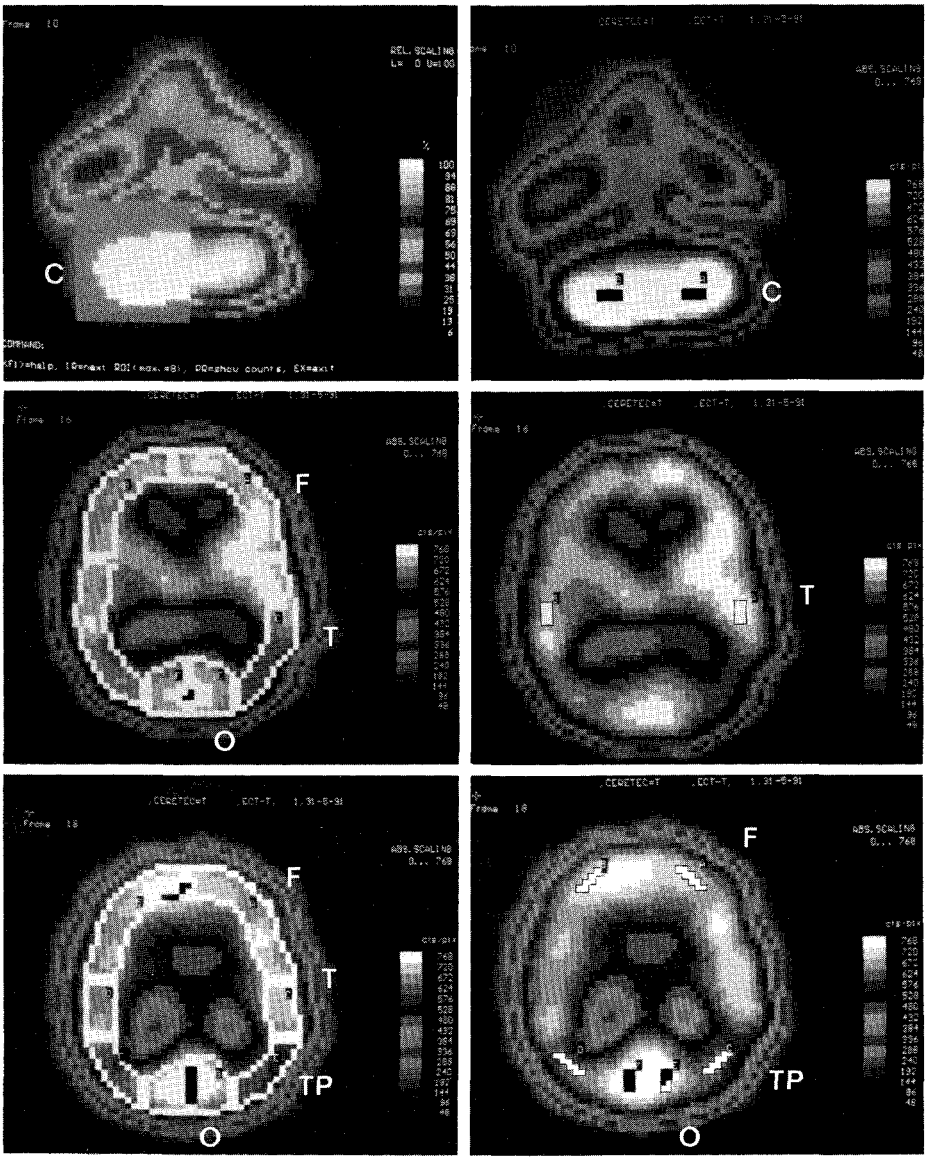


*Figure 3.1.1 An example of a SPECT scan (Siemens camera) of a normal control. Figures on the left hand panel: regions of interest drawn with reference to normal anatomy; the boundary of the cerebellar reference slice (C) is defined by activity higher than 70% of the maximum value in this slice. Figures on the right hand panel: 2x4 pixels rectangular ROI's. C=cerebellum, F=frontal, T=temporal, P=parietal, TP=temporo-parietal, O=occipital*

***Table 3.1.2*** *Results of 3 different methods of semi-quantitative rCBF measurements in 60 control subjects*

| | rCBF values (%)* | | | Difference in rCBF values between methods (%) | | |
|---|---|---|---|---|---|---|
| | Anatomical ROI multi-slice (A) | Anatomical ROI single-slice (B) | Rectangular ROI single-slice (C) | A-B | C-A | C-B |
| Frontal right | 84.7 ± 5.6 | 84.8 ± 7.2 | 88.8 ± 9.6† | -0.1 ± 3.0 | 4.1 ± 6.0 | 4.0 ± 4.8 |
| Frontal left | 83.9 ± 5.6 | 83.5 ± 7.3 | 87.6 ± 8.5† | 0.4 ± 3.3 | 3.8 ± 4.7 | 4.1 ± 3.7 |
| Temporal right | 84.3 ± 4.6 | 84.2 ± 4.8 | 87.9 ± 6.8† | 0.1 ± 1.5 | 3.6 ± 5.2 | 3.7 ± 5.5 |
| Temporal left | 81.7 ± 5.7 | 81.9 ± 5.7 | 85.9 ± 7.0† | -0.2 ± 1.8 | 4.1 ± 5.3 | 4.0 ± 5.3 |
| Temporo-parietal right | 79.9 ± 7.2 | 80.1 ± 7.6 | 86.9 ± 8.9† | -0.1 ± 1.3 | 6.9 ± 7.8 | 6.8 ± 7.9 |
| Temporo-parietal left | 76.8 ± 6.4 | 77.1 ± 6.7 | 86.9 ± 8.9† | -0.3 ± 1.9 | 10.1 ± 8.9 | 9.7 ± 8.9 |
| Occipital right | 89.6 ± 6.1 | 89.9 ± 6.7 | 95.0 ± 10.5† | -0.3 ± 2.5 | 5.3 ± 9.3 | 5.1 ± 9.3 |
| Occipital left | 88.8 ± 6.1 | 89.1 ± 6.5 | 96.5 ± 10.0† | -0.3 ± 2.5 | 7.6 ± 7.2 | 7.3 ± 6.4 |

*Values represent mean percentage ± SD of rCBF relative to that of the cerebellar reference slice, adjusted for type of SPECT camera
†Mean values are significantly higher than those of (A) and (B) by analysis of variance with repeated measures, adjusted for type of SPECT camera ($p < 0.001$)

***Table 3.1.3*** *Results of 3 different methods of semi-quantitative rCBF measurements in 48 Alzheimer patients*

| | rCBF values (%)* | | | Difference in rCBF values between methods (%) | | |
|---|---|---|---|---|---|---|
| | Anatomical ROI multi-slice (A) | Anatomical ROI single-slice (B) | Rectangular ROI single-slice (C) | A-B | C-A | C-B |
| Frontal right | 81.0 ± 5.8 | 80.3 ± 6.5 | 83.2 ± 8.2‡ | 0.6 ± 2.9 | 2.3 ± 5.5 | 2.9 ± 5.1 |
| Frontal left | 80.6 ± 6.7 | 80.0 ± 7.1 | 85.4 ± 9.6† | 0.6 ± 2.9 | 4.8 ± 6.0 | 5.5 ± 5.7 |
| Temporal right | 77.3 ± 7.7 | 76.8 ± 8.0 | 81.0 ± 9.3† | 0.5 ± 1.5 | 3.7 ± 5.6 | 4.2 ± 5.8 |
| Temporal left | 74.8 ± 6.4 | 74.3 ± 6.9 | 78.0 ± 8.1† | 0.5 ± 1.7 | 3.2 ± 5.7 | 3.7 ± 5.6 |
| Temporo-parietal right | 72.0 ± 9.5 | 72.5 ± 9.9 | 78.0 ± 11.9† | -0.5 ± 2.1 | 6.0 ± 7.3 | 5.5 ± 7.3 |
| Temporo-parietal left | 68.9 ± 10.0 | 69.3 ± 10.5 | 78.0 ± 11.9† | -0.4 ± 2.4 | 9.1 ± 8.9 | 8.8 ± 9.2 |
| Occipital right | 86.7 ± 5.9 | 87.3 ± 6.1 | 93.3 ± 9.5† | -0.6 ± 2.1 | 6.5 ± 9.0 | 5.9 ± 8.8 |
| Occipital left | 86.3 ± 6.9 | 86.7 ± 8.4 | 94.9 ± 9.0† | -0.5 ± 3.2 | 8.7 ± 8.8 | 8.2 ± 9.6 |

*Values represent mean percentage ± SD of rCBF relative to that of the cerebellar reference slice, adjusted for type of SPECT camera
† ‡Mean values are significantly higher than those of (A) and (B) by analysis of variance with repeated measures, adjusted for type of SPECT camera ($p < 0.001$ and $p < 0.01$, respectively)

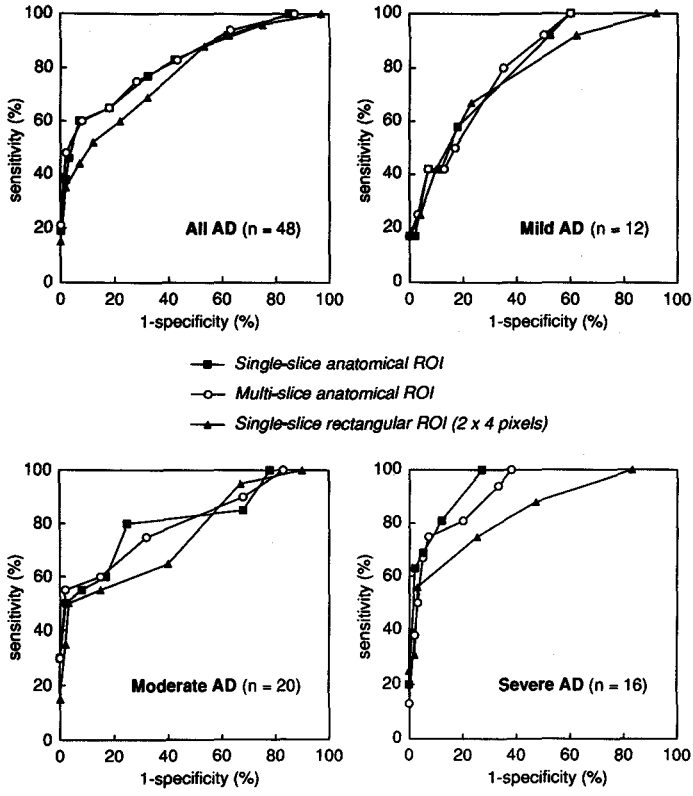


***Figure 3.1.2*** *Receiver-operator-characteristic curves to analyze the discriminative ability of semi-quantitative rCBF values in 60 controls and 48 Alzheimer patients based on rCBF values of the temporal ROI's*

After analysis with logistic regression, every combined left and right ROI was significantly associated with the diagnosis AD (Table 3.1.4). When all regions were simultaneously entered into a full multivariate model, only the temporal ROI remained significantly associated with the diagnosis AD for all 3 ROI methods investigated (Table 3.1.4). Receiver-operator-characteristic curves plotted for all AD patients based on the analysis of the temporal ROI showed a lower discriminative ability for the rectangular ROI method, compared to both methods with ROI's corresponding to the normal anatomy (Figure 3.1.2). However, when dementia severity is taken into account, no major differences in sensitivity and specificity between the 3 ROI methods are found in mild and moderate AD. In contrast, worst diagnostic performance was observed for the rectangular ROI method in patients with severe AD (Figure 3.1.2).

***Table 3.1.4*** *The discriminative value of semi-quantitative rCBF measurements for controls and Alzheimer patients in 3 different regions of interest analyzed with logistic regression**†*

| | Anatomical ROI multi-slice | | Anatomical ROI single-slice | | Rectangular ROI single-slice | |
|---|---|---|---|---|---|---|
| | Odds ratio | 95% CI | Odds ratio | 95% CI | Odds ratio | 95% CI |
| Regions separately entered in model | | | | | | |
| Frontal | 1.12 | 1.04-1.22 | 1.14 | 1.06-1.22 | 1.09 | 1.03-1.14 |
| Temporal | 1.26 | 1.13-1.39 | 1.23 | 1.12-1.36 | 1.10 | 1.04-1.17 |
| Temporo-parietal | 1.13 | 1.06-1.20 | 1.09 | 1.03-1.15 | 1.08 | 1.03-1.13 |
| Occipital | 1.08 | 1.01-1.16 | 1.08 | 1.01-1.15 | 1.04 | 1.0-1.09 |
| Regions combined into full model | | | | | | |
| Frontal | 0.96 | 0.82-1.08 | 0.96 | 0.84-1.07 | 1.05 | 0.98-1.11 |
| Temporal | 1.37 | 1.12-1.66 | 1.44 | 1.17-1.75 | 1.10 | 1.01-1.19 |
| Temporo-parietal | 0.99 | 0.84-1.14 | 0.93 | 0.79-1.05 | 1.05 | 0.99-1.11 |
| Occipital | 0.94 | 0.80-1.06 | 0.98 | 0.87-1.08 | 0.99 | 0.92-1.05 |

*Odds ratios per percent change in rCBF (mean of right and left) are used to estimate the association of a regional perfusion value with the diagnostic outcome, normal control or Alzheimer patient, with logistic regression, adjusted for age, sex, and SPECT camera
†Significant prediction of the clinical diagnosis Alzheimer's disease when an odds ratio of 1.0 is not included in the confidence interval

## Discussion

We compared 3 methods of ROI definition in a semi-quantitative rCBF study with $^{99m}$Tc HM-PAO SPECT in controls and AD patients: multi-slice and single-slice ROI's with reference to normal anatomy of the brain; and single-slice rectangular ROI's (2x4 pixels). No major differences in the mean semi-quantitative rCBF were found between the anatomically defined ROI in the single-slice method when compared to that of the multi-slice method. In contrast, the semi-quantitative rCBF values measured with the rectangular method resulted in significantly higher mean semi-quantitative rCBF values than that measured in the anatomically defined ROI's. These findings were similar in controls and in AD patients. Analysis with logistic regression followed by the construction of receiver-operator-characteristic curves showed superior discriminative ability between controls and severe AD patients of the temporal anatomically defined ROI's when compared to that of the rectangular ROI method. No major differences in discriminative ability between the 3 methods were found in mild and moderate AD.

ROI's are designed to reduce the enormous amount of 3-dimensional data in SPECT scanning contained in thousands of pixels, but there is no agreement on the choice of the location, the size and shape of an ROI. In general, to be optimally sensitive for detection of rCBF decrease in a particular area of the brain, the ROI should be constructed conform the dimensions of this rCBF deficit. Abnormalities in rCBF may remain undetected when the analysis is limited to 1 slice as in the single-slice method with anatomically constructed ROI's, or when very small ROI's are employed, as in the geometric ROI method. However, an accurately placed small geometric ROI is potentially more sensitive for small areas of rCBF change than larger ROI's with reference to normal anatomy of the brain. On the other hand, these small ROI's may be imprecise because of their predetermined and fixed location. Thus, the validity and precision of a certain ROI method designed to detect changes in rCBF depends on both the nature of the rCBF deficit and on the shape, size and localization of the constructed ROI. The results of rCBF measurements with the 3 investigated ROI methods suggest that in studies of aging and AD analysis of the rCBF in the anatomically defined temporal ROI in one slice would suffice, as shown in the receiver-operator-characteristic curves. This may be explained by the diffusely distributed neuropathological abnormalities in AD. When rCBF reflects neuronal function, as has been suggested by a close coupling of rCBF and neuronal metabolism in PET studies,[17,18] rCBF decrease is expected to be diffusely distributed in AD.[19-21] In studies involving patients with

probable AD and control subjects without neurologic disease, the limitation of the analysis to one slice is a great benefit in terms of cost-effectiveness. It remains to be established if these results hold for patients where focal rCBF abnormalities can be expected, such as in those with vascular dementia,[22,23] as problems of measurement validity and precision may then play a role.

Analysis with receiver-operator-characteristic curves enables a comparison between methods with respect to discriminative ability between Alzheimer patients and controls. The superior discriminative ability of the method with reference to normal anatomy when compared to that of the geometric ROI's, is possibly a result of an additional rCBF decrease in severe AD in cortical areas beyond the boundaries of the rectangular ROI's and may have several consequences for research studies. The comparison of controls and AD patients representing a broad range of severity in studies with rectangular ROI's may tend to underestimate the differences between these groups. Therefore, it is suggested that the anatomically ROI method should be preferred in studies investigating rCBF characteristics of AD, for example in longitudinal studies of predictors of decline. On the other hand, when SPECT is used as a diagnostic adjunct for the diagnosis of mild AD, any of the 3 investigated methods may be used. The choice of the ROI method will therefore depend on the purpose for which SPECT is used.

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*Chapter 3.2*

# The diagnostic value of SPECT with $^{99m}$Tc HM-PAO in Alzheimer's disease: a population-based study

## Introduction

The prevalence of Alzheimer's disease (AD) increases with advancing age[1] and about two million patients in the European Community and a similar number of persons in the United States are affected by this disease.[2] Diagnostic accuracy is of primary importance with an increasing number of AD patients and several diagnostic criteria are currently available.[3,4] However, considerable overlap exists between AD patients, especially those with mild AD, and non-demented elderly individuals in cognitive performance,[5] radiographic findings,[6] and neurochemical[7] and neuropathological changes.[8,9] Recent interest has focused on regional cerebral blood flow (rCBF) measurements with single photon emission computed tomography (SPECT) with technetium-99M labeled hexamethyl-propylene amine oxime ($^{99m}$Tc HM-PAO) for the diagnosis of AD. SPECT studies have shown a reduction of rCBF in the temporo-parietal cortex in mild and moderately severe AD patients when compared to age-matched healthy control subjects.[10-13] Encouraging results of SPECT in the diagnosis of AD have been reported[10,11,14] and some authors suggest the routine administration of SPECT in the diagnostic evaluation of AD.[12,15,16] However, there are problems with the interpretation of these results since the selection procedures for controls in these studies are likely biased towards healthier individuals, resulting in reference groups that are not a reliable reflection of those in the general population that are at risk for the disease. Therefore, in the present study we compared patients with probable AD with controls originating from a population-based study to investigate the possible contribution of SPECT for the diagnosis of AD, especially for mildly affected patients.

## Subjects and methods

### Patients

Forty-eight patients referred from the metropolitan area of Rotterdam to the outpatient clinic of the Departments of Neurology and Geriatric Medicine, University Hospital Rotterdam Dijkzigt, for the evaluation of memory complaints in the period 1989 to 1991, fulfilled NINCDS-ADRDA criteria for probable AD[3] and were included in this study. Mean age was 72.2 years (range 50 to 89) and dementia severity was assessed with the Global Deterioration Scale (GDS).[17] Patients with scores of 3 on the GDS were classified as having mild dementia (n=12), patients with the score 4 as moderate dementia (n=20), and patients with scores 5 or higher as severe dementia (n=16). The mean Mini Mental State Examination (MMSE)[18] score was 19.9 and ranged from 9 to 25 (Table 3.2.1).

*Table 3.2.1 Entry characteristics of 60 controls and 48 Alzheimer patients*

| | Controls (n=60) | All AD patients (n=48) | Mild AD (n=12) | Moderate AD (n=20) | Severe AD (n=16) |
|---|---|---|---|---|---|
| Sex | | | | | |
| male | 27 | 22 | 7 | 7 | 8 |
| female | 33 | 26 | 5 | 13 | 8 |
| Age | | | | | |
| mean (in years) | 74.1 ± 0.8 | 72.2 ± 1.2 | 70.8 ± 2.0 | 71.4 ± 2.1 | 74.3 ± 2.1 |
| range (in years) | 62-86 | 50-89 | 56-78 | 50-81 | 56-89 |
| GDS | | | | | |
| 3 | - | 12 | 12 | 0 | 0 |
| 4 | - | 20 | 0 | 20 | 0 |
| 5 or 6 | - | 16 | 0 | 0 | 16 |
| MMSE | | | | | |
| mean | 28.3 ± 0.2 | 19.9 ± 0.7* | 22.3 ± 0.8* | 19.1 ± 1.2* | 17.7 ± 1.3* |
| range | 25-30 | 9-25 | 19-25 | 9-25 | 11-24 |

*Significantly different from controls by t-test ($p < 0.01$)

### Controls

Controls for the SPECT study were recruited from a random sample of 111 persons from the Rotterdam Elderly Study that participated in an additional magnetic resonance

imaging (MRI) study (response rate for the MRI study: 87%) and were matched for age and sex to the AD patients (Table 3.2.1). The Rotterdam Elderly Study is an ongoing single center prospective follow-up study of the total population aged 55 years and over from the suburb Ommoord in Rotterdam. The total eligible population of this study comprises 11,854 persons and the refusal rate in those aged 65 to 84 years was 18 percent. Rationale and design of the Rotterdam Elderly Study have been described elsewhere.[19] A careful screening procedure for dementia was employed.[20] Written informed consent was obtained from all subjects (controls and patients). Twenty-one of the 111 controls were excluded on the basis of a neurologic disease, such as a history of stroke or transient ischemic attacks, Parkinson's disease and AD. The remaining 90 persons were eligible for the SPECT study. Twenty-four of these 90 refused participation and one died before the SPECT study could be performed. SPECT was performed in the remaining 65 persons, 5 of these scans were not available for analysis because of technical reasons. A total of 60 persons was therefore included in the control group. A comparison of these 60 persons with the 30 of the 90 eligible subjects that were not included in the study showed no significant differences in various cardiovascular parameters (history of myocardial infarction, hypertension, systolic and diastolic blood pressure, plasma fibrinogen, cholesterol, high density lipoprotein, and factor VII and factor VIII).

**SPECT**

Regional CBF studies were performed with $^{99m}$Tc HM-PAO. Technetium-99M labeled HM-PAO was prepared by adding 1,110 megabecquerel (MBq) freshly eluted sodium pertechnate to a vial containing freeze-dried 0.5 mg *d,l*-HM-PAO, 7.6 microgram stannous chloride dihydrate, and 4.5 mg sodium chloride (Ceretec). From this mixture, 740 MBq was intravenously administered within 30 minutes after preparation. SPECT was carried out 10 to 20 minutes later to avoid possible interference from cerebral uptake of free sodium pertechnate. Data acquisition was performed with either a single-head rotating gamma-camera (model Siemens) with a low energy all purpose collimator or a 3-headed rotating gamma camera (model Picker). Total acquisition angle was 360°, 60 projections of 30 seconds each, with a 64x64 matrix and spatial resolution of approximately 12 mm for the Siemens camera, and 128x128 matrix and approximately 7 mm spatial resolution for the Picker camera. Transversal (parallel to orbito-meatal line) slices were reconstructed with a ramp filter, after correction for attenuation, by calculation of the geometric means.

**Regions of interest**

Analysis of the results of rCBF measurements was performed on a personal computer with GAMMAPC 1.41 software (Dr O. Nickel, Department of Nuclear Medicine, University of Mainz, Germany). The matrix of images acquired with the Picker camera was reduced from 128x128 to 64x64. Regions of interest were drawn blinded to the clinical diagnosis by the following standardized procedure. The reference slice included one cerebellar hemisphere that contained a pixel with the highest counts of the entire cerebellum. After drawing a region by hand roughly around this hemisphere, the computer defined the reference region by activity higher than 70% of the maximum value in this slice (Figure 3.2.1). The boundary of this reference region corresponds to the anatomical boundary of the cerebellum. The mean activity of this region of interest in counts per pixel was regarded as a reference value. All other slices were displayed in a color scale set relative to this value. The regions of interest corresponding to frontal, temporal, temporo-parietal, parietal and occipital cortex, were drawn by hand with reference to an anatomical atlas[21] (Figure 3.2.1). The temporo-parietal region was defined in the transition area of the temporal and parietal cortex where no reliable differentiation between these two areas could be made. The outer border of these regions of interest was placed at the first line of pixels with a fast decline in activity, assuming that this reflects the transition from brain tissue to cerebrospinal fluid. The inner border was set to a fixed distance of 2 cm from the outer border to approximate the area of grey matter in the brain. With this method our intra-observer variability in terms of mean counts per pixel in a specific region of interest was 0.8%. With the exception of the outermost slices, all slices were included in the analysis. Semi-quantitative rCBF was defined as the average counts per pixel within a region of interest divided by that of the cerebellar reference slice and multiplied with 100 (percentage).

**Statistical analysis**

Semi-quantitative rCBF in corresponding regions of interest was compared between AD patients and controls. Linear regression analysis was performed to adjust for differences in age, sex and type of SPECT scanner. The predictive power of the semi-quantitative rCBF in each individual region of interest for the classification normal control or AD patient was assessed in a multivariate logistic regression model, with adjustment for age, sex and type of SPECT camera and expressed as an adjusted odds ratio with a 95% confidence interval. All regions of interest significantly predicting the diagnostic classification in this model were simultaneously entered into a second logistic

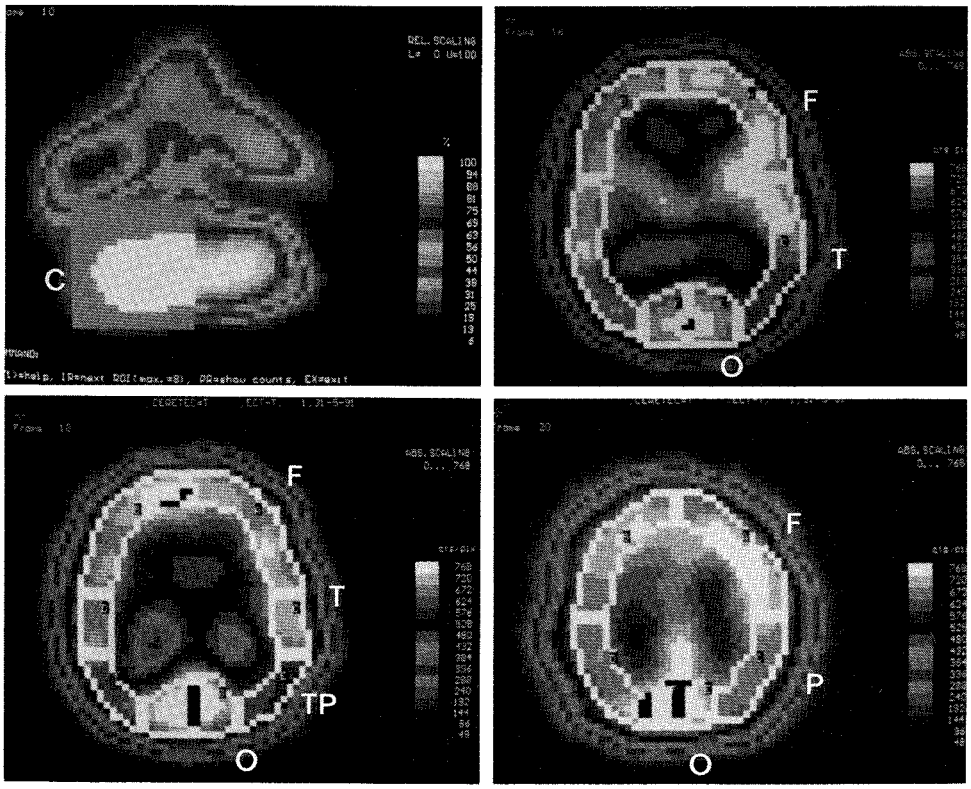


***Figure 3.2.1*** *Regions of interest (ROI's) were drawn with reference to normal anatomy by a standardized procedure. Examples of SPECT (Siemens camera) with $^{99m}$Tc HM-PAO from a control subject are shown for ROI's from the cerebellum (C), from the frontal ROI (F), the temporal ROI (T), the parietal ROI (P), the temporo-parietal ROI (TP), and the occipital ROI (O)*

model. Regions of interest that were no longer significant in the second logistic model were excluded. Thereafter, receiver-operator-characteristic curves were constructed for patients with mild, moderate and severe AD separately on the basis of the results of the logistic regression analysis.[22] The logistic model calculated the predicted probability of being a patient with AD for all subjects and subsequently generated the sensitivity and specificity for a series of probability cut-off points. In receiver-operator-characteristic curves, the true-positive rate (sensitivity) is plotted against the false-positive rate (1 minus specificity) and the discriminative ability of a test is shown by the position of the full curve: the farther upward and to the left the curve lies in the figure, the better is the test. After determining the sensitivity corresponding to a specificity of 90%, the incremental ruling-in and ruling-out gain was calculated and plotted against the prior probability in mild AD, as estimated by the clinician.[21] Post-test probability is calculated by adding the incremental gain to the prior probability that the disease is present in case of a positive test result or by adding the incremental gain to the prior probability that the disease is not present in case of a negative test. Specificity was defined at 90% because in this study emphasis is given to the value of SPECT to confirm AD.

## Results

Entry characteristics of AD patients and controls are presented in Table 3.2.1. In Figure 3.2.2, regional differences of semi-quantitative rCBF between AD patients and controls are shown for combined right-left pairs of regions of interest. The lowest rCBF value was observed in the temporo-parietal cortex for patients and controls, while the greatest rCBF decrease in AD patients compared to controls was in the temporal region of interest. The relationship between dementia severity measured with the Global Deterioration Scale and semi-quantitative rCBF is shown in Table 3.2.2. In mild AD patients a significant decrease of rCBF was found only in temporal and temporo-parietal regions. No significant differences between right and left regions of interest were observed. In moderate and severe AD all investigated regions of interest were significantly decreased, with the exception of the occipital cortex in the group with severe AD.

In Table 3.2.3, decreased rCBF was defined as a semi-quantitative rCBF value lower than 2 standard deviations from the mean of controls. Significantly more patients with AD had a decreased rCBF in the temporal, temporo-parietal, parietal and frontal regions

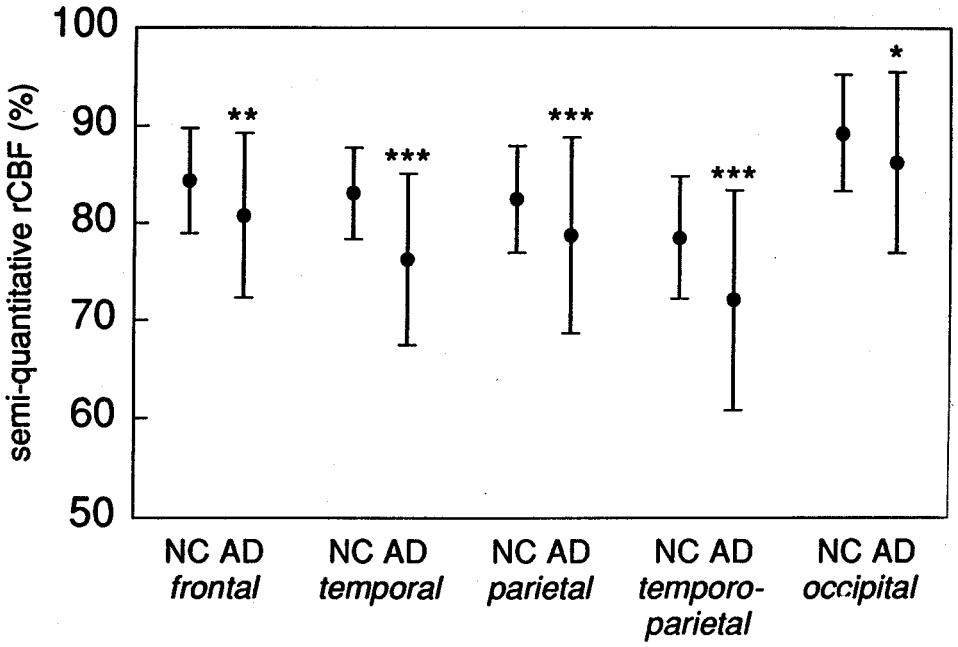


*Figure 3.2.2 Semi-quantitative rCBF (percentage relative to mean rCBF of the cerebellar reference slice) in 60 controls and 48 Alzheimer patients, * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$. Values represent mean ± 1 standard deviation of both right and left regions of interest*

of interest.

After analysis with logistic regression, every individual region of interest, with the exception of the left occipital lobe, was significantly associated with the diagnosis AD (Table 3.2.4). No consistent right-left differences were observed and in subsequent analyses the average of the combined left and right regions of interest was included. When all regions of interest were simultaneously entered into the full multivariate logistic model, only the temporal cortex remained significantly associated with the diagnosis of AD (OR = 1.37, 95% CI 1.24 to 1.51, per 1% change in rCBF) (Table 3.2.4). As a result, only this region was included in the further analysis.

*Table 3.2.2 Mean semi-quantitative regional cerebral blood flow in 60 controls and 48 Alzheimer patients**

| Region | Controls (n=60) | Mild AD† (n=12) | Moderate AD† (n=20) | Severe AD† (n=16) |
|---|---|---|---|---|
| Frontal | | | | |
| Mean | 84.1 ± 5.5 | 81.7 ± 5.7 | 80.5 ± 6.9‡ | 78.7 ± 6.1§ |
| Right | 84.5 ± 5.6 | 82.1 ± 5.9 | 80.6 ± 7.0‡ | 78.9 ± 6.3§ |
| Left | 83.7 ± 5.6 | 81.5 ± 5.8 | 80.4 ± 7.2‡ | 78.4 ± 6.4§ |
| Temporal | | | | |
| Mean | 82.9 ± 4.7 | 78.1 ± 5.3 | 75.2 ± 6.8# | 74.8 ± 5.4# |
| Right | 84.2 ± 4.6 | 80.2 ± 5.4‡ | 76.4 ± 7.4# | 75.8 ± 5.6# |
| Left | 83.7 ± 5.7 | 78.0 ± 6.2§ | 76.1 ± 7.5# | 75.9 ± 6.4# |
| Parietal | | | | |
| Mean | 82.3 ± 5.5 | 79.8 ± 5.5 | 77.8 ± 7.2‡ | 77.8 ± 7.1‡ |
| Right | 83.6 ± 5.4 | 81.6 ± 5.4 | 78.6 ± 7.1§ | 78.6 ± 7.2‡ |
| Left | 81.0 ± 6.5 | 77.8 ± 6.2 | 77.0 ± 8.2‡ | 77.2 ± 7.6‡ |
| Temporo-parietal | | | | |
| Mean | 78.5 ± 6.3 | 73.4 ± 6.9‡ | 71.5 ± 8.3# | 71.4 ± 7.3# |
| Right | 80.1 ± 7.2 | 75.5 ± 7.7‡ | 73.7 ± 8.7# | 72.9 ± 8.2# |
| Left | 76.9 ± 6.4 | 71.3 ± 7.2‡ | 69.5 ± 9.2# | 70.0 ± 7.7# |
| Occipital | | | | |
| Mean | 89.0 ± 6.0 | 86.1 ± 6.6 | 85.0 ± 7.4‡ | 86.9 ± 6.7 |
| Right | 89.4 ± 6.1 | 86.7 ± 6.6 | 85.0 ± 7.6‡ | 87.2 ± 6.7 |
| Left | 88.6 ± 6.1 | 85.3 ± 6.7 | 84.9 ± 7.6‡ | 86.5 ± 7.4 |

*Values represent mean percentage ± SD of rCBF relative to that of cerebellum, adjusted for age, sex, and SPECT camera
†Classified according to the Global Deterioration Scale (GDS), mild Alzheimer's disease (AD), GDS=3; moderate AD, GDS=4; severe AD, GDS ≥ 5 or 6
‡ § #Significantly different from controls by linear regression ($p < 0.05$, $p < 0.01$, $p < 0.001$, respectively)

Receiver-operator-characteristic curves showed that discriminative ability of SPECT was best for severe AD patients. The difference in discriminative ability of SPECT between mild and moderate AD was small (Figure 3.2.3). When the specificity was set at 90%, the corresponding sensitivity for the temporal region of interest was 42% in mild AD, 56% in moderate AD, 79% in severe AD and 60% for all AD patients. Based on these results, the incremental ruling-in and ruling-out gain for those with mild AD was plotted against the prior probability of having the disease (Figure 3.2.4). The contribution of SPECT to the diagnosis of AD proved to be poor when the test result was negative, varying from 3% to 11%. On the other hand, the gain from a positive test ranged from 7% to 34% with the optimal gain at a prior probability of having the disease of 30% to 40% (Figure 3.2.4). For example, at prior probability for AD of 50% the post-test probability was 39% after a negative test result and 81% after a positive test result.

***Table 3.2.3*** *Proportion of patients with Alzheimer's disease and controls with decreased semi-quantitative rCBF*

| | Decreased rCBF* | |
|---|---|---|
| | Control subjects (n=60) | Alzheimer patients (n=48) |
| Frontal | 10 (17%) | 17 (35%)† |
| Temporal | 8 (13%) | 30 (63%)‡ |
| Parietal | 7 (12%) | 22 (46%)‡ |
| Temporo-parietal | 7 (12%) | 26 (54%)‡ |
| Occipital | 9 (15%) | 14 (29%) |

*Decreased rCBF is defined as lower than 2 SD from the mean of controls, for combined right and left regions of interest
† ‡Significantly different from controls by Chi-square test ($p < 0.05$, $p < 0.001$, respectively)

***Table 3.2.4*** *The relation between rCBF and the clinical diagnosis of Alzheimer disease in 60 controls and 48 Alzheimer patients, analyzed with logistic regression**

| | Regions separately | | Right and left values combined into full model | |
|---|---|---|---|---|
| Region | Odds Ratio | 95% CI | Odds Ratio | 95% CI |
| Frontal | | | | |
| Right and left | 1.12 | 1.04-1.22 | 0.97 | 0.91-1.03 |
| Right | 1.13 | 1.07-1.23 | | |
| Left | 1.10 | 1.02-1.19 | | |
| Temporal | | | | |
| Right and left | 1.26 | 1.13-1.39 | 1.37† | 1.24-1.51 |
| Right | 1.24 | 1.12-1.36 | | |
| Left | 1.22 | 1.12-1.34 | | |
| Parietal | | | | |
| Right and left | 1.09 | 1.02-1.17 | 0.97 | 0.91-1.03 |
| Right | 1.10 | 1.03-1.18 | | |
| Left | 1.07 | 1.01-1.14 | | |
| Temporo-parietal | | | | |
| Right and left | 1.13 | 1.06-1.20 | 1.0 | 0.93-1.07 |
| Right | 1.11 | 1.04-1.17 | | |
| Left | 1.12 | 1.05-1.19 | | |
| Occipital | | | | |
| Right and left | 1.08 | 1.01-1.16 | 0.95 | 0.89-1.01 |
| Right | 1.09 | 1.02-1.17 | | |
| Left | 1.08 | 1.01-1.15 | | |

*Odds ratios per percent change in rCBF are used to estimate the association of a regional perfusion value with the diagnostic outcome, normal control or Alzheimer patient, with logistic regression, adjusted for age, sex, and SPECT camera
†Significant prediction of the clinical diagnosis Alzheimer's disease when an odds ratio of 1.0 is not included in the confidence interval

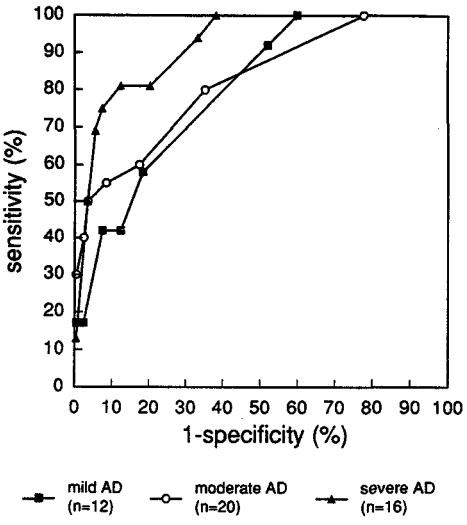


*Figure 3.2.3 Analysis of the discriminative ability of SPECT with receiver-operator-characteristic curves in 60 controls and 48 Alzheimer patients (mild, n=12; moderate, n=20; severe, n=16)*

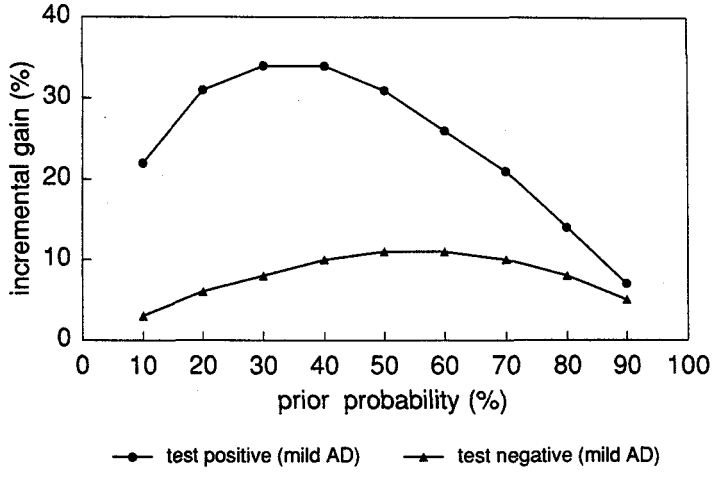


*Figure 3.2.4 Expected incremental ruling-in and ruling-out gain in percentage plotted against the prior probability for mild Alzheimer's disease (n=12) as estimated by the clinician. Curves are computed at sensitivity level of 42% and specificity level of 90%*

## Discussion

Several methodological issues need consideration before discussing the results of this study. Strenuous efforts were made to compose a control group that is representative for those in the general population at risk for the disease. Since 30 of the 90 eligible individuals were not included in the study, selection in the recruitment of controls resulting in a group biased towards healthier individuals can theoretically still be possible. If this bias played a role, it might have resulted in an overestimation of the difference in rCBF measurements between Alzheimer patients and controls. However, no evidence could be found that the non-participation was selective. Another potential problem is the inaccuracy of the clinical diagnosis of AD.[23,24] The inclusion of non-Alzheimer individuals in the patient group could have resulted in an underestimation of the true difference in rCBF between AD patients and controls. Therefore, we carefully defined our group of AD by means of the NINCDS-ADRDA criteria, and the results apply to these criteria as they are currently employed in clinical routine.

We compared AD patients with population controls to estimate the diagnostic characteristics of SPECT with $^{99m}$Tc HM-PAO. The control individuals were recruited from a population-based study; we excluded only those with a neurologic disease including AD and no restrictions were made with cognitive screening tests. In patients with AD, rCBF was decreased in all regions of interest when compared to that of controls, and temporal rCBF proved to be the best discriminating region. If analysis is restricted to the temporal cortex, sensitivity and specificity of the SPECT scan as a diagnostic test for AD were strongly dependent on dementia severity. Although sensitivity of SPECT is best in severe AD, the need for a diagnostic adjunct is strongest in those with mild AD. Analysis of the practical usefulness of SPECT by means of the incremental ruling-in and ruling-out gain provides quantification of how much SPECT scanning will change the prior view of the clinician that the disease is present. We limited this analysis to patients with mild AD. Since SPECT as a diagnostic tool is aimed to confirm a clinical suspicion of AD rather than to exclude this disease, stringent criteria for a positive test were applied and specificity was set at 90%. Consequently, little is gained when the test result is negative. On the other hand, there is a more substantial gain from a positive test result, reaching a maximum of 34% increase in the probability of having AD. This gain may be clinically relevant with a 40% to 60% prior probability, resulting in an increase in the probability from 40% to 74% and from 60% to 86%, respectively. Thus, a benefit from a positive SPECT result in mild AD is expected only

when considerable doubt about the diagnosis exists. However, the problem of low sensitivity remains, since SPECT as a diagnostic test will only be positive in 42% of the mild AD patients which is a severe limitation in terms of cost-effectiveness.

Earlier SPECT studies of rCBF in AD patients have paid little attention to the control group. No attempts have been made to recruit controls from a random sample of the general population. Various selection criteria have been applied, and the number of persons in the control groups have been small.[10,11,14,15,25-28] As a result, selection of controls is likely biased towards healthier individuals which may explain the following discrepancies between our study and previous reports. First, in our study temporal rCBF was the variable with the largest difference between controls and AD patients. By contrast, most other investigators have found the largest rCBF decrease in parietal and temporo-parietal cortex of AD patients when compared to controls.[10,14,15,29-31] Although our controls were non-demented, they had a wide range of cognitive function and a history of various illnesses such as cardiovascular disease as would be expected in the general population. Individuals with these characteristics were excluded in several other studies.[14,15,25,26] Persons with various cerebrovascular risk factors reportedly have a decreased bilateral parietal rCBF compared to age-matched controls without these risk factors.[32] Moreover, it is known that the temporo-parietal cortex (watershed area) proves to be particularly sensitive to hypoxia, hypotension, or both.[33,34] Nevertheless, the possibility that low temporo-parietal rCBF in some of our controls is an early sign of developing AD can not be excluded and should be investigated in follow-up studies. Second, sensitivity and specificity figures from previous SPECT and PET studies were better than those of our study.[10,11,14,35,36] In addition to differences in selection of controls, this may be explained by the inclusion of more severely diseased patients in some studies when compared to those of our study.[31,32]

An apparent discrepancy in results may arise when the decision of clinical administration of SPECT in the diagnosis of AD is based on the predictive value of the test alone. In a recent report,[37] the authors concluded that $^{99m}$Tc HM-PAO SPECT is useful in the diagnostic evaluation of patients with memory and cognitive abnormalities, based on the finding that a bilateral temporo-parietal rCBF defect in patients referred for cognitive impairment had a predictive value of 82% for AD. Their high predictive value corresponds with the probability of having mild AD after a positive test in patients with a prior probability of 50-60% in our study (Figure 3.2.4). After our recalculation based on the results published in their study, sensitivity for all patients with this temporo-parietal defect is about 65%, which is in the range of that in our study (overall sensitivity

of 60%). The authors would probably have arrived at a different conclusion, had they taken dementia severity and all diagnostic characteristics into account, including sensitivity, specificity and predictive value.

We conclude that the inclusion of controls recruited from a randomly sampled population-based study lowered the diagnostic accuracy of SPECT with $^{99m}$Tc HM-PAO in AD as compared to previous studies. In particular, our findings suggest that the use of SPECT as a diagnostic adjunct in patients with mild AD should be limited to those in whom considerable doubt about the diagnosis exists.

## Acknowledgement

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*Chapter 3.3*

# Vascular risk factors and regional cerebral blood flow in a population-based study: the Rotterdam Elderly Study

## Introduction

Determinants of regional cerebral blood flow (rCBF) in elderly individuals have been investigated with positron emission tomography (PET) and single photon emission computed tomography (SPECT). A decrease of rCBF with age has been reported in the limbic and cortical association areas with PET,[1-3] and a rCBF decline in frontal cortex and subcortical structures was observed with SPECT.[4] Few subjects over eighty years were included in these studies and it is unknown whether the observed relation of rCBF and age extends into the eighth decade of life. In addition to age, risk factors for cerebrovascular disease have been associated with changes of overall CBF. Studies comparing persons with vascular risk factors to age-matched controls without these risk factors showed that overall CBF was decreased in the former group.[5,6] However, few studies investigated the individual contribution of various risk factors and their interdependence with rCBF, although hypertension,[5] blood viscosity,[7] and smoking[8] have been suggested to play a role. Furthermore, few attempts have been made to study rCBF in relation to age and vascular risk factors in the general population. To further elucidate the association of age and vascular risk factors with rCBF, we sampled subjects from a population-based study, and studied rCBF with SPECT using technetium99M-labeled hexamethyl-propylene amine oxime ($^{99m}$Tc HM-PAO).

## Subjects and methods

### Subjects

Subjects were selected from participants of the Rotterdam Elderly Study, a single center prospective follow-up study conducted among the total population aged 55 years and over of the suburb Ommoord in Rotterdam, The Netherlands. Written informed consent was obtained for participation in this study as well as for the SPECT

investigation. Rationale and design of the Rotterdam Elderly Study have been described elsewhere.[9] In brief, the objective of the study is to investigate determinants of chronic and disabling cardiovascular, neurogeriatric, locomotor and ophthalmologic diseases. The total eligible population comprises 11,854 persons, with 6,494 persons between the ages of 65 and 85 years. Persons residing in homes for the elderly are included. The refusal rate in the persons aged 65 to 84 years was 18 percent at the time of the present study. Participants are extensively interviewed at home and receive further investigations during two visits at a special research center. Subjects for the present study were recruited from a group of 111 persons from the Rotterdam Elderly Study that participated in a magnetic resonance imaging study, of which the response rate was 87%. A total number of 66 subjects were included in the present study, aged 63 to 89 years.

**Measurements**

At a home interview subjects received a computerized questionnaire to obtain information on current health status, medical history, drug prescriptions and actual use, smoking behavior and level of education. A history of cerebrovascular event was considered positive when the diagnosis had been made by a physician and the diagnosis of myocardial infarction was based on ECG. Subjects were divided in 3 groups of smoking behavior: current smokers, former smokers, and those who had never smoked.

At the research center several measurements were performed. The average of two blood pressure measurements, separated by a count of the pulse rate, taken in sitting position at the right upper arm with a random-zero sphygmomanometer was used for the analysis.[10] Hypertension was defined as a systolic blood pressure of 160 mmHg or over and/or diastolic blood pressure of 95 mmHg or over, and/or as the use of antihypertensive drugs. A venipuncture was performed, with minimal stasis, through a 21 gauge butterfly needle (surflo winged infusion set, Terumo, Belgium). Blood samples were collected into siliconized Vacutainer tubes (Becton & Dickinson, France) containing clotting activator and separator for serum, or 0.129 M sodium citrate for plasma. Serum was separated by a one-stage centrifugation for 10 min at 1,600 g. From the citrate tubes platelet poor plasma was prepared by a two-stage centrifugation; first for 10 minutes at 1,600 g at 4 °C and subsequently for 10 minutes at 10,000 g at 4 °C. All samples were quickly frozen in liquid nitrogen and then stored at -80 °C before assay. Serum total cholesterol was determined with an automated enzymatic procedure.[11] High density lipoprotein cholesterol level was measured similarly, after precipitation of the non HDL fraction with phosphotungstate-magnesium. Plasma fibrinogen level was assessed

according to the Clauss method (Diamed AG, Switzerland).[12] Factor VIIc and factor VIIIc activity were assayed by means of Automated Coagulation Laboratory (ACL) (Instrumentation Laboratory, IJsselstein, The Netherlands), with the aid of factor VII and factor VIII deficient plasma (Ortho Diagnostic Systems, Beerse, Belgium), with Thromborel S (Behringwerke, Germany) and Thrombosil I (Ortho Diagnostic Systems, Beerse, Belgium) as reagents, respectively. Plasma obtained from 40 healthy men was pooled and served as reference for the measurements of factor VIIc and factor VIIIc. Factor VIIc and factor VIIIc levels of the donors were all within normal range and no differences between reference pools could be detected.

Ultrasonography of both carotid arteries was performed with a 7.5 MHz linear array transducer using a Duplex scanner (ATL Ultramark IV, Advanced Technology Laboratories, Bethel, Washington, USA). According to the Rotterdam Elderly Study scanning protocol, a careful search was performed for the intima-lumen interface on the near wall, the lumen-intima interface and the media-adventitia interface on the far wall of the distal common carotid artery.[13] The distances between the interfaces represent the lumen diameter and the intima-media thickness, respectively.[14,15] When optimal longitudinal image was obtained, it was frozen on the R wave of the electrocardiogram and stored on video tape. This procedure was repeated three times for both sides. Subsequently, the carotid artery was evaluated for the presence of atherosclerotic plaques, defined as focal widening relative to adjacent segments, with protrusion into the lumen. The actual measurements were performed off-line. The frozen images on video tape were digitized and displayed on a screen using additional dedicated software. This procedure has been described in detail previously.[15] In short, the interfaces of the distal common carotid artery were marked with a cursor over a length of 10 mm. The beginning of the dilatation of the distal common carotid artery served as a reference point for the start of the measurement. This method permits the determination of mean values as well as maximal values for intima-media wall thickness and lumen diameter. The average of the intima-media wall thickness and lumen diameter of each of the 3 frozen images was taken as a measure for current wall thickness of the distal common carotid artery and lumen diameter, respectively. In addition, for each subject total intima-media wall thickness was calculated: (left side + right side)/2.

**SPECT**

Regional CBF studies were performed with $^{99m}$Tc HM-PAO. Technetium-99M labeled HM-PAO was prepared by adding 1,110 megabecquerel (MBq) freshly eluted sodium

pertechnate to a vial containing freeze dried 0.5 mg *d,l*-HM-PAO, 7.6 microgram stannous chloride dihydrate, and 4.5 mg sodium chloride (Ceretec). From this mixture, 740 MBq was intravenously administered within 30 minutes after preparation. SPECT was carried out 10 to 20 minutes later to avoid possible interference from cerebral uptake of free sodium pertechnate. Data acquisition was performed with either a single-head rotating gamma-camera (model Siemens) with a low energy all purpose collimator, or a 3-headed rotating gamma camera (model Picker). Total acquisition angle was 360°, 60 projections of 30 seconds each, with a 64x64 matrix and spatial resolution of approximately 12 mm for the Siemens camera, and 128x128 matrix and approximately 7 mm spatial resolution for the Picker camera. Transversal (parallel to orbito-meatal line) slices were reconstructed with a ramp filter, after correction for attenuation, by calculation of the geometric means.

**Regions of interest**

Analysis of the results of rCBF measurements was performed on a personal computer with GAMMAPC 1.41 software (Dr O. Nickel, Department of Nuclear Medicine, University of Mainz, Germany). The matrix of images acquired with the Picker camera was reduced from 128x128 to 64x64. Regions of interest were drawn blinded by the following standardized procedure. The reference slice included one cerebellar hemisphere that contained a pixel with the highest counts of the entire cerebellum. After drawing a region by hand roughly around this hemisphere, the computer defined the reference region by activity higher than 70% of the maximum value in this slice (Figure 3.3.1). The boundary of this reference region corresponds to the anatomical boundary of the cerebellum. The mean activity of this region of interest in counts per pixel was regarded as a reference value. All other slices were displayed in a color scale set relative to this value. The regions of interest corresponding to frontal, temporal, temporo-parietal, parietal and occipital cortex, were drawn by hand with reference to an anatomical atlas[16] (Figure 3.3.1). The temporo-parietal region was defined in the transition area of the temporal and parietal cortex where no reliable differentiation between these two areas could be made. The outer border of these regions of interest was placed at the first line of pixels with a fast decline in activity, assuming that this reflects the transition from brain tissue to cerebrospinal fluid. The inner border was set to a fixed distance of 2 cm from the outer border to approximate the area of grey matter in the brain. With this method our intra-observer variability in terms of mean counts per pixel in a specific region of interest was 0.8%.

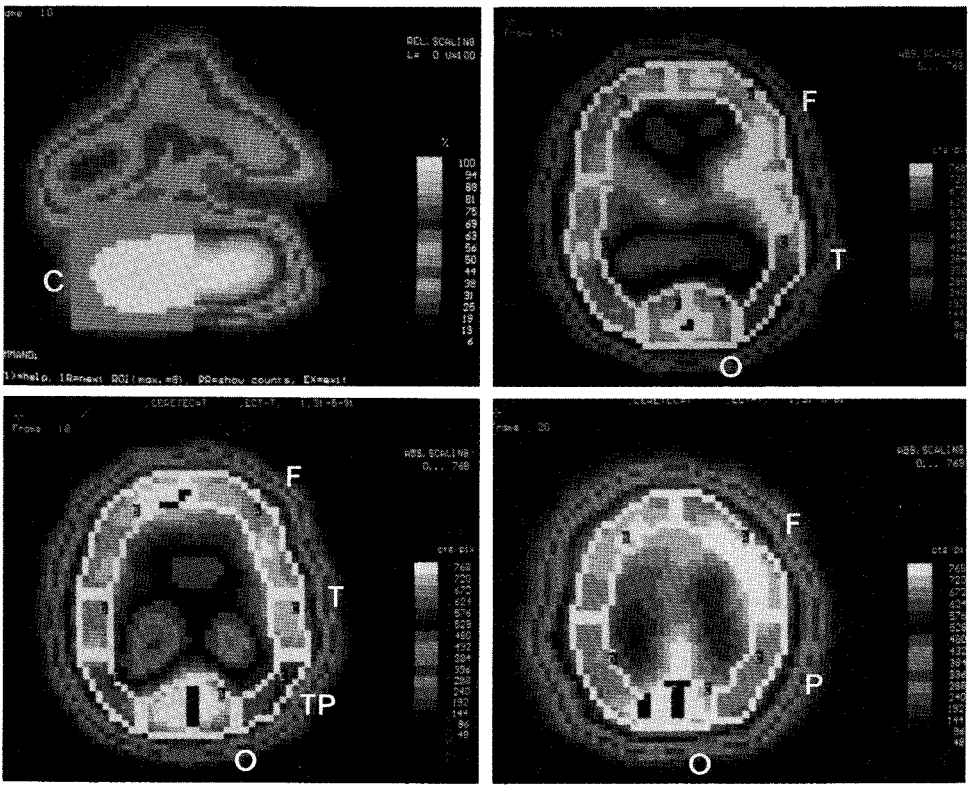


*Figure 3.3.1 Regions of interest (ROI's) were drawn with reference to normal anatomy by a standardized procedure. Examples of SPECT (Siemens camera) with $^{99m}$Tc HM-PAO from a control subject are shown for ROI's from the cerebellum (C), from the frontal ROI (F), the temporal ROI (T), the parietal ROI (P), the temporo-parietal ROI (TP), and the occipital ROI (O)*

With the exception of the outermost slices, all slices were included in the analysis. Semi-quantitative rCBF was defined as the average counts per pixel within a region of interest divided by that of the cerebellar reference slice and multiplied with 100 (percentage).

**Statistical analysis**

The association of vascular risk factors and rCBF was investigated with multiple linear regression analysis, with adjustment for age, sex and type of SPECT camera. The relation of age and rCBF was separately evaluated with linear regression, adjusted for sex and type of camera, with the cumulative addition of vascular risk indicators, and then indicators for atherosclerosis to the same regression model. Analysis of the differences between quartiles of the distribution of a determinant and rCBF was performed with indicator variables. Regression coefficients (with 95% confidence interval, 95% CI) are reported as a measure of the strength of the association of determinants and rCBF.

## Results

The distribution of various demographic and vascular characteristics under study was very similar in the 66 subjects studied with SPECT to that in all subjects examined in the Rotterdam Elderly Study in the same age range. A decrease in values of rCBF with advancing age was observed in all cortical regions studied (Figure 3.3.2). The regression coefficients were significant for temporal (-0.29% rCBF per year, 95% CI [-0.47,-0.11]), parietal (-0.27, [-0.47,-0.07]), and temporo-parietal (-0.25, [-0.47,-0.03]) regions of interest, but not for the frontal cortex (-0.20, [-0.42,0.02]).

A positive diagnosis of myocardial infarction was not significantly related to rCBF (Table 3.3.1). Higher diastolic blood pressure was associated with higher values of rCBF in all cortical regions and reached a level of statistical significance in the temporo-parietal region (Table 3.3.2). The same trend was observed for systolic blood pressure and rCBF but the relations were not significant (Table 3.3.2). A threshold effect was observed for the relation of diastolic blood pressure and temporo-parietal rCBF. Subjects in the lowest quartile of the distribution of diastolic blood pressure (≤ 60 mmHg) had significantly less temporo-parietal rCBF than subjects above the 25th percentile (difference -5.1% rCBF, 95% CI [-9.2%,-1.1%]) (Figure 3.3.3).

*Table 3.3.1 The association between positive history of myocardial infarction and rCBF**

| Variable | Frontal | Parietal | Temporo-parietal | Temporal |
|---|---|---|---|---|
| Myocardial infarction (n=9) | 86.3 ± 1.8 | 82.9 ± 2.1 | 78.9 ± 1.9 | 83.6 ± 1.6 |
| No myocardial infarction (n=57) | 83.6 ± 0.8 | 81.2 ± 0.7 | 77.2 ± 0.8 | 82.3 ± 0.7 |
| Difference (% rCBF) [95% CI] | 2.7 [-1.3,6.7] | 1.7 [-1.9,5.3] | 1.7 [-2.3,5.7] | 1.3 [-1.9,4.5] |

*Values represent mean ± SEM of right and left rCBF in percentage relative to that of cerebellum, adjusted for age, sex, and type of SPECT camera

*Table 3.3.2 The association between blood pressure and rCBF**

| Variable | Frontal | Parietal | Temporo-parietal | Temporal |
|---|---|---|---|---|
| Systolic blood pressure (per mmHg) | 0.04 [-0.04,0.12] | 0.03 [-0.03,0.09] | 0.06 [-0.02,0.14] | 0.05 [-0.01,0.11] |
| Diastolic blood pressure (per mmHg) | 0.01 [-0.13,0.14] | 0.08 [-0.04,0.20] | 0.16 [0.04,0.28] | 0.05 [-0.05,0.15] |

*Multiple linear regression coefficients in % rCBF per mmHg, adjusted for age, sex, and type of SPECT camera, with 95% confidence intervals (n=66)

Similar results were observed when subjects using antihypertensive drugs were excluded. The pattern of increasing rCBF with higher blood pressures was confirmed with the dichotomized definition of hypertension, but these values were not significant (Table 3.3.3).

*Table 3.3.3 The association between hypertension and rCBF**

| Variable | Frontal | Parietal | Temporo-parietal | Temporal |
|---|---|---|---|---|
| Hypertension† (n=19) | 84.0 ± 1.0 | 83.3 ± 1.2 | 79.7 ± 1.3 | 83.7 ± 1.0 |
| No hypertension (n=47) | 82.3 ± 1.0 | 82.0 ± 0.9 | 78.0 ± 0.9 | 82.0 ± 0.7 |
| Difference (% rCBF) | 1.7 | 1.3 | 1.7 | 1.7 |
| [95% CI] | [-1.3,4.8] | [-1.4,4.1] | [-1.3,4.8] | [-1.3,4.8] |

*Values represent mean ± SEM of right and left rCBF in percentage relative to that of cerebellum, adjusted for age, sex, and type of SPECT camera
†Defined as systolic blood pressure ≥ 160 mmHg and/or diastolic blood pressure ≥ 95 mmHg, and/or use of antihypertensive medication (n=19 with hypertension)

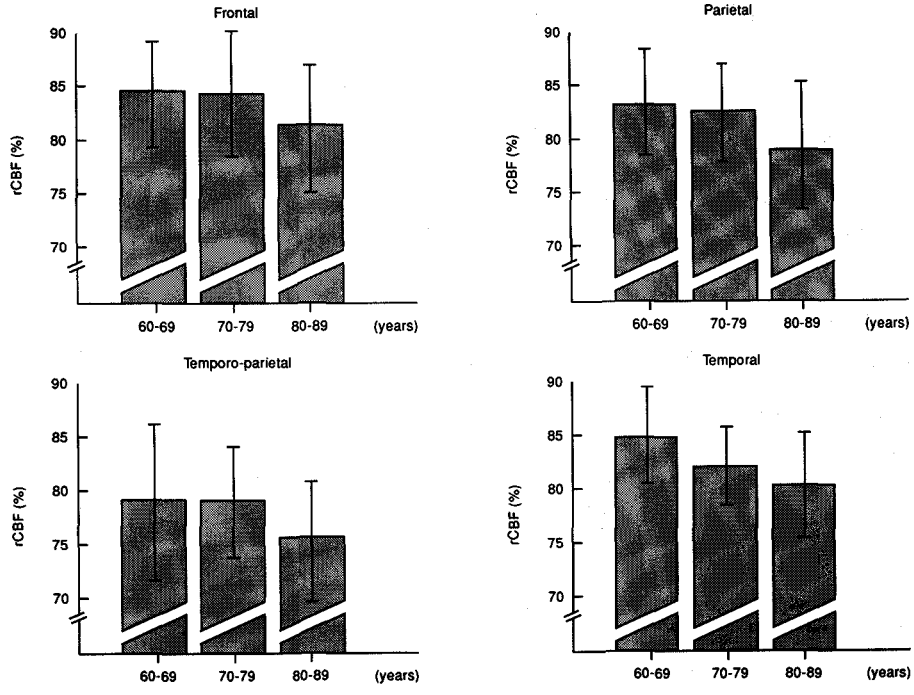


*Figure 3.3.2 Mean values of rCBF in percentage relative to cerebellum of frontal, parietal, temporo-parietal, and temporal rCBF, per age category (60-69, 70-79, and 80-89 years, respectively)*

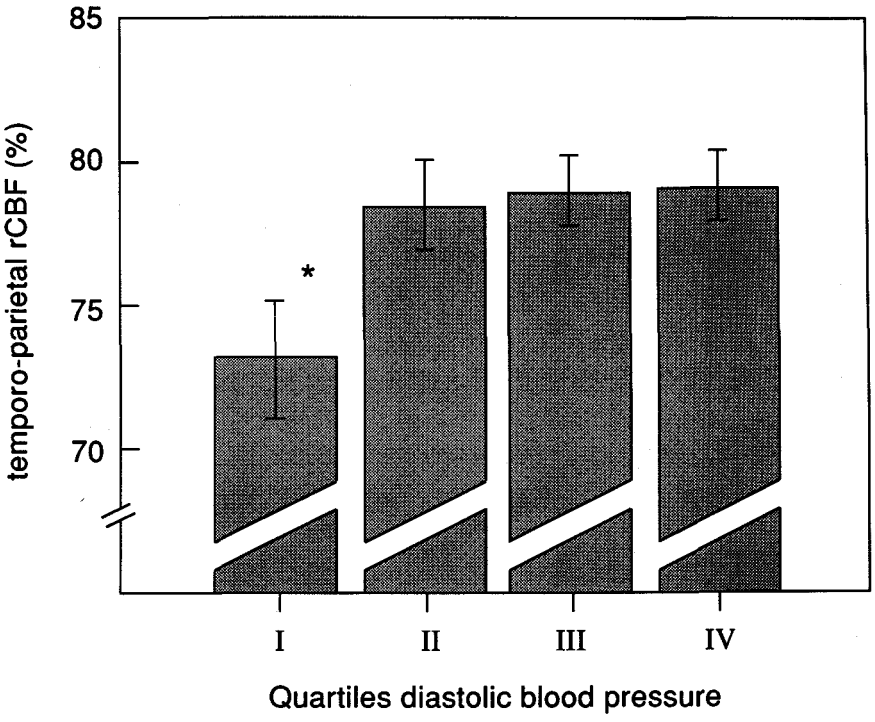


***Figure 3.3.3*** *Mean temporo-parietal rCBF values in percentage relative to cerebellum are shown for quartiles of diastolic blood pressure distribution. The 1st quartile is significantly different from the other quartiles with linear regression, adjusted for age, sex, type of SPECT camera ($p < 0.01$)*

Analysis of hemostatic factors revealed an inverse association of fibrinogen with rCBF in all regions, that was significant in the parietal cortex (Table 3.3.4). This relation persisted after adjustment for previous stroke or myocardial infarction. Detailed analysis suggested a threshold effect for fibrinogen in the highest quartile of the fibrinogen distribution. Persons above the 75th percentile of this distribution (≥ 3.2 g/l) had significantly lower parietal rCBF than those below the 75th percentile (difference -3.0%

rCBF, 95% CI [-5.7,-0.2]) (Figure 3.3.4). Since current smoking is associated with increased levels of fibrinogen, these analyses were adjusted for current smoking. Smoking itself, defined in 3 groups of smoking behavior, was not related to rCBF. No significant associations were found between factor VIIc, factor VIIIc, and rCBF (Table 3.3.4).

*Table 3.3.4 The association between plasma fibrinogen, factor VIIc, factor VIIIc and rCBF**

| Variable | Frontal | Parietal | Temporo-parietal | Temporal |
|---|---|---|---|---|
| Fibrinogen (per g/l)† | -0.1 [-2.4,2.1] | -2.0 [-4.0,-0.04] | -1.8 [-4.0,0.4] | -0.5 [-2.4,1.3] |
| Factor VIIc activity (per $10^{-1}$IU/ml)‡ | 0.06 [-0.01,0.13] | 0.06 [-0.02,0.14] | 0.05 [-0.03,0.13] | 0.05 [-0.01,0.11] |
| Factor VIIIc activity (per $10^{-1}$IU/ml)‡ | 0.01 [-0.01,0.03] | 0.00 [-0.00,0.00] | 0.01 [-0.01,0.03] | 0.01 [-0.01,0.03] |

*Multiple linear regression adjusted for smoking, age, sex, and type of SPECT camera, with 95% confidence intervals
†Adjusted for current smoking; regression coefficients in % rCBF per g/l (n=63)
‡Subjects taking currently anticoagulant drugs excluded; regression coefficients in % rCBF per $10^{-1}$ IU/ml (n=53)

Neither cholesterol nor high density lipoprotein cholesterol levels were significantly related to rCBF (Table 3.3.5). Measurements of the carotid artery revealed a significant inverse association of intima-media wall thickness of the carotid artery and parietal rCBF (Table 3.3.6). However, this relation was no longer significant when patients with stroke (n=3) were excluded (regression coefficient -0.04 % rCBF per mm, 95% CI [-0.10,0.01]).

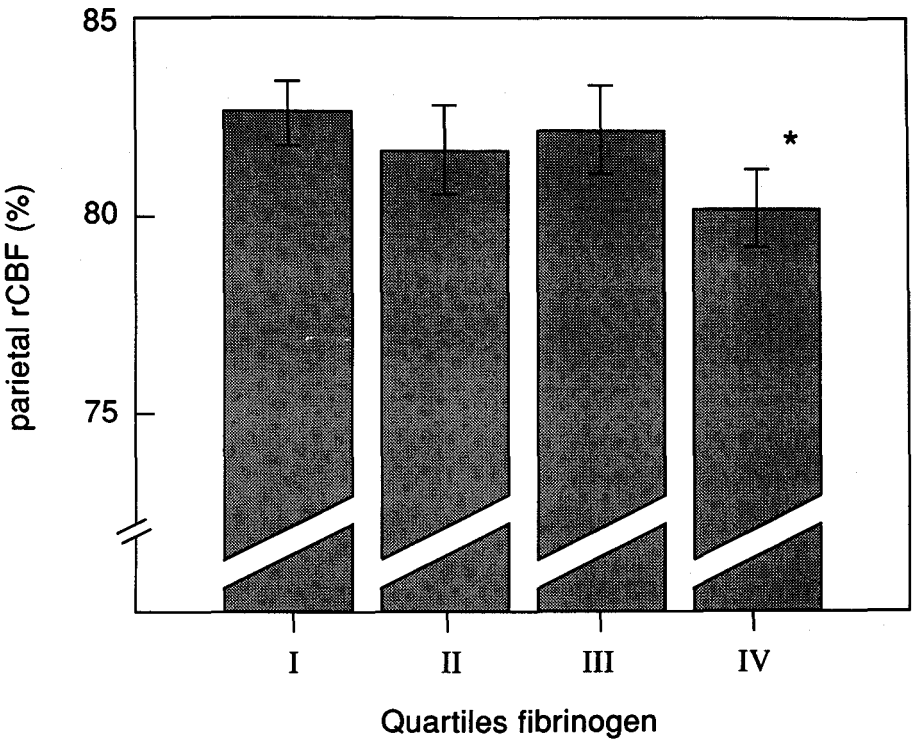


*Figure 3.3.4 Mean parietal rCBF values in percentage relative to cerebellum are shown for quartiles of fibrinogen distribution. The 4th quartile is significantly different from the other quartiles with linear regression, adjusted for age, sex, and type of SPECT camera, and smoking ($p < 0.05$)*

*Table 3.3.5 The association between plasma lipid levels and rCBF**

| Variable | Frontal | Parietal | Temporo-parietal | Temporal |
|---|---|---|---|---|
| Cholesterol (per mmol/l) | 0.3 [-0.8,1.4] | 0.2 [-0.9,1.2] | 0.1 [-1.0,1.2] | -0.4 [-2.7,1.9] |
| HDL (per $10^{-1}$ mmol/l) | 0.4 [-3.9,4.6] | -0.5 [-4.4,3.3] | -3.0 [-7.1,1.2] | -2.0 [-5.5,1.4] |

*Multiple linear regression coefficients in % rCBF per mmol/l, adjusted for age, sex, and type of SPECT camera, with 95% confidence intervals (n=64)

*Table 3.3.6 The association between carotid artery characteristics and rCBF**

| Variable | Frontal | Parietal | Temporo-parietal | Temporal |
|---|---|---|---|---|
| Intima-media vessel wall thickness (per mm) (n=61)† | -0.03 [-0.10,0.04] | -0.06 [-0.12,-0.00] | -0.07 [-0.13,0.00] | -0.04 [-0.09,0.02] |
| Lumen diameter (per mm) (n=53) | -0.00 [-0.02,0.02] | -0.01 [-0.02,0.01] | -0.01 [-0.03,0.01] | -0.00 [-0.02,0.01] |

*Multiple linear regression coefficients in % rCBF per mm, adjusted for age, sex, and type of SPECT camera, with 95% confidence intervals
†Intima-media vessel wall thickness refers to (left side+right side)/2

No significant relations between the lumen of the carotid artery and rCBF were found (Table 3.3.6), or between plaques in the carotid artery or bifurcation and rCBF (Table 3.3.7).

*Table 3.3.7 The association between plaques in the carotid artery and rCBF**

| Variable | Frontal | Parietal | Temporo-parietal | Temporal |
|---|---|---|---|---|
| Plaques carotid artery (n=62)† | 1.0 [-2.91,4.81] | -0.50 [-3.97,2.97] | -0.40 [-4.18,1.53] | 1.32 [-1.91,2.97] |
| Plaques bifurcation (n=53)‡ | -2.82 [-6.11,0.47] | -2.10 [-5.12,0.92] | -2.05 [-5.34,1.24] | -1.57 [-4.31,1.17] |

*Multiple linear regression coefficients are differences in % rCBF between persons without plaques and persons with plaques, respectively, adjusted for age, sex, and type of SPECT camera, with 95% confidence intervals
†Plaques present in either left or right common carotid artery
‡Plaques present in either left or right carotid bifurcation

The effect of various vascular risk factors and indicators of atherosclerosis on the relation between age and rCBF was evaluated in a separate regression analysis. The regression coefficients for the relations of age and rCBF were essentially similar when various vascular risk factors (major cardiovascular event such as myocardial infarction or stroke, hypertension, fibrinogen and smoking, factor VIIc, factor VIIIc) were added cumulatively into the regression model (Table 3.3.8). However, major changes of regression coefficients were observed when indicators of atherosclerosis (intima-media wall thickness of the carotid artery and plaques in the bifurcation of the carotid artery) were added into the regression model (Table 3.3.8).

***Table 3.3.8*** *The association between age, vascular risk indicators, indicators for atherosclerosis and rCBF**

| Variable | Frontal | Parietal | Temporo-parietal | Temporal |
|---|---|---|---|---|
| **Age** (years)† | -0.20 [-0.42,0.02] | -0.27 [-0.47,-0.07] | -0.25 [-0.47,-0.03] | -0.29 [-0.47,-0.11] |
| **Vascular risk indicators‡** | | | | |
| + Major CV event§ | -0.20 [-0.42,0.02] | -0.27 [-0.47,-0.07] | -0.25 [-0.47,-0.03] | -0.29 [-0.47,-0.11] |
| + Hypertension# | -0.20 [-0.42,0.02] | -0.27 [-0.47,-0.07] | -0.25 [-0.47,-0.03] | -0.30 [-0.48,-0.12] |
| + Fibrinogen¶ | -0.20 [-0.44,-0.04] | -0.19 [-0.41,0.03] | -0.18 [-0.42,0.06] | -0.27 [-0.47,-0.07] |
| + Factor VIIc♮ | -0.16 [-0.42,0.10] | -0.14 [-0.40,0.12] | -0.23 [-0.51,0.05] | -0.28 [-0.50,-0.06] |
| + Factor VIIIc♮ | -0.15 [-0.41,0.11] | -0.17 [-0.43,0.09] | -0.24 [-0.54,0.06] | -0.29 [-0.51,0.07] |
| **Atherosclerosis indicators‡** | | | | |
| + Wall thickness of carotid artery (mm)♭ | -0.11 [-0.39,0.17] | -0.07 [-0.33,0.19] | -0.08 [-0.38,0.22] | -0.21 [-0.45,0.03] |
| + Plaques in either carotid bifurcation | -0.09 [-0.39,0.21] | -0.00 [-0.32,0.32] | 0.03 [-0.37,0.43] | -0.15 [-0.45,0.15] |

*All regression coefficients reported are for age, in percentage rCBF relative to cerebellum per year, with 95% confidence intervals
†Multiple linear regression of age and rCBF adjusted for sex, and type of SPECT camera (n=66)
‡Multiple linear regression of age and rCBF adjusted for the presence of vascular risk indicators or indicators for atherosclerosis (data for some patients were missing); The plus indicates that the variable is added to the regression model while the previous variables are kept in the model as well
§History of major cardiovascular event, myocardial infarction or stroke
#Defined as systolic blood pressure ≥ 160 mmHg and/or diastolic blood pressure ≥ 95 mmHg, and/or use of antihypertensive medication
¶Adjusted for current smoking
♮Subjects taking currently anticoagulant drugs excluded
♭Intima-media vessel wall thickness refers to (left side+right side)/2

## Discussion

Age and vascular risk factors were studied in relation to rCBF in a sample from the general population of 60 years and over. An age related decrease in rCBF was observed in the parietal, temporo-parietal and temporal cortex. Analysis of various vascular risk factors revealed that high levels of fibrinogen were independently associated with decreased rCBF in the parietal cortex. Intima-media wall thickness of the carotid artery was inversely related to parietal rCBF only when stroke patients were included. In addition, low diastolic blood pressure was found to be related to decreased rCBF in the temporo-parietal region.

Most studies report a decline of rCBF with advancing age.[1-3,17] An early study by Kety et al, employing the nitrous oxide method, suggested that there is a rapid fall of global CBF from childhood through adolescence followed by a more gradual but progressive reduction throughout the remaining age span.[18] The observation in our study that rCBF shows an age related decline from the 6th to 8th decade therefore extends earlier findings of rCBF and age from PET studies[1-3,6] and a HM-PAO SPECT study,[4] although younger persons were included in these studies and no attempts have been made to sample subjects from the general population. However, the possibility exists that the presence of age-related vascular disease and vascular risk factors[19] in our population-based study, where these age-related changes are likely to be encountered, explains the relation of rCBF and age. Dastur et al reported that there was no decline of CBF with age unless asymptomatic vascular disease was present, and it was suggested that decreases in overall CBF are not the consequence of chronological aging per se but rather of arteriosclerosis.[20] This hypothesis was not supported by later findings with PET that CBF showed a decline with age in persons not compromised by subclinical vascular disease.[2] Furthermore, changes in rCBF were observed in aged individuals without vascular risk factors, while those with vascular risk factors showed an accelerated decline in CBF,[5] and independent contributions of aging and vascular risk factors for rCBF were suggested.[21]

In the present study we found that the contribution of age to rCBF decline was relatively independent of the presence of vascular risk indicators. However, when additionally was controlled for indicators of atherosclerosis, a large decrease or even a reversal of the relationship of age and rCBF was observed. These findings raise serious doubts about age as an independent determinant of rCBF decrease from the 6th to 8th decade of life, and they suggest that this relationship is explained for the major part by

the presence of both vascular risk factors and atherosclerotic manifestations, evidenced by the presence of plaques in the carotid artery and intima-media vessel wall thickness. These findings are in agreement with previous reports of Dastur et al[20] and Shenkin et al.[22]

Fibrinogen has been identified as a major risk factor for cardiovascular disease and stroke.[23,24] Moreover, an association was found between overall CBF and fibrinogen in a heterogeneous sample of patients with vascular disease and controls[25] and an inverse relation of fibrinogen and blood velocity of the middle cerebral artery was reported.[26] In our study, the observed inverse and independent relation of fibrinogen with parietal rCBF in a population sample is in agreement with these findings. The relationship appeared to be attributable to a threshold effect of the highest quartile of the fibrinogen distribution, suggesting that high fibrinogen may be a risk factor for parietal rCBF deficits. The mechanisms of fibrinogen as a risk factor for ischemic vascular events and as determinant of rCBF are not fully understood. Conversion to fibrin, platelet aggregation and adhesion, altered blood viscosity, and fibrinogen vessel wall interactions have all been implicated,[27] and each or combinations of these mechanisms may be responsible for decreased parietal rCBF. On the other hand, no support was found for the hypothesis that elevated coagulation factors are related to rCBF, although coagulation factors are risk factors for cardiovascular disease[28] and factor VIIc is associated with atherosclerotic disease.[29] We had no readily explanation for the fact that only in the parietal cortex the effect of fibrinogen was significant. The negative association of fibrinogen with rCBF in all cortical areas examined could indicate that too few subjects were included in this study to fully examine this relationship. The possibility may exist, however, that it reflects the vulnerability to ischemia of the watershed areas in the parieto-occipital region, which is the furthest from the origins of the anterior, middle and posterior cerebral arteries.[30] Some support for this contention comes from a study that shows compromised rCBF in bilateral parietal regions in persons with vascular risk factors as compared to those without these risk factors.[6]

With respect to increased intima-media wall thickness of the carotid artery, it may be argued that this does not truly reflect the atherosclerotic process. Atherosclerosis is viewed as a disorder which is restricted to the intimal layer of the arterial vessel wall.[31] Ultrasound technique can not discriminate between the intima layer and the media layer of vessel wall. However, evidence that increased intima-media wall thickness may be regarded as indicator for atherosclerosis may come from several studies, in which ultrasonographically assessed increased intima-media wall thickness of the common

carotid artery has been associated with elevated levels of cardiovascular risk factors.[32,33] Moreover, increased carotid intima-media wall thickness and carotid plaques have been associated with presence of atherosclerotic vessel abnormalities in coronary arteries,[34] abdominal aorta[35] and peripheral arteries.[36] In this study, the inverse association of intima-media wall thickness of the carotid artery and parietal rCBF approached significance when stroke patients were excluded. These results suggest that atherogenic mechanisms may be related to parietal rCBF, and that this relation is enhanced by the presence of clinical symptoms. Support for this hypothesis comes from several studies. Similar observations were reported for the relation of hyperlipidemia and rCBF. Patients with transient ischemic attacks with elevated levels of cholesterol or triglycerides had decreased rCBF when compared to patients with normal serum lipid levels, while a non significant trend was observed when neurologically normal volunteers were investigated.[37] It was also demonstrated that decline in rCBF accompanies development and progression of aorto-cerebral atherosclerosis prior to the appearance of signs and symptoms of cerebrovascular disease.[38] Furthermore, carotid artery stenosis in asymptomatic individuals is a well defined risk factor for transient ischemic attacks and stroke.[39-41]

Independent factors associated with carotid atherosclerosis include coronary artery disease, hypertension, smoking and low HDL cholesterol,[42,43] whereas elevated LDL and fibrinogen levels are associated with atherosclerosis progression.[44,45] The relation of parietal rCBF with both fibrinogen and carotid artery intima-media wall thickness could point to a common underlying mechanism, which is supported by the physiological role of fibrinogen in carotid atherosclerosis.[44] In turn, these may be, at least in part, independent relations. Statistical analysis in this study would favour the latter view since the association of intima-media wall thickness of the carotid artery and parietal rCBF was independent of fibrinogen levels and vice versa.

Autoregulation of the vascular supply to the brain is a mechanism to keep rCBF essentially constant over a broad range of cerebral perfusion pressure, ranging from approximately 60 to 140 mmHg.[46] When the cerebral perfusion pressure falls below 40 mmHg, rCBF is invariably reduced and the parieto-occipital watershed borders are the sites of lowest supratentorial perfusion.[47,48] The threshold effect of a diastolic blood pressure below 60 mmHg and low temporo-parietal rCBF in our study is in agreement with these findings and supports the concept that this region is particularly vulnerable to changes in cerebral perfusion pressure. Indeed, a recent study suggested that the temporo-parietal cortex may be particularly sensitive to hypoxia, hypotension, or both.[49]

In conclusion, this study suggests that the observed general decline in rCBF from the

6th to 8th decade of life is in large part dependent on the presence of both vascular risk factors and indicators of atherosclerosis. In addition, both thrombogenic and atherogenic mechanisms may be related to parietal rCBF. There is some evidence that low temporo-parietal rCBF may be associated with low diastolic blood pressure.

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# Chapter 4

# Pharmacological therapy for Alzheimer's disease

*Chapter 4*

# Pharmacological therapy for Alzheimer's disease

## Introduction

The increasing number of patients with dementia is an important problem confronting both society and medical science. Alzheimer's disease accounts for more than 60% of the total number of patients suffering from dementia and is characterized by a slowly progressive decline in language, orientation, and visuo-spatial abilities.[1,2] There are at least 50,000 patients with this disease in the Netherlands[3] and approximately two million in the United States,[1] and estimations indicate that in the year 2040 these numbers will have doubled.[4] Currently, no treatment is available for the cognitive symptoms of Alzheimer's disease. The development of effective therapeutic strategies in addition to etiologic and pathogenetic studies is therefore one of the important goals of dementia research. A variety of therapeutic approaches are currently under investigation. First, these strategies include the development of pharmaceuticals that confer only symptomatic benefit, such as with neurotransmitter substitution therapy or with metabolic stimulation. Second, new areas of research seek to understand and ultimately modify putative causal mechanisms that could result in retarding the progression of the disease, although at present definitive treatment remains elusive. We will briefly describe methodological issues in the conduct of clinical trials and then proceed to review symptomatic and causal therapeutic strategies with special reference to the improvement of cognitive symptoms of Alzheimer's disease.

## Methodological issues in clinical trials

The randomized parallel study design is generally regarded as the "gold standard" for research of the efficacy of treatment. The validity of therapeutic results in the widely used cross-over study may be affected by period-effects or carry-over effects.[5,6] Period-effects are for example progression of the disease or learning after repeated

neuropsychological testing that may occur in early Alzheimer's disease.[7] Although it is statistically possible to test for carry-over effects, most studies include too few subjects to generate sufficient power to test for the assumption.[8] One of the main advantages of the cross-over trial is the increased precision of estimation of the outcome parameters and thereby the need of fewer subjects.[9]

Various pharmaceuticals show an inverted U-shaped dose-response curve with ineffective high and low doses. In addition, the optimal individual dose shows considerable variability which led to the development of a study design that consists of two phases. In the first phase a dose titration is performed to find the optimal dose, while in the second phase the drug efficacy is evaluated through comparison of the presumptive "best-dose" with placebo. After the dose-finding phase, patients that do not show a predetermined response can be excluded to participate in the second phase, which is called an "enrichment-population" design. Although the possibility to find an antidementia effect is increased with this procedure, the generalization of the results is diminished.

Diagnostic misclassification is an important problem in clinical Alzheimer trials because no biological marker is available for the disease. The careful application of current diagnostic criteria is therefore necessary to avoid the inclusion of patients with another disease that potentially leads to dilution of the clinical drug effect. Although the development of the DSM-III[10] and NINCDS-ADRDA criteria[11] has improved diagnostic accuracy for Alzheimer's disease, a recent study showed that considerable disagreement may occur between medical specialists.[12] The introduction of standard measurement instruments has facilitated the comparison of clinical trials but the variation in results can, at least in part, be attributed to differences in dementia severity.

Outcome measures for therapeutic efficacy are generally focused on the principal domains of cognitive decline. It is important, however, to evaluate non-cognitive variables including attention and mood since they possibly interact with cognitive functions and have a prominent impact on the quality of life. A well defined hypothesis about the pharmacologic mechanism of action with a presumptive clinical effect can lead to the evaluation of selective neuropsychological functions in certain instances. A potential drug effect in the development of new pharmaceuticals may be indicated by improvement in specific neuropsychological functions; for the patient and caregiver, on the other hand, improvement in functions of activities of daily living are of decisive importance. The same holds true for disturbances in behavior and the

occurrence of psychiatric problems such as depression and psychosis that have received less therapeutic investigative attention. In most current therapeutic evaluations, measurements of activities of daily living, behavior assessment scales, and ratings of global function are included.

## Symptomatic therapy

*Acetylcholine*

In the last decade improvement of cholinergic function has received most attention in the attempt to improve cognitive function in Alzheimer's disease. In the 1970's, it was discovered that Alzheimer patients had decreased levels of choline acetyltransferase (a cholinergic marker) in the hippocampus and cerebral cortex[13] that correlated with cognitive decline.[14] A few years later neuronal cell loss was shown in the nucleus basalis of Meynert, an important source for cortical cholinergic projections.[15] Furthermore, blockade of cholinergic receptors with scopolamine caused cognitive impairment in healthy volunteers that could partially be reversed by physostigmine.[16] Based on these findings the "cholinergic hypothesis" was proposed suggesting that the cognitive abnormalities including memory impairment in Alzheimer's disease could largely be attributed to cholinergic dysfunction.[17,18] In analogy with dopamine substitution in Parkinson's disease, compounds that enhanced central cholinergic function would improve symptoms of Alzheimer's disease.

Three approaches have been used for cholinomimetic therapy: the administration of metabolic precursors, inhibition of the enzyme acetylcholinesterase that degrades acetylcholine, and stimulation with agonists of the receptors situated post-synaptically to the degenerating cortical projections (Table 4.1). Each of these therapeutic approaches will be discussed.

(1) Precursor mono-therapy with choline or lecithin (phosphatidylcholine) has not been successful, either in short-term,[19,20] or in relatively long-term treatment.[21,22] A possible explanation for these findings is that precursors do not enhance central cholinergic activity.

(2) Cholinesterase inhibitors such as physostigmine and tetrahydro-aminoacridine (tacrine) may increase the synaptically available acetylcholine. A multitude of trials have been performed with physostigmine, a compound that is limited by its short

*Table 4.1 Therapeutic strategies for Alzheimer's disease*

| *Therapeutic strategy* | *Experimental compounds* |
|---|---|
| I. Symptomatic therapy | |
| 1. Acetylcholine | |
| A. Precursors | lecithin, choline |
| B. Cholinesterase inhibitors | physostigmine, tacrine, HP029 |
| C. Receptor agonists | RS-86, bethanechol, AF102B nicotine |
| 2. Noradrenaline | clonidine |
| 3. Serotonin | zimeldine, mCPP |
| 4. Dopamine | L-dopa, selegiline |
| 5. Neuropeptides | somatostatin, vasopressin, naltrexon |
| 6. GABA | THIP, $\beta$-carbolines |
| 7. Metabolic enhancers | hydergine, piracetam, pramiracetam, |
| II. Causal therapy | |
| 1. Amyloid metabolism | intervention in amyloid formation |
| 2. Excitoxin hypothesis | NMDA-antagonists, calcium blockers |
| 3. Oxy-radical scavengers | selegiline, acetyl-l-carnitine, lazaroids |
| 4. Growth stimulants | nerve growth factor, ganglioside GM-1 |

half-life, narrow therapeutic window and cholinergic side-effects. Double-blind studies have reported that physostigmine improves verbal memory and visuo-spatial abilities and a decrease of intrusions (spontaneous false answers),[23-27] while others studies have not substantiated these findings.[28,29] In negative studies, it is possible that the level of attained central cholinergic activity plays a role since an association was found between cognitive improvement and cholinesterase inhibition in the spinal fluid.[24]

Trials with tacrine have shown controversial results thus far,[30-37] and several studies have received criticism with regard to methodological issues (Table 4.2).

*Table 4.2 Clinical trials with tacrine*

| *Study (first author)* | *Study-design* | *Dose-titration** | *"Enrichment"* | *ADL* | *Result* |
|---|---|---|---|---|---|
| Summers[30,31] 1986 | cross-over | best-dose (maximal 200 mg) | no | no | +† |
| Chatellier[32] 1990 | cross-over | to maximal dose (125 mg) | no | no | - |
| Gauthier[33] 1990 | cross-over | to maximal dose (100 mg) | no | yes | - |
| Eagger[34] 1991 | cross-over | to maximal dose (150 mg) | no | yes | +/-‡ |
| Weinstein[35] 1991 | parallel | to maximal dose (100 mg) | no | yes | - |
| Davis[36] 1992 | parallel | best-dose (40 or 80 mg) | yes | yes | + |
| Farlow[37] 1992 | parallel | fixed-dose (20, 40 or 80 mg) | no | yes | + |

*Dose in mg tacrine per day
†Results questioned after FDA inspection[31]
‡Significant improvement in Mini-Mental State Examination, not in neuropsychological tests and ADL-measurements

Recently, it was concluded that there is no scientific evidence to treat patients with tacrine.[38-41] Criticism has focused on trial design, dose-titration, and choice of outcome measures. Few studies use the parallel design and are not subject to the disadvantages of the cross-over trial. Although at least some degree of response variability is to be expected with tacrine, most studies titrate to the maximally tolerated dose, that is not necessarily the best-dose. At best, it seems that tacrine could afford limited improvement in an early stage of the disease, because the effect of cholinesterase inhibitors is based on a functional reserve of cholinergic neurons that declines with progression of the disease. Moreover, the response variability and liver toxicity of tacrine limit the number of patients for potential treatment. In the

meantime, other cholinesterase inhibitors are under investigation including HP-029, and less toxic compounds are in development.

(3) The therapeutic rationale for receptor agonists is supported by the finding that post-synaptic receptors are relatively intact.[42] Cholinergic receptor agonists such as RS-86[43] and intravenously administered betanechol[44] have not shown convincing symptomatic benefit. A possible explanation for these findings is that agonists have an aspecific effect on subgroups of muscarinic receptors.[45] Selective agonists, for example the M1 agonist AF102B, could theoretically lead to better therapeutic results.[46] Attempts have been made to stimulate nicotinic receptors because the density of these receptors in cortical structures is decreased in Alzheimer's disease. In addition, through presynaptic receptors nicotine can stimulate the release of acetylcholine. Two trials show an increase in attention[47] and decrease of intrusions, but also a dose-related appearance of anxiety and depression.[48]

*Monoamines*

A decreased activity in central noradrenergic[49] and serotonergic[50] systems has been found in Alzheimer patients, and dopamine activity is possibly decreased as well.[45,51] Although noradrenaline and serotonin are the focus of many preclinical studies of memory functions and learning, the effect of monoamines in Alzheimer's disease is not consistent. In clinical studies, treatment with clonidine and the second generation $\alpha_2$-adrenergic receptor agonist guanfacine, was not effective.[52,53] Blockade of presynaptic serotonin uptake with zimeldine had no effect on cognitive or affective functions.[54] Furthermore, m-chlorophenyl-piperazine (mCPP), a serotonergic receptor agonist, negatively influenced memory and mood in Alzheimer patients and control subjects.[55] Although dopaminomimetic therapy yielded small improvement in aspects of cognitive function in non-demented parkinsonians,[56,57] no successful results were reported for patients with Alzheimer's disease.[58] However, short-term treatment with selegiline, a selective monoamine-oxidase-B inhibitor in relatively low dosages that blocks dopamine catabolism, suggested improvement in verbal memory and attention.[59]

*Neuropeptides*

The rationale for treatment of Alzheimer's disease with most neuropeptides is based on evidence from preclinical studies, including the improvement of learning and memory in animal models.[60] In Alzheimer dementia, changes in central neuropeptide

concentrations have been reported.[61] Most clinical trials, however, have reported negative results and a relationship with the advancement of neuronal cell loss was suggested.[62,63] A trial with a synthetic vasopressin analogon did not show clinical improvement.[64] ACTH-related compounds have an effect on attention and motivation in animal experiments,[65] but a study with ORG 2766, a synthetic analogon of ACTH 4-9, did not show clinical benefit.[66] Central somatostatin concentration are diminished in Alzheimer's disease and the reduction of serotonin and metabolites in spinal fluid reportedly correlate with the severity of dementia.[67] No significant improvement was found after treatment with the somatostatin analogon L363,586.[68] Opiate antagonists have been investigated in Alzheimer's disease because endorphines and enkephalines can impair memory functions.[69] A trial with naltrexon was without clinical effect.[70]

*GABA*

Gamma-amino-butyric acid (GABA) has an inhibitory effect on neuronal function and decreased activity in the temporal cortex,[71] and decreased levels in cerebrospinal fluid[72] are shown in Alzheimer patients. An attempt to improve cognitive function with tetrahydro-isoxazolpyridine (THIP) was not successful.[73] The benzodiazepine agonist diazepam, on the other hand, impairs cognitive function in healthy individuals, possibly through GABA activation.[74] A relative overactivation of GABA in the presence of reductions in other neurotransmitters could contribute to the symptoms of Alzheimer's disease.[75] The $\beta$-carbolines, inverse benzodiazepine agonists that antagonate GABA-ergic transmission, will therefore be tested in clinical trials.

*Metabolic enhancers*

Numerous clinical trials have been conducted to evaluate the putative effect of metabolic enhancers with unknown pharmacologic mechanisms of action. It is suggested for some compounds that they stimulate neuronal metabolism and increase cerebral perfusion, such as for codergocrine (hydergine).[76] Hydergine is the only compound marketed in the U.S.A. for symptomatic treatment of Alzheimer dementia, but its clinical effect is controversial. A recent study with 80 patients concluded that hydergine is ineffective in Alzheimer's disease.[77] This confirms the findings of other studies that hydergine and related compounds have no clinical effect in memory impairment and dementing disorders.[78]

Nootropics are a group of psychotropic drugs of which piracetam was first discovered as GABA derivative. The precise mechanism of action is unknown but

may be related to facilitation of cholinergic function.[79] In animal models of dementia these compounds are effective in the reversal of scopolamine or hypoxia induced amnesia, but the validity of these animal models has been questioned.[80] Piracetam at high dosages increases cerebral metabolism in Alzheimer patients,[81] but its clinical effect is minimal[82] or absent.[83] The same results were found with oxiracetam.[84] To accommodate the inverted U-shaped dose-response curve in most nootropics, a recent clinical trial employed a two-phase "enrichment-population" design. No improvement was found in cognition, mood, or ADL functions.[85] Sabeluzole, a pharmaceutical with no interaction with memory related neurotransmitters,[86] is currently under evaluation in a multi-center trial. The therapeutic potential of nootropics will receive further attention in mildly affected Alzheimer patients and in age-associated memory impairment. A large number of compounds is in development and will be evaluated in clinical studies.

## New areas of research: causal therapy

*Amyloid metabolism*

The neuropathologic hallmarks of Alzheimer's disease, neuritic plaques and neurofibrillary tangles, have characteristic locations in neocortex, hippocampus, amygdala and subcortical nuclei projecting to the cortex.[87,88] It has been suggested that the deposition of the 42 amino acids containing $\beta$-amyloid protein ($\beta$AP) in discrete plaques is an early event in the pathogenesis of Alzheimer's disease.[89] However, it remains unknown whether the formation of amyloid depositions arises as a primary consequence of the degenerative process or whether these structures may appear secondarily as a part of the complex pathogenetic cascade. The $\beta$AP is released through proteolytic activity from the transmembrane part of the 695 amino acids containing amyloid precursor protein, the precursor of $\beta$AP.[90] Several variants of amyloid precursor protein have been found, including one with a 56 amino acids containing insertion with protease inhibiting activity of the Kunitz type.[91] The neuritic plaques contain several proteases, including elastase and ubiquitine, and protease inhibitors such as $\alpha_1$-antichymotrypsine. As yet, there is little understanding of the significance of these proteases and protease inhibitors in the pathogenesis of amyloid formation, but abnormal genetic expression and abnormal degradation of the amyloid

precursor protein could play a role. The neurofibrillary tangles contain intracellular accumulated structures that can be visualized with electronmicroscopy as "paired helical filaments". One of the proteins found in these structures is tau.[92] Abnormal phosphorylation of tau may contribute to the formation of neurofibrillary tangles, but this process remains largely unknown.

Intervention in the complex cascade of amyloid formation could possibly prevent amyloid depositions, for example with the development of selective protease inhibitors. Furthermore, modifications of the genetic expression of the amyloid precursor protein with DNA- or RNA-binding proteins or with promotor specific proteins are considered as possible interventions. The development of transgenic mouse models expressing an extra amyloid gene is important to test potential therapeutic drugs. Encouraging results were recently reported, but two of these papers were retracted,[93,94] while the other transgenic mouse does not provide a model for testing drugs at this stage.[95] New insights in amyloid formation are necessary before rational intervention is possible.

*Excitotoxin hypothesis*

Excitatory amino acids including glutamate and aspartate are neurotransmitters of pyramidal neurons in cerebral cortex and hippocampus that play a role in memory and learning.[96] In Alzheimer's disease, these neurons are involved in the neuropathology and contain neurofibrillary tangles.[97] The glutamatergic dysfunction could contribute to the symptoms of Alzheimer's disease and a relation of glutamate neurotoxicity with biochemical and structural changes has been suggested.[98] Excitatory amino acids are potentially neurotoxic in instances of overactivation, and the N-methyl-D-aspartate (NMDA) receptor has been suggested to play a crucial role.[99] Hyperstimulation of these receptors, for example through overproduction or decreased uptake of glutamate, can lead to intracellular influx of chloride and calcium and may result in neuronal death.[100] Inhibition of excitatory amino acid activity has the potential of neuroprotection as was shown in animal models of ischemia and hypoglycaemia.[101] If excitotoxicity plays a role in the neuronal degeneration of Alzheimer's disease, excitatory amino acid antagonists could retard the progression of the disease. Other potential avenues for therapeutic intervention are modulation of receptor function through the glycine recognition site of the NMDA receptor complex, and calcium antagonists can block the calcium entry into the cell that occurs with activation of the NMDA receptor. Clinical trials with NMDA antagonists could

contribute to the understanding of the role of excitotoxicity in the pathogenesis of Alzheimer's disease. At present, these compounds have substantial side-effects which is a limitation for their clinical application.

*Anti-oxidants*

Under normal circumstances the cell produces free oxy-radicals, superoxides, and peroxides, that are scavenged by superoxide dismutase to prevent potential toxicity. A defect in this protection mechanism or overproduction of incompletely reduced oxygen molecules can lead to cell injury, for example through peroxidation of membrane lipids. A recent study suggests that selegiline retards the progression of Parkinson's disease, possibly through inhibition of the presumptive oxidative effects associated with MAO-B activity.[102] MAO-B activity in the cerebral cortex is increased in normal aging and in Alzheimer patients,[103,104] and MAO-B activity is decreased in the nucleus basalis in Alzheimer's disease.[103] The hypothesis that oxidative stress plays a role in the neuronal degeneration of Alzheimer's disease is under investigation with long-term administration of selegiline. Other compounds that could produce neuroprotection through this mechanism are acetyl-l-carnitine[105] and the lazaroid pharmaceuticals that inhibit lipid peroxidase.[106]

*Growth factors*

New therapeutic possibilities are sought with the development of nerve growth factors that have a function in neuronal growth and survival. Several studies show that nerve growth factor has a trophic function for cholinergic neurons.[107,108] In aged rats, nerve growth factor improves visual memory and decreases atrophy of cholinergic neurons.[109] Administration of nerve growth factor in an early phase of Alzheimer's disease could decrease cell loss, but the possibility that the pathologic process is enhanced can not be excluded.[110] Recently, guidelines were provided by a workgroup of the National Institute on Aging for the clinical application of nerve growth factor including the conduct of future studies on toxicity, dosage, and best way of administration.[111] Other growth stimulating factors such as ganglioside GM-1 are under investigation without definitive results. A study of GM-1 treatment of mild to moderately severe patients with Alzheimer's disease showed no overall symptomatic benefit.[112]

## Conclusion

Symptomatic treatment of Alzheimer's disease with neurotransmitter substitution or metabolic stimulation has yielded disappointing results so far. Several new compounds are in development or are tested for clinical efficacy which may lead to a palliative therapy in the future. This therapeutic strategy does not interfere in the underlying pathogenetic process which stresses the need for the development of interventions in causal mechanisms. Methodological aspects are important for the interpretation of trial results. Several studies have been criticized for issues related to trial design, dose-titration, and choice of adequate outcome measures. The increasing knowledge in recent years of the neurobiology and molecular genetics of Alzheimer's disease has stimulated several hypotheses on the etiology. Various mechanisms are now suggested to play a role in the neuronal degeneration of Alzheimer's disease, including amyloid metabolism, excitatory amino acids, growth factors, and oxidative stress. The development of treatment strategies based on these insights may lead to intervention that retards or halts the progression of the disease and will contribute to the understanding of the etiology.

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# Chapter 5

# Studies of therapy

*Chapter 5.1*

# Clinical trials in dementia: learning effects with repeated testing

## Introduction

Clinical trials of putative antidementia drugs, have increased dramatically during the past decade. Study designs, evaluating the effect of experimental agents on cognition, often employ various treatment conditions, such as dose-finding or multiple drug levels versus placebo.[1,2] Repeat testing involved in these designs is, as much as possible, performed with alternate forms in an attempt to create equivalent conditions. It is unknown, however, whether multiple testing is immune to practise effects.

Residual learning abilities have been reported in patients with Alzheimer's disease, even in advanced stages of this disorder.[3] For example, studies of procedural learning yielded comparable acquisition curves in Alzheimer patients and matched controls.[4,5] These data suggest that Alzheimer patients may show learning in certain instances, when tested repeatedly.

Current concerns about repeat testing and learning effects have been addressed by randomization of placebo and drug treatment. Nevertheless, the possible confounding role of learning on outcome measures remains uncertain. Learning effects may affect measurement accuracy by increasing overall variance.[6] To further evaluate this problem, we selected a broad sample of both verbally and visuospatially mediated tests as well as of attention, both manually administered and computerized, for repeat administration to mimic a baseline, placebo, and drug condition.

## Methods

Seventeen patients (7 men, 10 women), with the diagnosis of Alzheimer's disease,[7] and National Institute of Neurological and Communicative Disorders and Stroke-Alzheimer's Disease and Related Disorders Association criteria of probable Alzheimer's disease,[8] were randomly selected from participants of drug trials at the National Institute of

Neurologic Disorders and Stroke, and consented to participate in this study. Patients had a mean (± SEM, range) age of 64 ± 1.7 years (55 to 77), education 16 ± 0.7 years (12 to 20); duration of dementia symptoms was 4 ± 0.7 years (1 to 11). Dementia severity was assessed with the Mattis Dementia Rating Scale[9] and ranged from mild (129) to severe (73), with a mean score of 104 ± 3.5. Sixteen healthy subjects (10 men, 6 women), matched for age (mean 64 ± 1.7; 49 to 73) and education (mean 17 ± 0.9; 12 to 20), served as controls. Intellectual and memory status was evaluated at study onset with the Wechsler Adult Intelligence Scale-Revised[10] and the Wechsler Memory Scale (Table 5.1.1).[11]

*Table 5.1.1 Demographic variables**

| | Alzheimer patients | Control subjects |
|---|---|---|
| Age | 64 ± 1.7 (55- 77) | 64 ± 1.7 ( 49- 68) |
| Education | 16 ± 0.7 (12- 20) | 17 ± 0.9 ( 12- 20) |
| Symptom duration | 4 ± 0.7 ( 1- 11) | |
| WAIS-R verbal IQ | 82 ± 2.9 (62-106) | 120 ± 3.3 ( 92-144) |
| WAIS-R performance IQ | 75 ± 3.1 (62-105) | 121 ± 2.8 (101-135) |
| WAIS-R full scale IQ | 79 ± 2.6 (62-106) | 123 ± 3.2 ( 97-141) |
| Wechsler Memory Scale | 70 ± 3.1 (52-100) | 128 ± 2.9 (103-143) |
| Mattis Dementia Rating Scale | 104 ± 3.5 (73-129) | 142 ± 0.4 (140-144) |

*Values are the means ± SEM (range)

Neuropsychological function was assessed with tests of verbal and visual memory, as well as attention. The battery consisted of both manually administered tests and computerized evaluations. Computerized testing was performed with a touch-sensitive television screen.[12] Subjects were tested at baseline with alternate forms of the same tests in random order at weekly intervals for a total of 3 sessions. Duration of testing was approximately 90 minutes and occurred at the same time for all assessment sessions.

Verbal memory was assessed with the following neuropsychological tests: Verbal Learning,[13] Logical Memory,[11] Paired Associate Learning,[11] Selective Reminding (computerized).[12] Visual memory was evaluated with the following items: Benton Visual Retention Test,[14] Misplaced Objects (computerized),[12] Delayed Non-Matching to Sample (computerized).[12] In addition, a novel experimental Visual Learning Test was administered: 5 trials with immediate and 1 with delayed recall. Five targets (indicated by a crossed out dot) on a 5 by 5 dotted matrix, had to be located after a 10 second exposure to the target design on a blank matrix. Attention was measured with Telephone Dialing (computerized).[12] Data were analyzed with repeated measures analysis of variance (BMDP P2V)[15] with the following design: first, both patients and controls combined over trial 1, 2, and 3, to determine order of test trial and group interaction effects. If the order of test trial effect was significant, a post-hoc analysis (BMDP P2V) was performed to determine learning curves for groups separately. In case of significant group interactions, a post-hoc analysis for groups was performed separately to determine differential learning effects. Effects on performance across trials were evaluated by calculating a symmetric change score.[16] Results are reported as means ± SEM. Correlations between demographic variables and change scores were evaluated by Pearson's correlational analysis (BMDP P7D).

## Results

Alzheimer patients were significantly impaired compared to controls on virtually all functions assessed (Table 5.1.2). Evaluation of learning effects with repeated measures analysis of variance showed that patients were unable to significantly enhance performance from the initial trial (trial 1) to subsequent trials (trial 2 and 3). In contrast, normal age-matched controls evidenced consistent learning effects in 3 of 9 neuropsychological tests: improvement took place in two tasks of visual memory, Misplaced Objects 1st try ($p < .05$) and the Benton Visual Retention Test (BVRT) immediate recall ($p < .05$), as well as in one test of verbal memory, Logical Memory delayed recall ($p < .05$).

More subtle learning effects, sought through correlational analysis between demographic variables and change scores (trial 1 to 3), suggested a significant association in Alzheimer patients between dementia severity (Mattis Dementia Rating Scale) and percentage change in Verbal Learning (immediate recall; $r = .51$, $p < .05$), and Visual

*Table 5.1.2 Mean test scores*

| | Alzheimer patients | | | Control subjects | | |
|---|---|---|---|---|---|---|
| | Trial 1 | Trial 2 | Trial 3 | Trial 1 | Trial 2 | Trial 3 |
| Verbal Memory | | | | | | |
| Verbal Learning total§ | 15.5 ± 1.3* | 16.2 ± 1.8 | 15.8 ± 1.7 | 34.2 ± 1.0 | 33.7 ± 1.0 | 33.8 ± 0.9 |
| Verbal Learning delay | 0.5 ± 0.3* | 0.4 ± 0.3 | 0.4 ± 0.3 | 4.8 ± 0.6 | 4.1 ± 0.7 | 5.0 ± 0.6 |
| Logical Memory total | 1.4 ± 0.3* | 1.6 ± 0.4 | 2.0 ± 0.3 | 9.4 ± 0.8 | 9.5 ± 0.8 | 10.3 ± 0.7 |
| Logical Memory delay | 0.5 ± 0.2* | 0.4 ± 0.2 | 0.3 ± 0.1 | 7.3 ± 1.0 | 7.8 ± 0.7 | 9.0 ± 0.6‡ |
| Paired Associates total | 3.5 ± 0.8* | 3.4 ± 0.5 | 3.7 ± 0.7 | 16.5 ± 0.9 | 15.9 ± 0.8 | 15.5 ± 0.9 |
| Paired Associates delay | 1.4 ± 0.3* | 1.1 ± 0.2 | 1.2 ± 0.2 | 5.5 ± 0.5 | 5.8 ± 0.4 | 5.5 ± 0.4 |
| Selective Reminding total | 11.4 ± 1.9* | 12.4 ± 1.8 | 12.4 ± 1.9 | 49.1 ± 3.4 | 48.9 ± 3.4 | 49.9 ± 3.5 |
| Selective Reminding delay | 1.3 ± 0.4* | 1.4 ± 0.4 | 1.4 ± 0.5 | 11.3 ± 0.7 | 10.9 ± 1.0 | 10.7 ± 0.9 |
| Visual Memory | | | | | | |
| Misplaced Objects 1st try | 3.4 ± 0.6* | 3.0 ± 0.4 | 3.5 ± 0.6 | 11.9 ± 0.9 | 13.6 ± 0.8 | 14.4 ± 0.8‡ |
| Misplaced Objects 2nd try | 5.3 ± 0.8* | 5.0 ± 0.6 | 5.5 ± 0.9 | 15.6 ± 0.7 | 16.4 ± 0.7 | 16.6 ± 0.7 |
| Visual Retention total | 2.9 ± 0.4* | 3.8 ± 0.5 | 3.4 ± 0.5 | 6.0 ± 0.4 | 6.8 ± 0.3 | 7.1 ± 0.2‡ |
| Visual Retention delay | 2.6 ± 0.4* | 2.6 ± 0.5 | 2.9 ± 0.5 | 6.4 ± 0.4 | 6.6 ± 0.4 | 6.6 ± 0.4 |
| Visual Learning total | 11.9 ± 1.2* | 12.8 ± 1.6 | 13.6 ± 1.7 | 20.5 ± 1.7 | 21.7 ± 1.5 | 21.6 ± 1.9 |
| Visual Learning delay | 2.2 ± 0.3* | 2.4 ± 0.3 | 2.2 ± 0.4 | 4.8 ± 0.5 | 4.3 ± 0.5 | 4.3 ± 0.6 |
| Delayed Non Match Sample | 7.6 ± 1.3* | 7.9 ± 1.2 | 8.3 ± 1.2 | 19.7 ± 0.8 | 18.8 ± 0.8 | 18.9 ± 1.7 |
| Attention | | | | | | |
| Telephone Dialing 3 numbers | 2.3 ± 0.2† | 2.2 ± 0.2 | 2.3 ± 0.2 | 3.0 ± 0.2 | 3.0 ± 0.0 | 3.0 ± 0.0 |
| Telephone Dialing 7 numbers | 2.9 ± 0.6* | 3.4 ± 0.5 | 3.2 ± 0.5 | 6.7 ± 0.1 | 6.6 ± 0.1 | 6.8 ± 0.1 |
| Telephone Dialing 10 numbers | 2.3 ± 0.5* | 2.3 ± 0.4 | 2.2 ± 0.5 | 6.9 ± 0.4 | 7.4 ± 0.4 | 7.0 ± 0.4 |

*Alzheimer patients baseline (trial 1) significantly different from baseline controls at $p < 0.001$
†Alzheimer patients baseline (trial 1) significantly different from baseline controls at $p < 0.05$
‡Significant performance increase in controls across trial 1, 2 and 3 at $p < 0.05$
§Total scores represent total immediate recall

Learning (immediate recall; $r = .59$, $p < .01$). Thus, the lower the cognitive impairment, the higher the capacity to increase performance across trials. No consistent relation was found for any of the other demographic variables and change scores in patients or in the control group.

## Discussion

No alterations were found in performance in Alzheimer patients across test trials; in contrast, control subjects demonstrated learning in one third of all neuropsychological tasks administered. Although alternate randomized test forms were used in each of the 3 trials, controls increased scores in tests of verbal and visual memory, in manual as well as computerized tests. Thus learning seemed not confined to a certain cognitive domain or to a specific testmodality. On the other hand, the possibility exists that ceiling effects in neuropsychological testing in controls (e.g. Visual Learning, Telephone Dialing) and floor effects in testing in Alzheimer patients (e.g. delayed recall scores of most tests) play a role in the absence of learning in these tests.

Learning potential in Alzheimer patients is suggested on the basis of reported abilities in procedural learning and implicit memory,[4,5,17] and normal rates of forgetting as compared to controls.[18,19] Accordingly, it could be expected that these residual capacities of memory and learning confound outcome measures in clinical trials. However, in the design employed in this study, chosen to model the widely used cross-over design with a comparison of baseline, drug and placebo, no learning could be ascertained in the patient group. Although some learning capacity may in fact be retained, the time interval of one week and the use of alternate forms could be sufficient to prevent Alzheimer patients from learning on these tests. On the other hand, learning could be a confounding factor when the number of test trials exceeds the number of available forms, which makes repetition of the same form necessary. Indeed, Alzheimer patients show better performance when tested repeatedly with same test items than with alternate items over a relatively long period of time,[20] and improve in a variety of cognitive domains when the same form is repeated.[3]

Subtle learning effects were suggested in the patient group by the association of symptom severity with change in verbal as well as visual learning. Encoding and learning may thus be preserved to a certain extent in patients with mild Alzheimer's disease. However, the patient number performing in the range of mild dementia is too small in

the present study to address this issue in terms of seeking a subset showing consistent learning.

The present results indicate that alternate forms of neuropsychological tests are not subject to learning in patients with Alzheimer's disease, in particular in those with moderately severe impairment. However, the suggestion of relative preservation of some learning capacity in patients with mild Alzheimer's disease and consistent learning in controls suggest that mental status could play an important role in the acquisition of material and the ability to increase performance. Thus, subjects with no or minimal cognitive impairment may show learning effects, which can decrease measurement precision in clinical trials.[6] Accordingly, researchers using repeat testing in controls or mild Alzheimer's disease, should be cautious for the possible confounding role of learning effects, even when alternate test forms are used.

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*Chapter 5.2*

# Nootropic drugs in Alzheimer's disease: symptomatic treatment with pramiracetam

## Introduction

Nootropics are a class of psychotropic drugs that enhance learning acquisition and reverse learning impairment in the experimental animal.[1] As yet, there has been no established mechanism of action for these drugs, but their toxic potential appears to be low.[1,2] The initial compound discovered was piracetam, but several more potent nootropic drugs have since been developed,[3,4] including pramiracetam.[5]

Behavioral pharmacologic studies with pramiracetam have yielded positive results in several animal models of learning and memory.[2] These models include reversal of electroconvulsive shock induced amnesia, improved performance in a delayed spatial alternation task, and reversal of learning deficits produced by hippocampal stimulation in rodents as well as facilitation of visual discrimination learning in primates. The mechanism of action of nootropic drugs remains poorly understood, although for some, cholinomimetic effects may contribute to the cognitive enhancing effects.[6] Pramiracetam, for example, increases high affinity choline uptake in rat hippocampus[7,8] and may stimulate hippocampal cholinergic neuronal impulse flow.[7] Pramiracetam shows no effect on other central neurotransmitter levels and does not bind to their receptors.[7]

To date, although it has been difficult to document the ability of any nootropic to alleviate symptoms of Alzheimer's disease (AD),[9,10] previous clinical trials failed to employ adequate dose-finding in the individual patient, despite the characteristic presence of an inverted U-shaped dose-response curve for these drugs in the experimental animal.[4,11,12] Moreover, preclinical studies often reveal considerable intersubject variability with respect to the optimally effective dose. Clinical trials using a broad range of doses in an initial dose-finding phase, followed by a replication phase for those patients evidencing a best-dose, might help compensate for both these problems. As a test of this possibility, we evaluated the antidementia efficacy of pramiracetam, based on its activity in animal models and its reported effects on cholinergic function.

## Patients and methods

Ten patients (3 men, 7 women) who satisfied NINCDS-ADRDA criteria[13] for probable AD consented to participate in this study after full disclosure of its potential risks and benefits. Mean (± SEM and range) age was 66 ± 2.3 years (55 to 77), education 14 ± 1.4 years (8 to 20), and symptom duration 4 ± 0.7 years (1 to 7). Dementia severity, assessed with the Mattis Dementia Rating Scale,[14] ranged from moderately severe to mild (68 to 125), with a mean score of 101 ± 5.2. Wechsler Adult Intelligence Scale-Revised[15] yielded a mean full scale IQ of 74 ± 3.6 (62 to 83), and Wechsler Memory Scale[16] an MQ of 70 ± 3.9 (59 to 96). All patients were free of centrally active medications for at least 4 weeks prior to study.

Pramiracetam was evaluated in 2 phases under double-blind placebo-controlled conditions. In an initial dose-finding phase, baseline assessments were obtained after 1 week treatment with placebo. Pramiracetam was then introduced at an initial dose of 300 mg per day, and increased by weekly increments of up to 50% until a daily dose of 4000 mg per day was attained. The duration of this phase was 5 weeks, but for safety purposes the initial 3 patients were treated for 8, 7 and 6 weeks respectively. Neuropsychological testing was performed weekly by means of the cognitive portion of the Alzheimer's Disease Assessment Scale (ADAS).[17] The non-cognitive portion of the ADAS was not included in the assessment of drug efficacy because the rating is relatively subjective. Patients found to have a "best-dose" (the largest ADAS scale reduction of at least 4 points) then entered a 2nd, replication phase, during which they received 2 weeks placebo and of pramiracetam treatment in random order. Placebo was given for at least 1 week immediately preceding and following active drug administration.

Drug efficacy was evaluated by a blinded rater during baseline, placebo and best-dose treatment periods with alternate forms of the following neuropsychological tests: cognitive ADAS,[17] Verbal Selective Reminding Test[18] (15 word version), Digit Span,[15] Word Fluency,[19] and Logical Memory.[16] Visual memory was assessed with a novel Visual Learning Test using 5 trials with immediate and 1 with delayed recall. Five targets (indicated by a crossed out dot) on a 5 by 5 dotted matrix, have to be located after a 10 second exposure to the target design on a blank matrix. In addition, the Dementia Mood Assessment Scale,[20] a modified version of the Instrumental Activities of Daily Living Scale,[21] and a novel Visual Selective Reminding Test (developed as an analog to the Verbal Selective Reminding Test; 15 pictures) were administered. In comparison to 8 healthy subjects (2 men, 6 women: mean age 66 ± 1.8, 57 to 71; mean education 15 ±

1.2, 12 to 20), matched for age, sex and education to those patients that completed the replication phase, AD patients were significantly impaired in most outcome measures (Table 5.2.1).

Pramiracetam levels were measured[22] in plasma during the dose-finding and replication phases as well as simultaneously in plasma and CSF after 2 additional weeks of best-dose treatment in 7 patients to assess blood-brain-barrier penetration. Lumbar punctures were performed 3.5 hours after the 1st morning drug dose. Pramiracetam side effects were monitored every 2 weeks with ECG and blood samples.

Drug effects on brain energy metabolism were studied in 2 patients using 18F-Fluorodeoxyglucose and Scanditronix-15B Positron Emission Tomography (PET) camera. Patients were scanned (using deoxyglucose autoradiographic method applied to PET)[23] without drug and after 2 weeks of best-dose treatment. During the 2nd scan the head was precisely repositioned in the same position by using the mask employed in the 1st scan. 18F-Fluorodeoxyglucose (5mc) was injected 180 min after drug ingestion and scanning began 30 min later. Irregular regions of interest were defined with the help of an anatomical atlas[24] for cortical and subcortical structures in 9 contiguous slices of the 1st scan and copied on to the corresponding slices of the 2nd scan. In patient 1 absolute quantification was not obtained (arterial blood samples not available) and differences between scans were calculated in regions of interest/whole brain ratios.

Neuropsychological data are reported as means ± SEM and analyzed with ANOVA techniques (BMDP P7D)[25] and repeated measures analysis of variance (BMDP P2V).[25] Correlations of spinal fluid pramiracetam levels and pretreatment psychometric values with drug therapy change scores[26] in the replication phase were evaluated by Pearson's correlational analysis (BMDP P7D).[25]

## Results

During the 1st phase of this study, 8 of the 10 patients were found to have a best-dose of pramiracetam (Table 5.2.2). In these individuals, improvement in ADAS scores, where lower values reflect better performance, ranged from -8.3 to -4.3 (Table 5.2.3). ADAS score changes in the 2 remaining patients ranged from -0.6 to 7. The degree of change did not correlate with untreated dementia severity, as measured by the Mattis Dementia Rating Scale.

***Table 5.2.1*** *Effect of pramiracetam on cognitive function**

| | Controls | Patients | | |
|---|---|---|---|---|
| | Baseline | Baseline | Placebo | Drug |
| General cognitive function | | | | |
| ADAS cognitive | 2.9 ± 0.6 | 30.8 ± 4.2† | 29.9 ± 4.3 | 29.1 ± 4.0 |
| ADAS non-cognitive | 0.5 ± 0.2 | 4.5 ± 1.7‡ | 4.9 ± 1.3 | 4.9 ± 1.5 |
| Activities of Daily Living | | 35.7 ± 9.3† | 35.9 ± 9.3 | 35.6 ± 9.3 |
| Mood Assessment | | 26.6 ± 4.9† | 24.0 ± 5.8 | 25.0 ± 5.7 |
| Verbal Memory | | | | |
| Verbal Selective Reminding total | 43.6 ± 4.4 | 15.4 ± 2.3† | 12.9 ± 2.5 | 12.5 ± 2.1 |
| Verbal Selective Reminding delay | 5.5 ± 1.2 | 0.0 ± 0.0† | 0.4 ± 0.4 | 0.0 ± 0.0 |
| Digit Span forward | 10.4 ± 0.7 | 8.1 ± 1.0 | 8.3 ± 0.8 | 7.6 ± 0.8 |
| Digit Span backward | 7.9 ± 0.9 | 3.3 ± 0.5† | 3.1 ± 0.9 | 3.8 ± 0.5 |
| Logical Memory immediate recall | 8.7 ± 1.5 | 1.1 ± 0.2† | 1.5 ± 0.4 | 1.4 ± 0.4 |
| Logical Memory delayed recall | 7.1 ± 1.5 | 0.4 ± 0.3† | 0.3 ± 0.2 | 0.3 ± 0.2 |
| Verbal Fluency total | 43.0 ± 4.0 | 10.4 ± 3.4† | 10.1 ± 3.6 | 9.3 ± 4.1 |
| Verbal Fluency perseverations | 1.5 ± 0.9 | 1.9 ± 1.0 | 1.8 ± 0.9 | 1.0 ± 0.9 |
| Visual Memory | | | | |
| Visual Selective Reminding total | 52.4 ± 3.1 | 14.8 ± 3.5† | 14.0 ± 2.5 | 16.5 ± 3.2 |
| Visual Selective Reminding delay | 11.6 ± 0.6 | 1.1 ± 0.8† | 0.5 ± 0.5 | 1.4 ± 0.9 |
| Visual Learning total | 17.4 ± 2.5 | 10.9 ± 2.5 | 14.1 ± 2.3 | 14.4 ± 3.1 |
| Visual Learning delay | 4.4 ± 0.7 | 1.4 ± 0.3‡ | 1.9 ± 0.6 | 2.9 ± 0.6§ |

*Values are the means ± SEM
†Baseline AD patients significantly different from baseline controls at $p < 0.001$
‡Baseline AD patients significantly different from baseline controls at $p < 0.05$
§Drug significantly different from baseline and placebo at $p < 0.05$

*Table 5.2.2 Pramiracetam levels in plasma and CSF**

| | Best dose (mg/day) | Plasma (μm/ml) | CSF (μm/ml) | CSF/plasma ratio |
|---|---|---|---|---|
| Patient | | | | |
| 1 | 2,000 | 7.5 | 1.0 | 0.13 |
| 2 | 3,200 | 12.7 | 4.1 | 0.32 |
| 3 | 1,600 | 9.1 | 2.0 | 0.22 |
| 4 | 2,400 | 11.4 | 1.4 | 0.12 |
| 5 | 3,200 | 14.5 | 3.8 | 0.26 |
| 6 | 2,000 | 6.8 | 1.1 | 0.16 |
| 7 | 1,200 | 1.3 | 0.5 | 0.38 |
| Mean ± SEM | 2,229 ± 288 | 9.0 ± 3.4 | 2.0 ± 0.5 | 0.23 ± 0.04 |

* Values are from simultaneously obtained venous blood and lumbar CSF samples from all but one patient evidencing a best dose of pramiracetam. The missing patient had a best dose of 4,000 mg/d and completed the study but was unsuitable for lumbar punction

During the 2nd, replication phase, all 8 patients evidenced a significant improvement in the Visual Learning delay task ($p < .05$). There was, however, no improvement on any other neuropsychological test (Table 5.2.1). Indeed, the ADAS score change from placebo to drug treatment averaged only -0.8 ± 1.1 (Table 5.2.3). Moreover, there was no correlation between the change in ADAS scores from baseline to best-dose in the dose-finding phase and alteration from placebo to drug in the replication phase.

Evaluation of individual patient data indicated that in the replication phase only 2 individuals (patient 1 and 2) had a more than 4 point reduction in their ADAS score with drug treatment (Table 5.2.3). Patient 1 also showed improvement in 3 other neuropsychological tests (Visual Selective Reminding total and delay, Visual Learning total and delay, and Logical Memory immediate recall). Analysis of individual dose-response curves in the dose-finding phase revealed no consistent inverted U-shaped pattern.

PET scans using 18F-Fluorodeoxyglucose were completed in the 2 patients evidencing more than a 4 point improvement in the ADAS scale. Relative metabolic values (ratio of cerebral metabolic rates for glucose for regions of interest to the whole brain) showed

*Table 5.2.3 Effect of pramiracetam on cognitive ADAS scores*

| | Dose-finding phase* | | | Replication phase | | |
|---|---|---|---|---|---|---|
| Patient | Baseline | Best-dose | Change | Placebo | Best-dose | Change |
| 1 | 40.3 | 32.0 | -8.3 | 36.3 | 32.0 | -4.3 |
| 2 | 21.0 | 15.6 | -5.4 | 21.6 | 16.3 | -5.3 |
| 3 | 35.6 | 30.4 | -5.2 | 35.7 | 35.0 | -0.7 |
| 4 | 20.7 | 16.3 | -4.4 | 18.7 | 21.7 | +3.0 |
| 5 | 38.0 | 32.4 | -5.6 | 34.0 | 35.0 | +1.0 |
| 6 | 21.0 | 16.7 | -4.3 | 21.3 | 21.0 | -0.3 |
| 7 | 23.4 | 18.3 | -5.1 | 18.7 | 21.0 | +2.3 |
| 8 | 52.3 | 45.3 | -7.0 | 53.0 | 50.7 | -2.3 |
| Means ± SEM | 31.5 ± 4.2 | 25.9 ± 3.8 | -5.7 ± 0.5 | 29.9 ± 4.3 | 29.1 ± 4.0 | -0.8 ± 1.1 |

* Two patients did not evidence an improvement of 4 points or more. Baseline scores and best scores in dose finding were 34.0 and 35.7 for patient 9 and 7.3 and 6.7 for patient 10

no consistent differences between the untreated and pramiracetam treated states: these differences from frontal, temporal, and parietal cortex, and from striata and thalamus ranged from -7.0 to 4.3 in patient 1, and from -8.4 to 4.6 in patient 2. Absolute metabolic values obtained in patient 1 showed an increase in glucose consumption (ranging from 9% to 20%) while on drug as compared to placebo.

Measurement of pramiracetam levels revealed a consistent oral dose-plasma level relationship (Figure 5.2). CSF/plasma partition coefficients for the group ranged from 0.12 to 0.38 (Table 5.2.2). No consistent relation between cognitive change scores and spinal fluid pramiracetam levels or pretreatment neuropsychological tests could be established.

Side effects occurring during pramiracetam administration were few and mild. Two patients described headache at 400 to 1200 mg/day, and one experienced sleepiness, decreased appetite and dizziness for 2 days when placebo was begun after treatment with the highest drug dose (4000 mg). Pramiracetam had no effect on vital signs, standard hematologic and blood chemistry values or on the ECG.

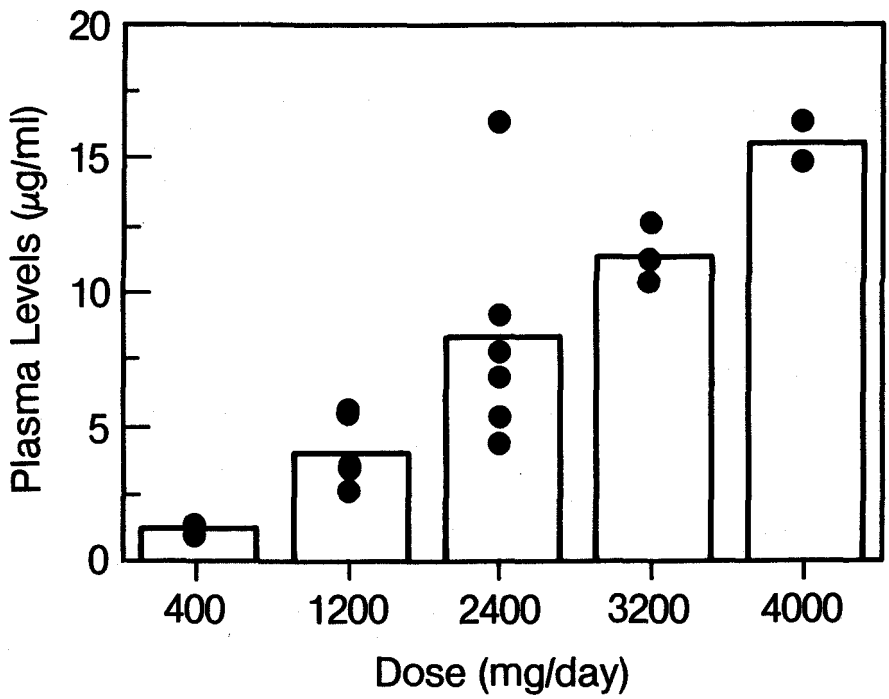


*Figure 5.2 Effect of pramiracetam on plasma drug levels*

## Discussion

We could not demonstrate any clinically significant improvement in cognition, mood or activities of daily living in the pramiracetam treated AD patients admitted to this study. Indeed, among the numerous outcome measures employed, statistically significant benefit occurred only in the delay scores from a visual learning task. Since similar tests showed no such change, these seemingly positive results presumably reflect the chance variations that are frequently observed when using multiple measures. Our findings thus failed to confirm the encouraging inferences drawn from an earlier open-label trial using much lower pramiracetam doses,[27] and extended those of previous controlled studies of other nootropics indicating that agents of this type confer no consistent symptomatic benefit in AD[28,29] and in Parkinson's disease.[30]

Strenuous efforts were made to improve the design in this clinical trial so the possibility of demonstrating antidementia efficacy would be maximally increased. In particular, we attempted to deal with the potential problems of narrow range of efficacy and wide intersubject variability by means of a 2 phase study, initially involving the ascertainment of a putative best-dose for each patient over a wide range of drug dosages. At 1st glance it would seem surprising that our "enrichment" strategy of admitting only patients showing an "effect" of pramiracetam in the initial dose-finding phase to the subsequent replication phase actually seemed to function counterproductively: while 80% of those entering the study were found to have a best-dose and thus to evidence a presumptive antidementia effect, only 25% of this subgroup achieved a similar response during the replication phase. Presumably, a 4 point change in the cognitive portion of the ADAS simply reflects the variability normally attending the repeated administration of this test under similar conditions over a relatively long period. Future applications of this enrichment strategy using the ADAS may thus require more rigorous criteria for admission into the replication phase.

The failure of this study to demonstrate antidementia activity also does not appear attributable to pharmacokinetic issues. Pramiracetam doses administered here, calculated on a mg/kg basis, were in the range found to benefit behavioral function in the experimental animal (2). Moreover, pramiracetam's access to the central nervous system of our patients, as estimated by a mean CSF/plasma partition coefficient of 0.23, approximates the coefficient of 0.15 found in rodent studies (unpublished data).

The PET scan results supported the possibility that pramiracetam may have no intrinsic activity in Alzheimer dementia. In neither of two cases was there any consistent

regional variation in relative metabolic values. Moreover, pramiracetam induced no marked increase in absolute metabolic values in one of these individuals. The present results contrast with earlier observations suggesting that relatively high doses of a closely related nootropic, piracetam, enhance regional glucose metabolism in AD patients.[31] In view of our PET findings, however, it is hardly surprising that pramiracetam, at doses up to 4000 mg, afforded no clinically evident symptomatic benefit.

It is tempting to speculate that animal models[32,33] currently in use to screen potential antidementia compounds may not be valid predictors of therapeutic efficacy in AD. To the extent now possible, it will be important to validate these models as well as to develop alternatives of greater predictive value. In the meantime, clinical trials of pharmaceuticals selected solely on the basis of activity in current preclinical models of learning and memory may no longer be tenable.

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*Chapter 5.3*

# Serotonergic mechanisms in Alzheimer's disease: preliminary results of a controlled clinical trial with lisuride

## Introduction

Lisuride is a semisynthetic ergot derivative and a potent ligand for both serotonergic and dopaminergic receptors in the central nervous system. Pharmacologic preclinical studies of lisuride have shown a serotonergic antagonistic effect in low doses.[1] A decrease in serotonin (5-HT) synthesis and turnover was reportedly caused by lisuride, probably mediated through cessation of spontaneous firing of 5-HT neurons by 5-HT receptor stimulation.[2,3] Intravenous infusions of lisuride in the rat produce a suppression of neurons in the raphe dorsalis, the major ascending serotonergic pathway of the brain.[4] Receptor binding studies show that lisuride has high affinity for the $5\text{-HT}_{1a}$ receptor.[5,6]

Studies in animal models of learning and memory suggest that serotonergic mechanisms may play an important role in information processing. Although results have been inconsistent, the general observation is that interventions designed to elevate levels of 5-HT throughout the brain impair cognitive function.[7] In turn, experiments with 5-HT antagonists in aged rats demonstrated improvement of memory acquisition and retrieval.[7] It is further suggested that 5-HT activity exhibits an inhibitory influence on other neurotransmitters, including the noradrenergic[8] and cholinergic systems.[9,10]

Clinical trials in Alzheimer's disease have attempted to restore serotonergic neurotransmission, based on evidence that serotonergic activity is impaired in this disease.[11-15] A recent study with m-chlorophenyl-piperazine (mCPP), a 5-HT receptor agonist, reported worsening of memory and behavioral functions in patients with Alzheimer's disease.[16] These results, together with preclinical findings, suggest that inhibition of serotonergic neurotransmission might confer benefit to the symptoms of Alzheimer's disease. To obtain some preliminary data on this hypothesis, a controlled clinical trial with lisuride was carried out in 16 Dutch patients with mild to moderately severe Alzheimer's disease.

## Subjects and methods

Sixteen patients (10 men, 6 women) who satisfied NINCDS-ADRDA criteria for probable Alzheimer's disease[17] consented to participate in this study after full disclosure of its potential risks and benefits. Approval was obtained from the Medical Ethics Committee of the University Hospital Rotterdam Dijkzigt and of the Erasmus University Rotterdam. Subjects were selected from participants of the Rotterdam Elderly Study, a single center prospective follow-up study conducted among the total population aged 55 years and over of the suburb Ommoord in Rotterdam, The Netherlands. Rationale and design of the Rotterdam Elderly Study have been described elsewhere.[18] In addition, patients were referred from the metropolitan area of Rotterdam to the outpatient clinic of the Departments of Neurology and Geriatric Medicine, University Hospital Rotterdam Dijkzigt. Inclusion criteria for the study were: Mini-Mental State Examination score[19] higher or equal to 15 and lower or equal to 24, Hachinski ischemic score[20] maximum 4, and blood pressure between 100 and 170 mmHg systolic and 60 to 100 mmHg diastolic. Dementia severity, assessed with the Global Deterioration Scale[21] ranged from mild to moderately severe (3 to 6). On admission to the study, all patients had been free of centrally active drugs for at least 1 month.

Lisuride was administered in a randomized double-blind, placebo-controlled, parallel group design. Baseline assessments were obtained after an initial 2-week single-blind treatment with placebo. Patients were then randomized to either lisuride or placebo, and started the first study phase, a dose-finding phase of 4 weeks, with an initial dose of 0.075 mg per day increased by weekly increments of 0.075 mg, divided in 3 daily doses, until a dose of 0.3 mg per day was attained. The "best-dose", defined as an optimal ratio between efficacy and tolerance, was determined with the Clinical Global Impression Scale.[22] When no clinical change was observed, the highest dose of 0.3 mg per day was administered as the best-dose. All patients then entered the second study phase, an efficacy phase of 8 weeks with treatment of the best-dose.

Outcome measures were evaluated at baseline, at the end of the dose-finding phase at the maximal dose, and after 8 weeks treatment of the best-dose. Assessment of global cognitive and non-cognitive function, included the Clinical Global Impression Scale, the Dementia Mood Assessment Scale[23] and the Standardized Investigation of Dementia (SIDAM).[24] The Mini-Mental State Examination is part of the SIDAM and is reported additionally. Neuropsychological evaluations were performed with the following tests: modified version of the California Verbal Learning Test,[25] Word Fluency,[26] and Visual

Vigilance Task. In the Visual Vigilance task the patient must perceive a darker forming square from white noise in one of 4 squares of a computer monitor as quickly as possible. In addition, 3 computerized tests of visual memory from the Cambridge Neuropsychological Test Automated Battery (CANTAB) were included: Delayed matching-to-sample, Conditional visuospatial associative learning, and Working memory test.[27-29] Psychomotor function was assessed with 2 tests of the psychoexperimental test battery, including Tapping and Peg switching.[30] Side effects and blood pressure were monitored at weekly intervals during the dose-finding phase, and at 4 weekly intervals during the efficacy phase, and ECG at baseline was repeated at termination of the study.

Outcome measures of cognitive and non-cognitive function are reported as means ± SEM. Percentage change scores[31] from baseline to best-dose treatment were compared between groups with t-tests. The association between change scores of psychometric performance and the SIDAM baseline score in the lisuride treated group was evaluated with linear regression analysis.

## Results

Entry characteristics of the lisuride and placebo treated patients are presented in Table 5.3.1. No significant differences between groups in pretreatment baseline scores were found in neuropsychological outcome measures, but significantly higher scores on the SIDAM ($p < 0.01$) and the MMSE ($p < 0.05$) were observed at baseline in the lisuride treated group (Table 5.3.2).

During the dose-finding phase of this study, 6 patients had 0.3 mg per day and 1 patient 0.225 mg of lisuride as their best-dose. The single patient with a best-dose of 0.225 mg had improved attention and increased initiative at this dose and evidenced tiredness at the maximal dose of 0.3 mg per day. Assessment with the Clinical Global Impression Scale for individual patients at the highest dose showed no differences between groups. No change was observed in 4 lisuride patients and in 4 placebo patients, worse impression in 1 lisuride patient and in 3 placebo patients, and better impression in 1 lisuride patient and in 2 placebo patients.

During the efficacy phase, patients treated with lisuride showed significantly better performance ($p < 0.05$) compared to placebo treated patients in the modified version of the California Verbal Learning Test (Table 5.3.3). In contrast, patients with lisuride showed a decrease in scores of two tests of attention (Tapping and Visual Vigilance

*Table 5.3.1 Entry characteristics of Alzheimer patients for the lisuride and placebo group at screening**

| | Lisuride (n=7) | Placebo (n=9) |
|---|---|---|
| Sex | | |
| Male | 4 | 6 |
| Female | 3 | 3 |
| Age (years) | 74.4 ± 5.0 (65-79) | 74.6 ± 1.7 (65-82) |
| Education (years) | 9.9 ± 4.1 (6-18) | 8.3 ± 1.8 (6-10) |
| Symptom duration (years) | 4.7 ± 2.8 (2-10) | 5.0 ± 2.0 (2-9) |
| Mini-Mental State Examination | 22.1 ± 2.7 (17-24) | 17.9 ± 3.9 (15-24) |
| GDS | 4.6 ± 1.0 (3-6) | 5.4 ± 0.7 (4-6) |

*Values are presented as means ± SD (range)

Task), while the placebo group increased performance (table 5.3.3). However, these changes were not statistically significant. Similar results were obtained when 3 patients with the highest Mini-Mental scores were excluded. No consistent alterations in any of the other tests of verbal and visual memory, attention, psychomotor function, and measures of global cognitive function or mood were observed (Table 5.3.2 and Table 5.3.3). A similar slight decrease in global cognitive function as measured with the SIDAM was present in both groups over time. Moreover, no differences were observed between lisuride and placebo treated patients for clinical impression. No change was observed in 3 lisuride patients and in 3 placebo patients, worse impression in 4 lisuride patients and in 5 placebo patients, and better impression in none of the lisuride patients and in 1 placebo patient. In addition, anecdotal reports by some patients and their families were in agreement with the generally negative results. No significant relations were found between percentage change scores and baseline SIDAM scores. Side effects were few and mild. Possible drug related side effects were tiredness in 1 patient at 0.3 mg lisuride per day and headache and dizziness in 1 other patient at 0.225 mg, for both patients only in the dose finding phase.

***Table 5.3.2*** *Effect of lisuride on measures of general cognitive function and mood**

| | Lisuride (n=7) | | | Placebo (n=9) | | |
|---|---|---|---|---|---|---|
| | Baseline | Highest dose | Best-dose | Baseline | Highest dose | Best-dose |
| General Cognitive function | | | | | | |
| SIDAM | 38.0 ± 3.0† | 37.7 ± 3.3 | 36.3 ± 4.2 | 23.0 ± 2.9 | 22.3 ± 2.4 | 21.8 ± 2.5 |
| Mini-Mental State Examination | 23.2 ± 1.3‡ | 22.9 ± 2.0 | 22.0 ± 1.8 | 16.9 ± 1.7 | 16.1 ± 1.4 | 14.8 ± 1.3 |
| Mood | | | | | | |
| Dementia Mood Assessment Scale | 20.9 ± 4.0 | 20.4 ± 3.3 | 24.3 ± 4.2 | 26.8 ± 3.8 | 25.8 ± 3.6 | 29.1 ± 4.3 |
| Clinical Impression | | | | | | |
| Clinical Global Impression Scale§ | | 29% | 57% | | 33% | 56% |

*Values are the means ± SEM
† ‡Baseline of lisuride treated patients significantly different from baseline of placebo treated patients at $p < 0.01$, and $p < 0.05$, respectively
§In percentage patients that appeared worse in clinical impression

*Table 5.3.3 Effect of lisuride on cognitive function**

| | Lisuride (n=7) | | | Placebo (n=9) | | |
|---|---|---|---|---|---|---|
| | Baseline | Highest dose | Best-dose | Baseline | Highest dose | Best-dose |
| Verbal Memory | | | | | | |
| California Verbal Learning Test‡ | 23.9 ± 4.2 | 23.2 ± 4.3 | 27.0 ± 4.0† | 17.0 ± 2.8 | 12.1 ± 3.2 | 14.1 ± 2.5 |
| Verbal Fluency | 14.9 ± 2.9 | 12.6 ± 3.0 | 15.9 ± 3.4 | 11.4 ± 2.3 | 10.7 ± 2.7 | 10.8 ± 1.9 |
| Visual Memory | | | | | | |
| Visuospatial Associative Learning‡ | 20.1 ± 3.8 | 12.1 ± 0.9 | 14.7 ± 2.4 | 18.4 ± 4.4 | 15.3 ± 3.8 | 11.1 ± 3.6 |
| Delayed Matching to Sample‡ | 15.9 ± 2.5 | 16.4 ± 1.9 | 16.3 ± 2.1 | 12.4 ± 2.2 | 11.4 ± 2.0 | 10.8 ± 2.0 |
| Working Memory Test | | | | | | |
| between errors | 49.9 ± 9.4 | 39.4 ± 8.8 | 28.6 ± 6.6 | 27.1 ± 9.8 | 29.4 ± 10.2 | 28.9 ± 9.8 |
| within errors | 3.1 ± 1.2 | 1.7 ± 1.1 | 1.6 ± 0.4 | 2.1 ± 0.8 | 1.2 ± 0.5 | 1.8 ± 0.8 |
| Attention | | | | | | |
| Visual Vigilance Task | 18.7 ± 2.9 | 22.3 ± 4.1 | 11.7 ± 3.1 | 18.7 ± 5.1 | 21.1 ± 4.2 | 22.5 ± 5.5 |
| Psychomotor | | | | | | |
| Tapping | 318.1 ± 11.1 | 295.6 ± 31.5 | 283.0 ± 48.8 | 240.4 ± 48.6 | 256.8 ± 36.1 | 300.0 ± 19.7 |
| Pegboard | 139.7 ± 8.1 | 134.7 ± 13.0 | 136.1 ± 14.4 | 104.3 ± 24.4 | 125.0 ± 15.1 | 118.3 ± 14.9 |

*Values are the means ± SEM
†Percentage change from baseline to best-dose significantly different from patients with placebo by t-test at $p < 0.05$
‡Scores are presented for total recall

## Discussion

Short-term treatment with lisuride in low doses in patients with mild to moderately severe Alzheimer's disease significantly improved performance on a verbal learning task, whereas no effects could be demonstrated in non-verbal cognitive tests. Clinical global impression was in agreement with an apparent lack of clinically significant therapeutic benefit, and indicated that more than half of both lisuride and placebo treated patients showed progressive worsening of their symptoms. The number of subjects in this study is small and statistical power to demonstrate a clinical effect is limited. Differences at baseline in test scores of screening tests were found but appear to be due to chance in our parallel study design and result from the small study number. This did not appear to influence the outcome of the study since similar results were obtained when subjects with the highest scores on these tests were excluded to allow comparison of groups more equal in baseline scores. Our preliminary findings thus suggest that a partial serotonergic agonist with predominant binding to the 5-$HT_{1a}$ receptor is unlikely to prove clinically useful in Alzheimer's disease, but may have effects on verbal memory.

The pharmacologic action of lisuride suggests an inhibitory effect on serotonergic neurotransmission in Alzheimer's disease, mediated through its binding at the 5-$HT_{1a}$ site. To attain this central pharmacologic effect, we administered lisuride in dosages up to 4 times the dose that was effective in the prevention of seizures.[32] Receptor modulation as a potential therapeutic strategy is dependent on the functional integrity of residual neurons. In Alzheimer's disease, receptor loss has been reported in 5-$HT_2$ sites, and to a lesser extent in 5-$HT_1$ sites.[33,34] We included patients in an early stage of the disease to maximize this potential therapeutic effect. However, it is possible that lisuride is active at both presynaptic (in dorsal raphe) and postsynaptic (in hippocampus and cerebral cortex) 5-$HT_{1a}$ receptors. While the presynaptic effect may well result in decreased serotonergic function, postsynaptic stimulation in hippocampal and cortical neurons may have the opposite effect (i.e. maintaining inhibitory serotonergic tone), attenuating its beneficial action. In addition to its high affinity for the 5-$HT_{1a}$ receptor, lisuride also binds with equal affinity to $D_2$ receptors and to 5-$HT_2$ and $D_1$ receptors with 10-100 times lower affinity.[35] Interactions between these receptors have been suggested. For example, it has been shown that the 5-$HT_2$ site may have an inhibitory effect on the 5-$HT_1$ receptor sites.[36] These findings may provide an explanation for the finding in this study that lisuride affords no clinically evident symptomatic benefit.

The functional significance of serotonergic mechanisms in Alzheimer's disease is not

fully understood. Impaired serotonergic function is shown in Alzheimer's disease, but no correlations of this impairment with neuropathologic findings and cognitive dysfunction could be established.[37] On the other hand, the serotonergic deficiency in this disease has been linked to behavioral disturbances, including depression, agressive behavior, and anxiety.[38-40] Neither clinical trials in Alzheimer's disease[41-43] nor evidence from the preclinical level have yet elucidated a well defined role of 5-HT in behavioral and mnemonic functions. Indeed, several issues complicate the interpretation of cognitive concomitants of serotonergic receptor modulation. At present, drugs may not be selective enough in their pharmacologic action. The clinical evaluation of new compounds, such as selective 5-$HT_{1a}$ receptor antagonists,[44] may provide more insight in the relevance of serotonergic mechanisms in Alzheimer's disease. The high concentration of 5-$HT_{1a}$ receptors in the hippocampus and entorhinal cortex,[45] structures intimately involved in memory and learning are in favour of further exploration of the significance of this receptor. Nevertheless, the clinical correlates of interactions with other neurotransmitter systems are still elusive, e.g. for cholinergic and noradrenergic neurons.[8-10] Furthermore, serotonergic impairment is regional in Alzheimer's disease and functional effects of serotonergic modulation may be accordingly different.

In conclusion, treatment of Alzheimer patients with lisuride had no consistent effect on cognitive functions or on global clinical change. It appears therefore unlikely that modulation of central serotonergic activity by this agent will alleviate symptoms of Alzheimer's disease. However, the study number was small in this preliminary study and the pharmacological profile with respect to receptor binding of lisuride may not be specific enough. The possibility can not be excluded that further treatment strategies designed to alter serotonergic neurotransmission are more successful.

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# Chapter 6

# General discussion

*Chapter 6*

# General discussion

## Introduction

Epidemiologic studies may address several types of research questions, related to the etiology, diagnosis and prognosis of the disease. In this thesis aspects of these 3 domains of research receive attention with respect to aging and Alzheimer's disease. In this chapter we will first focus on studies of cerebral blood flow (CBF) in aging and Alzheimer's disease measured with single photon emission computed tomography (SPECT) using technetium-99M labeled hexamethyl propylene amine oxime ($^{99m}$Tc HM-PAO) as tracer. In addition to methodological issues with respect to the internal and external validity of these studies, the diagnostic role of SPECT for Alzheimer's disease and vascular risk factors as possible etiologic determinants of regional cerebral blood flow (rCBF) will be discussed. We will then deal with the prognosis of Alzheimer's disease, in this thesis with reference to current and future therapeutic strategies, as well as with some methodological aspects related to the conduct of clinical trials.

## Regional cerebral blood flow measured with $^{99m}$Tc HM-PAO SPECT in aging and dementia

*Methodological considerations*

Several concerns of validity may arise for SPECT studies of Alzheimer's disease. Some of the problems that could have played a role in the SPECT studies presented in this thesis include diagnostic misclassification, selection bias, and the effects of non-participation. In addition, selection bias may be a problem in the definition of regions of interest in the analysis of SPECT studies. Each of these issues will be discussed.

*Diagnostic misclassification*

For Alzheimer's disease, one of the primary obstacles for researchers is the inaccuracy of the clinical diagnosis, since at present no biological marker for antemortem diagnosis is available. The introduction of the DSM-III criteria for primary degenerative dementia[1] and the NINCDS-ADRDA criteria for probable and possible Alzheimer's disease[2] has considerably improved diagnostic accuracy and standardization of clinical criteria, but diagnostic errors are still estimated to vary from 8-32%.[3] Despite huge current research efforts to find a biological marker in cerebrospinal fluid, cerebral cortex, or blood, no definitive results have been reported. Recent studies suggest a possible role for abnormally phosphorylated tau, elevated levels of ubiquitin,[4] neuronal thread protein,[5] or the amyloid precursor protein.[6] Studies investigating the contribution of SPECT to the diagnosis of Alzheimer's disease will be confronted with the problem of uncertain diagnosis, however to a certain extent. In the study evaluating the diagnostic characteristics of SPECT in this thesis (chapter 3.2), the clinical diagnosis of probable Alzheimer's disease[2] is taken as the gold standard. Notwithstanding the diagnostic error also present in our study, possibly resulting in an underestimation of the true difference in rCBF between Alzheimer patients and controls, the results apply to the criteria as they are currently used in clinical practise.

Diagnostic misclassification could also have occurred in the control group. We sampled controls from the population and excluded only those with a neurologic disease including subjects with a dementia syndrome and no restrictions were imposed with cognitive screening tests. Indeed, some overlap was present in the Mini Mental State Examination test for patients and controls, and a striking observation was made that the mean rCBF of the temporo-parietal cortex in controls was lower than the mean parietal rCBF of Alzheimer patients. The possibility that some patients with low temporo-parietal rCBF had preclinically developing Alzheimer's disease can not be excluded. This would have resulted in an underestimation of the difference in rCBF between Alzheimer subjects and controls.

*Selection bias for subjects*

Selection bias refers to any error that may occur in the process of identifying a study population. It could have played a role in the SPECT studies presented in this thesis: selection bias for control subjects (chapter 3.2) and for a sample from the general population (chapter 3.3).

In chapter 3.2, it was reasoned that an adequate comparison of Alzheimer patients for estimation of the diagnostic characteristics of SPECT should be performed with those at risk for the disease; therefore we sampled controls from the general population. It was hypothesized that previous studies included control subjects that were likely biased towards healthier individuals and that this could have resulted in an overestimation of the diagnostic potential of SPECT. Several results reported in this thesis supported this hypothesis, including the finding that temporal rCBF was the best discriminating variable while most other studies report largest differences between Alzheimer patients and controls in parietal and temporo-parietal cortex. It was speculated that this finding could be explained by the presence of various cerebrovascular risk factors in our control group from the population. Individuals with a history of various illnesses including cardiovascular disease were excluded in several other SPECT studies. Support for the contention that vascular risk factors may be related to parietal and temporo-parietal rCBF comes from the study presented in chapter 3.3. These findings may therefore explain the consistently higher figures of sensitivity and specificity of SPECT as a potential diagnostic tool in the diagnosis of Alzheimer's disease reported in the literature, as compared to our results. Three issues require further comment. The 1st issue refers to selection criteria for control subjects determined by the investigator, the 2nd and 3rd refer to the effect of non-response for controls and Alzheimer patients, respectively.

First, there is considerable debate about how to define normal aging of the nervous system and how to define a control group.[7,8] Differences in selection criteria for control populations may, at least in part, explain differences in study results, as for example in studies of cerebral glucose metabolism and normal aging.[8,9] A distinction was proposed of "usual aging" and "successful aging", the latter category representing an elite group without the apparent age-related changes, and recently a plea was made to investigate these groups separately.[10] We took as a point of departure that controls should represent those who, had they developed the disease, would have been selected as cases.[11] Subjects with a history of a neurologic disease were excluded from the control group, because interference may occur with the predicting variable, the rCBF. On the other hand, some nondemented subjects with a history of a neurologic disease that may compromise rCBF, are also at risk for Alzheimer's disease. In fact, these subjects may therefore be eligible for inclusion in the control group in certain instances, such as subjects with previous transient ischemic attacks. Exclusion of these subjects would result in an overestimation of the contrast between

groups. This would refer to only very few persons in our study but illustrates theoretical problems of selecting subjects for Alzheimer studies.

Second, selection bias for the control group could have resulted from non-response. Several subjects refused participation, but detailed analysis revealed that this was unlikely to be selective (chapter 3.2). Furthermore, the distribution of various vascular risk factors was similar to a sample considered representative for the general population in Ommoord. Thus, although the possibility of selection bias could not be fully excluded for the controls, these findings suggested that it is unlikely that selection bias played an essential role in this study.

Third, selection bias from non-response could have played a role for the Alzheimer subjects, recruited from the outpatient memory clinic from the University Hospital Rotterdam Dijkzigt. However, all patients in the period 1989 to 1991 referred for evaluation of memory complaints received a SPECT investigation. Thus, selection might have occurred in the referral pattern to the outpatient clinic, but it remains hard to predict how this would have affected the estimation of the difference in rCBF between Alzheimer patients and controls.

In chapter 3.3, the study of risk factors for atherosclerotic vessel disease as determinants of rCBF, we sought to sample a group of individuals representing a large spectrum of these possible determinants. This was performed by taking a sample from the general population in Ommoord. The non-response for all participants in the Rotterdam Elderly Study might have been selective, with less participation of more diseased subjects. In addition, selective refusal of subjects from the population of 111 subjects that participated in a magnetic resonance imaging study could be a source of selection bias. The potential problem refers to external validity, implying possibly compromised generalization of the results. However, this is not a primary concern in this study, since we sought to establish a relationship between age, vascular risk factors and rCBF. Thus no threat to the internal validity of the results was suggested on these grounds. Nevertheless, one may speculate that selective non-response may lead to an underestimation of the association of the determinants (vascular risk factors) with the outcome (rCBF).

*Sampling bias in regions of interest*

Regions of interest are constructed to have an anatomical reference for measurements of rCBF and to reduce the enormous amount of information generated by a cerebral SPECT study. The sampling of these regions could introduce bias since

areas of hypoperfusion outside the sampled area are not detected. Qualitative analysis would circumvent the problem of sampling bias only partially because no criteria for subjective judgement are generally accepted. We investigated 3 different methods for the definition of regions of interest as determining factors for the outcome of rCBF measurements (chapter 3.1). The regions of interest were constructed with reference to normal anatomy, including 1 method with multi-slices and 1 method using a single-slice, and a 3rd, rectangular region. It was suggested in this thesis that rectangular regions of interest are associated with decreased measurement precision and less discriminative ability for patients with Alzheimer's disease and control subjects, as shown with receiver-operator-characteristic curves, when compared to regions with reference to normal anatomy. However, these differences appeared to be dependent on dementia severity: with increasing dementia severity, there is an increasing risk of underestimating the difference between Alzheimer patients and control subjects with rectangular regions, while no differences between the 3 methods were observed for mild Alzheimer patients. With respect to discriminative ability, future SPECT studies using these rectangular regions of interest should be aware of the potential problems of this method, and may base the choice of method for analysis on the purpose for which SPECT is used.

The absence of a gold standard for rCBF assessment in humans, however, precludes a definitive interpretation of what may constitute a "best" method of analysis. Indeed, one of the concerns for regions of interest with reference to normal anatomy is the limited resolution of SPECT imaging. With the current spatial resolution, no reliable distinction can be made between cerebrospinal fluid and contiguous brain tissue. Thus, the partial volume effect described for PET studies,[12,13] can not be excluded as a confounding factor artificially reducing rCBF values for the method employed in our studies, in spite of the rigorously defined criteria for the outer borders of the regions of interest. Future technological advances and high resolution SPECT cameras may help to resolve this issue. In addition, quantification of rCBF measurements with SPECT may provide opportunities for more accurate assessment of rCBF.

## SPECT in the diagnosis of Alzheimer's disease

Several encouraging reports were published in recent years that rCBF might be

used as a diagnostic marker for Alzheimer's disease.[14-16] In particular, hypoperfusion in the parietal or temporo-parietal cortex has been suggested as a reliable discriminator between Alzheimer patients and normal aging. Four issues complicate the interpretation of results from previous SPECT studies: the composition of the control group, the severity of dementia, the interpretation of diagnostic characteristics, and finally, the methods used for the construction of regions of interest. Each of these issues may have a different and sometimes opposite effects on the discriminative ability of rCBF between patients and controls. First, our results suggest that use of controls from the general population may lower the etiologic contrast between Alzheimer patients and control subjects compared to previous reports, resulting in lower sensitivity and specificity (chapter 3.2). Previous studies thus tend to overestimate the contrast between groups. Second, we found a strong relationship of dementia severity and diagnostic performance, shown in receiver-operator-characteristic curves (chapter 3.2). The higher figures of sensitivity and specificity reported in previous studies compared to our findings may be explained by the use of more severe patients in some of these studies. Third, some principles of test interpretation were neglected in previous studies. The essence was summarized in an article from 1980 on this subject by Griner et al.[17] "Knowledge of test characteristics does not, per se, permit accurate interpretation of the test result. Such knowledge tells us only what proportion of patients with and without the disease in question will have a positive and negative result, respectively. Since the task of the clinician is to determine the presence or absence of the disease, the questions he or she must address are as follows: 1) Given a positive test, what is the probability that the disease is present? 2) Given a negative test, what is the probability that the disease is not present? ...The estimation of these post-test probabilities requires the integration of knowledge concerning test characteristics with the clinician's estimate of the likelihood of disease before the ordering of the test (prior probability or pretest probability)". Thus, integration of pre-test probability, dependent on the clinician, and post-test probability, dependent on the sensitivity and specificity of the test, is an essential step. Indeed, post-test probability is intimately dependent on and varies with the pre-test probability. Based on these guidelines, the results from our study suggested that the diagnostic value of SPECT in Alzheimer's disease is limited for patients with mild Alzheimer's disease: only in patients where considerable doubt exists about the diagnosis, with specificity set at 90%, clinically relevant diagnostic ruling-in gain can be expected (chapter 3.2). Fourth, the method of rectangular

regions of interest used in various studies may not be optimal for the discrimination of Alzheimer patients and control subjects, at least for patients with severe Alzheimer's disease. Especially longitudinal SPECT studies of Alzheimer's disease in search of predictors for decline in rCBF using rectangular regions of interest may tend to underestimate the difference between patients and controls.

## Determinants of cerebral blood flow

Cerebral blood flow is subject to changes in local neuronal metabolic requirements and adequate blood supply can be maintained under circumstances of hypotension and hypoxemia. Autoregulation is one of the mechanisms that enable the adequate maintenance of cerebral perfusion through rapid adjustments of cerebrovascular resistance in response to alterations in arterial perfusion pressure. Factors determining blood viscosity are also important for rCBF, including hematocrit and platelet aggregation. In addition, increasing age has been related to decreased rCBF in most studies. The relation of vascular risk factors and the occurrence of cerebrovascular disease such as stroke has fuelled interest in the study of these risk factors as determinants of rCBF. One approach to study this relation is the comparison of individuals with and without vascular risk factors for differences in rCBF, and several reports indicate compromised rCBF in the former group. Another possibility is the investigation of individual determinants of rCBF and the examination of their interdependence with multivariate regression analysis. Hypertension, blood viscosity, and smoking have been suggested as independent contributors to rCBF in most studies employing with the $^{133}$Xenon inhalation technique. Not all studies have examined the regional distribution of these putative relationships for individual risk factors. The Rotterdam Elderly Study provided an opportunity to study various vascular risk factors in relation to rCBF with $^{99m}$Tc HM-PAO SPECT in a sample from the population. The study of individuals from the population has the advantage that a broad range of these risk factors is represented. We extended previous concepts of the contribution of age to rCBF. However, the relation of age and rCBF was explained for the major part by the presence of both vascular risk factors and indicators of atherosclerosis. It is tempting to speculate that age by itself from the 6th to 8th decade of life is associated with only minor changes in rCBF and that extrinsic factors related to the aging process, but not aging as a factor per se,[18] are responsible

for the observed decline in rCBF. In addition, we identified high fibrinogen levels and intima-media wall thickness of the carotid artery as determinants for low parietal rCBF. Furthermore, low diastolic blood pressure seems to be associated with low temporo-parietal rCBF. Although these results would suggest that both thrombogenic and atherogenic mechanisms contribute to rCBF, not all relationships examined were in the same direction or were consistent with these concepts. The relatively low number of subjects could have played a role. In addition, it remains difficult to explain why the parietal rCBF would be most susceptible for alterations in thrombogenic and atherogenic status. Especially watershed areas in the parieto-occipital region, which is the furthest from the origins of the anterior, middle, and posterior cerebral arteries, may be most susceptible to decreases in rCBF,[19] providing a possible explanation. A technical limitation is that regions of interest with reference to anatomical borders of the cerebral cortex, may not be optimal for detection of changes in the watershed areas.

## Treatment strategies for Alzheimer's disease

*Methodological considerations*

In the conduct of clinical trials in Alzheimer's disease a host of methodological issues need careful attention. One of the important factors is the diagnostic screening of potential patients. Alzheimer's disease is most commonly diagnosed through the application of either the DSM-III criteria or the NINCDS-ADRDA criteria. As already mentioned, diagnostic accuracy of these criteria is imperfect, since no biological marker of disease exists at present. This poses a validity problem to trials because the inclusion of patients with another diagnosis dilutes the clinical effect and may lead to underestimation of the drug effect. Further, the selection of appropriate outcome measures is a controversial issue (chapter 4). There is a need for standardization of tests since little agreement exists on what tests should be included in a drug efficacy evaluation. An important stride in this direction was taken with the introduction of the Alzheimer's Disease Assessment Scale (ADAS).[20] Other aspects that need to be considered in the choice of outcome measures include length and complexity of the testbattery, floor and ceiling effects, and response variability in demented patients.[21] Recently, it was suggested that neuropsychological outcome

measures should concentrate on domains known to be affected by a particular compound, called the "testing toward the deficit" concept by the authors.[22] In addition, multiple test forms should be available to permit repeated administration of the tests. Even with multiple test forms there is the potential problem of learning effects that may act as period effects and affect the measurement precision of study results. In this thesis, significant learning effects with repeated test administration in several cognitive domains were found in control subjects but not in the Alzheimer patients as a group (chapter 5.1). More careful statistical analysis showed that improvement with repetition also may play a role in patients with mild Alzheimer's disease and it is suggested that mental status plays an important role in the acquisition of material and the ability to increase performance (chapter 5.1). Efficacy evaluations in controls and patients with mild Alzheimer's disease, should be aware of the possible confounding role of learning effects. The issue of learning effects is most important in the cross-over trial, whereas in the parallel trial design the period effect will be similar in both treatment arms and is not a statistical threat to the analysis. This is one of the reasons why generally the parallel design is preferred over the cross-over design. Especially for Alzheimer's disease this may be important since this disease is characterized by a gradual progression of symptoms. Although the cross-over study is attractive for its need of fewer subjects, carry-over effects and period-effects may confound the outcome measurements. It is possible to test for these confounding effects but most cross-over studies include too few subjects to generate enough power to perform these tests. Too few studies pay attention to these problems, which is demonstrated for example by the criticism on several of the recent tacrine trials (chapter 4). One of the issues raised is that some studies do not employ dose-finding for the individual patient, in spite of evidence of response variability in preclinical and clinical studies.

*Preclinical models*

The development of appropriate animal models for Alzheimer's disease and related disorders is important for the preclinical evaluation of potential therapeutic drugs. Current models include normal animals such as rodents, experimentally induced deficits in normal and aged animals, and aged nonhuman primates, but no animal model is yet universally accepted. There is a striking discrepancy between the clinical predictive ability of these models and results of clinical trials in humans, as shown in chapter 4. Sarter reviewed several of the invalidities associated with the current

models.[23] The first problem refers to the limited construct validity of these models: they do not reflect etiological or theoretical assumptions about the disease. Second, the face validity of current animal models is limited. The underlying cognitive functions of various behavioral changes remain unknown. For example, response latency in the passive avoidance task has been widely used as an outcome measure in the search for cognition enhancing agents, while the cognitive correlates, and thus the face validity of this task, has been questioned. The neuropsychological processes that are related to pharmacological modulation of neuronal mechanisms require further study. The predictive ability of an animal model will be dependent on both construct and face validity. With regard to construct validity, recently several studies reported transgenic mice models expressing an extra amyloid gene.[24-26] The development of these models is based on the hypothesis that in Alzheimer's disease accumulation of $\beta$-amyloid is responsible for the degenerative neuropathology, but it remains to be established whether deposition of $\beta$-amyloid is the cause or the consequence of neuronal death. In the meantime, 2 of these papers have been retracted while the 3rd model is not appropriate for testing drugs at this stage.

*Enhancement of metabolic function*

Nootropics are psychotropic drugs with unknown mechanism of action and have a characteristic inverted U-shaped dose-response curve. In this thesis we tested the hypothesis that employment of dose-finding over a broad range of doses and exclusion of patients for the replication phase not demonstrating a best-dose, could maximize the possibility of demonstrating an antidementia effect of pramiracetam (chapter 5.2). Despite these efforts, no improvement in cognitive function, mood, or functions of activities of daily living was observed in this study. These findings confirmed results from previous studies with piracetam and other nootropics. One of the explanations for the general failure of nootropics to alleviate symptoms of Alzheimer's disease is that current animal models are not valid predictors for therapeutic efficacy. Indeed, the rationale for pramiracetam administration in our study as well as the improvements in study design were entirely based on these preclinical models. Nevertheless, a PET study demonstrated an increase in regional glucose metabolism after piracetam treatment of Alzheimer patients in high doses.[27] The effect of nootropics is probably aspecific and may not result in improvement of cognitive functions. Based on the results of our study and similar studies with related nootropics, it may no longer be tenable to select pharmaceuticals solely on the basis

of preclinical models.

*Neurotransmitter modulation*

In analogy with the successful treatment of Parkinson's disease with dopaminomimetic agents, several hypotheses were generated about the treatment of Alzheimer's disease with neurotransmitter replacement. The cholinergic hypothesis has perhaps attracted most attention, since it was based on the presence of a well documented cholinergic deficit in Alzheimer's disease that showed an association with dementia severity and neuropathologic findings. Various other neurotransmitters and several neuropeptides have been shown deficient in Alzheimer's disease and numerous trials were conducted based on the replacement strategy. However, no consistent clinical benefit for Alzheimer patients was observed. For this reason, the cholinergic hypothesis, although still attractive, has been questioned. Two recent multi-center clinical trials with tacrine show positive effects on cognitive function,[28,29] while one of these studies also showed improvement in clinician-rated and caregiver-rated observations.[29] At present, it may be concluded that "from a scientific point of view, the very modest effects of short-term tacrine administration indicate that enhancement of acetylcholine transmission is inadequate to reverse the signs and symptoms of Alzheimer's disease".[30]

In this thesis, lisuride treatment in low doses of Alzheimer's disease is based on the hypothesis that inhibition of serotonergic neurotransmission in preclinical models helps improve memory function (chapter 5.3). Lisuride binds to various subtypes of serotonergic receptors, with highest affinity for the 5HT-1a receptor. The cognitive corollaries of serotonergic mechanisms in Alzheimer's remain poorly understood, and conflicting results have been reported in clinical trials. The design employed in this study was with parallel groups receiving either lisuride or placebo, with dose-finding over four doses to a maximum dose of 0.3 mg/day. The best-dose was defined as the optimal score on a clinical rating scale. Outcome measures included cognitive as well as non-cognitive functions, and global clinical impression. Although a statistically significant benefit was observed in a verbal learning task for lisuride treated patients as compared to the placebo group, the study showed no consistent improvement in cognitive or affective functions as well as in global clinical impression, confirming similar results in previous studies.

Several explanations may be considered for the limited success of lisuride treatment and of neurotransmitter specific therapy in general. The neurotransmitter

abnormalities and receptor alterations in Alzheimer's disease remain poorly understood. Moreover, their relation to specific neuropsychological functions is not clear. In addition, Alzheimer's disease is a multisystem disorder suggesting that treatment based on replacement of a single neurotransmitter will not be of major benefit. Therapeutic Alzheimer trials are further complicated by the inaccuracy of the clinical diagnosis and the heterogeneity of the disease. Variability and the existence of various subtypes has been suggested on clinical grounds, in neuropathological studies, and for neurochemical findings. Furthermore, linkage studies show that several different mutations may be responsible for the development of Alzheimer's disease,[31] and a genetic linkage study recently showed evidence for a familial Alzheimer's disease locus on chromosome 14.[32] Therefore, it becomes more likely that the possibility of demonstrating a drug effect may be impossible when all patients fulfilling the clinical diagnosis of Alzheimer's disease are included in a therapeutic trial. Another problem is that some drugs tested to date are aspecific in their pharmacologic mechanism. For example, cholinergic stimulation of subtypes of cholinergic receptors, including the postsynaptic excitatory M1 receptors and the inhibitory presynaptic M2 receptors, may yield a diminished net drug effect.[33] Lisuride may also stimulate several subtypes of serotonergic receptors while the functional importance of these receptors remains to be solved (chapter 5.3). Finally, several other factors contribute to the therapeutic failure of neurotransmitter replacement. These include the current insufficient animal models to predict clinical efficacy, the use of inadequate experimental designs in some studies, and the assurance to attain central pharmacologic drug activity. In future studies, it will be important to find factors that characterize potential responders for drug treatment in an early phase of the disease. The visualization of neurotransmitter receptors with neuroimaging might contribute to identifying these patients.

## Future research

Several of the designs employed in studies reported in this thesis are descriptive cross-sectional. Notwithstanding the advantages of these study designs, one of the major disadvantages of these studies is that only prevalent cases are studied and that the interpretations of etiologic mechanisms remain speculative. This is most evident

in the SPECT study of the relation of vascular risk factors, indicators of atherosclerosis and rCBF. To gain further insight in the development of vascular risk factors and determinants of atherosclerotic vessel disease and rCBF, epidemiologic longitudinal population-based studies are needed. In addition, dementia severity, identified as an important variable for the interpretation of the results of both SPECT studies of the diagnostic value of SPECT in Alzheimer's disease and the study of regions of interest, was investigated in a cross-sectional sample of subjects recruited from a population based study. Longitudinal studies of the follow-up type could add to our current knowledge by attenuating the effect of selective survival that may confound cross-sectional studies. Furthermore, future follow-up studies are needed to determine the predictive value of low temporo-parietal rCBF for Alzheimer's disease in cognitively normal elderly individuals from the population. It became evident from the studies in this thesis that careful attention should be payed to the selection procedures employed for both Alzheimer patients and control subjects, and that the methods used to analyze tomographic SPECT scans may have important consequences for the results and thus for the subsequent interpretation of the study findings.

Another important issue is the search for a biological marker, which is one of the primary goals of current Alzheimer's disease research. Although recent evidence has shown that mutations in the amyloid precursor gene may be responsible for some cases of familial Alzheimer's disease, the biological characterization of the disease would greatly facilitate the conduct of clinical studies of Alzheimer's disease.

The development of therapeutic strategies for the intercession in pathogenetic mechanisms is needed in addition to the search for a palliative treatment. Knowledge about the etiologic mechanisms that are responsible for the degenerative neuronal degeneration in Alzheimer's disease may yield clues for selective intervention that may retard the progression of the disease or even restore neuronal function. Several hypotheses about the cause of neuronal death have emerged in recent years, largely based on research at the preclinical level. The formation of amyloid depositions as a central event in the pathogenesis of Alzheimer's disease is gaining increased attention and may provide a site for therapeutic intervention. Recent advances include the discovery of a mutation in the $\beta$-amyloid precursor locus in several families, and the demonstration of a modified tau protein that exclusively correlates with a fraction containing only paired helical filaments. Other mechanisms suggested to play a role in the neuronal degeneration of Alzheimer's disease are excitotoxic effects of excitatory

amino acids, oxidative stress possibly mediated by the enzyme monoamine oxidase-B, and the lack of neuronal growth factors.

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# Chapter 7

# Summary

*Chapter 7*

# Summary

The diagnosis of Alzheimer's disease, the most common dementing disorder, is uncertain and no satisfactory treatment is available (**chapter 1**). This thesis deals with single photon emission computed tomography (SPECT) with technetium-99M labeled hexamethyl-propylene amine oxime ($^{99m}$Tc HM-PAO), an imaging technique to assess regional cerebral blood flow (rCBF) that has been suggested as a diagnostic aid for the clinical diagnosis of Alzheimer's disease (**chapter 2**). This suggestion was based on studies that showed distinctive rCBF decrements in cerebral cortex of patients who were diagnosed as having Alzheimer's disease compared to age-matched healthy control subjects.

Studies of methods used for the definition of regions of interest (ROI's) with SPECT are presented in **chapter 3.1**. In 60 control subjects and 48 patients with probable Alzheimer's disease, the difference was evaluated between ROI's with reference to normal anatomy using a single-slice and multi-slice method, and a geometrically shaped ROI. No differences were observed for the rCBF means between the single-slice and multi-slice method. The discriminative ability for controls and Alzheimer patients, evaluated with multivariate logistic regression and receiver-operator-characteristic curves, was similar across methods for mild Alzheimer patients. However, with increasing dementia severity the geometric ROI method showed lower performance compared to both anatomically defined methods. These results suggest that analysis of one slice would suffice in these study populations and that the choice of an ROI method is dependent on the purpose for which SPECT is used.

In **chapter 3.2**, the diagnostic accuracy of SPECT was studied in 48 patients with probable Alzheimer's disease and 60 controls recruited from a population-based study. Receiver-operator-characteristic curves showed that the discriminative ability of SPECT improved with increasing dementia severity. With specificity set at 90%, sensitivity figures were 42% in mild, 56% in moderate and 79% in severe Alzheimer's disease. Diagnostic gain for mild Alzheimer patients was substantial (maximum 34%) for a positive test result, when the prior probability varied around 50%, but poor for

a negative test result. The findings suggest that the practical usefulness of SPECT as a diagnostic adjunct in patients suspected of mild Alzheimer's disease is confined to the situation when, on clinical grounds, considerable doubt exists about the diagnosis.

**Chapter 3.3** addresses the association of various risk factors for atherosclerotic vessel disease and rCBF in a cross-sectional study. Sixty-six persons, aged 63 to 89 years, were recruited from the general population and studied with SPECT. Increasing age was significantly associated with a decrease in parietal, temporo-parietal and temporal rCBF, and the association approached significance for frontal rCBF. However, these relations appeared to be dependent on the presence of both vascular risk factors and indicators of atherosclerosis. Levels of plasma fibrinogen were independently and inversely associated with parietal rCBF, which appeared attributable to a threshold effect of high fibrinogen levels. Intima-media wall thickness of the carotid artery was also significantly and inversely related to parietal rCBF, but this association was no longer significant when subjects with a previous history of stroke were excluded. A threshold effect was observed for the relation of low diastolic blood pressure and low temporo-parietal rCBF. These findings suggest that the observed age-related decline in rCBF from the sixth to eighth decade of life is dependent on the presence of both vascular risk factors and atherosclerosis. In addition, low parietal rCBF may be related to both thrombogenic and atherogenic mechanisms, and low temporo-parietal rCBF may be associated with low diastolic blood pressure.

The development of effective treatment strategies for Alzheimer's disease is one of the major goals of dementia research. Attempts for symptomatic treatment with neurotransmitter substitution or metabolic stimulation have yielded disappointing results so far (**chapter 4**). Methodological issues related to trial design, dose-titration and choice of adequate outcome measures are important for the interpretation of trial results. Future interventions that retard or halt the progression of the disease may be based on new insights from various mechanisms that are now suggested to play a role in the neuronal degeneration in Alzheimer's disease, including amyloid metabolism, excitatory amino acids, growth factors, and oxidative stress (**chapter 4**).

The possible confounding role of learning effects during multiple test administration in clinical trials was evaluated in 17 mildly to severely affected patients with Alzheimer's disease and 16 controls (**chapter 5.1**). Mean scores of Alzheimer patients showed no stable improvement, but more subtle learning effects were suggested by an association of dementia severity and change scores in verbal and

visual learning. Controls evidenced learning in 3 of 9 tests. It is suggested that the degree of cognitive impairment may be related to learning capacity, which implies caution for learning effects in controls and possibly in patients with mild Alzheimer's disease.

The antidementia efficacy of the nootropic compound pramiracetam was evaluated in 10 patients with probable Alzheimer's disease (**chapter 5.2**). A 2-phase placebo-controlled enrichment-type design was employed, based on the characteristic inverted U-shaped dose-response curve of pramiracetam in preclinical models of learning and memory. Eight patients evidenced a best dose (defined as the largest ADAS scale reduction of at least 4 points) in the dose-finding, but in the subsequent replication phase only two again improved to a similar degree. Studies with positron emission tomography in two patients showed no definite change. It is therefore unlikely that pramiracetam in doses up to 4,000 mg confers symptomatic benefit to Alzheimer patients.

The hypothesis that inhibition of serotonergic neurotransmission with lisuride in low doses may have a positive effect on symptoms of Alzheimer's disease, based on its high affinity for the 5-$HT_{1a}$ receptor, was tested in **chapter 5.3**. Sixteen patients with probable Alzheimer's disease were included in a trial with a double-blind parallel group trial design with dose-finding of lisuride in doses up to 0.3 mg/day. Outcome measures included global clinical impression, as well as several measures of cognitive and non-cognitive function. Statistically significant improvement occurred in a task of verbal memory in patients treated with lisuride compared to those with placebo, but no consistent differences between groups emerged from this preliminary study. Indeed, the global clinical impression was in agreement with a general lack of clinical effect of lisuride. Therefore, modulation of serotonergic activity with lisuride in Alzheimer's disease is not likely to confer clinically demonstrable benefit to these patients.

In **chapter 6** methodological aspects of the studies presented in this thesis, as well as the interpretation of results and their implications are discussed from an epidemiologic point of view. In addition, the findings of these studies are discussed within the broad framework of current research of SPECT in degenerative dementia and therapeutic strategies of Alzheimer's disease, and directions for future research studies are suggested.

# Samenvatting

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De klinische diagnose van de ziekte van Alzheimer, de meest voorkomende vorm van dementie, kan op dit moment niet met zekerheid worden gesteld. Ook is er geen afdoende therapie beschikbaar (hoofdstuk 1). Single photon emission computed tomography (SPECT) met technetium-99M gemerkt hexamethyl-propylene amine oxime ($^{99m}$Tc HM-PAO) is een beeldvormende techniek voor het meten van regionale cerebrale perfusie (rCBF) en SPECT is gesuggereerd als diagnostisch hulpmiddel voor het stellen van de klinische diagnose van de ziekte van Alzheimer (hoofdstuk 2). Dit is gebaseerd op vermindering van rCBF bij patiënten met "probable Alzheimer's disease" volgens de NINCDS-ADRDA criteria, in vergelijking met gezonde controle personen van dezelfde leeftijd.

Methoden die gebruikt worden voor het definiëren van "regions of interest" (ROI's) werden bestudeerd in hoofdstuk 3.1. Het verschil werd nagegaan tussen ROI's gebaseerd op de normale anatomie met één of meerdere tomografische coupes, en een geometrische ROI, bij 60 controle personen en 48 patiënten met de ziekte van Alzheimer (hoofdstuk 3.1). Er werden geen verschillen gevonden tussen de methode waarin meerdere coupes werden geanalyseerd en de methode met één coupe. Het onderscheidend vermogen tussen controle personen en patiënten met de ziekte van Alzheimer, onderzocht met multivariate logistische regressie en receiver-operator-characteristic curves, toonde geen verschillen tussen de 3 methoden voor vroege Alzheimer patiënten. Met toenemende ernst van de dementie was het onderscheidend vermogen van de geometrische ROI methode echter lager in vergelijking met beide anatomisch gedefinieerde methoden. De resultaten geven aan dat analyse van één coupe voldoende is bij deze onderzoeksgroepen en dat de keuze van ROI methode afhangt van het doel waarvoor SPECT wordt gebruikt.

In hoofdstuk 3.2 is een onderzoek beschreven naar de diagnostische nauwkeurigheid van SPECT bij 48 patiënten met de ziekte van Alzheimer en 60 controle personen die werden gerecruteerd uit een bevolkingsonderzoek. Receiver-operator-characteristic curves toonden dat het onderscheidend vermogen van SPECT verbeterde met toenemende ernst van de dementie. Bij een specificiteit van 90% was de sensitiviteit 42% voor vroege Alzheimer patiënten, 56% voor matig ernstige en 79% voor ernstige patiënten. De diagnostische winst was aanzienlijk voor vroege Alzheimer patiënten (maximaal 34%) bij een positieve testuitslag, indien de

voorafkans op ziekte varieerde rond de 50%; de diagnostische winst was gering voor een negatieve testuitslag. Deze bevindingen maken aannemelijk dat het praktisch nut van SPECT als een diagnostisch hulpmiddel bij patiënten verdacht voor de vroege ziekte van Alzheimer beperkt is tot de situatie waarin, gebaseerd op klinische gegevens, aanzienlijke twijfel bestaat omtrent de diagnose.

In hoofdstuk 3.3 wordt het verband nagegaan tussen verscheidene risicofactoren voor atherosclerotische vaatafwijkingen en rCBF in een cross-sectioneel onderzoek. Zes-en-zestig personen van 63 tot 89 jaar werden gerecruteerd voor SPECT onderzoek uit de algemene bevolking. Toenemende leeftijd was geassocieerd met een afname in pariëtale, temporo-pariëtale en temporale rCBF, terwijl het verband het niveau van significantie benaderde voor frontale rCBF. Deze relaties, echter, bleken grotendeels afhankelijk van het voorkomen van zowel vasculaire risicofactoren als indicatoren voor atherosclerose. Het niveau van plasma fibrinogeen was onafhankelijk en omgekeerd gerelateerd aan pariëtale rCBF. Dit kan mogelijk worden toegeschreven aan een drempeleffect van hoge fibrinogeen waarden. De intima-media wanddikte van de arteria carotis communis was eveneens omgekeerd gerelateerd aan pariëtale rCBF, maar deze associatie was niet statistisch significant wanneer personen met een cerebrovasculair accident werden uitgesloten. Een drempeleffect werd gevonden voor de relatie van lage diastolische bloeddruk en pariëtale rCBF. Deze bevindingen suggereren dat de geobserveerde rCBF daling van het zestigste tot negentigste levensjaar afhankelijk is van zowel het vóórkomen van vasculaire risicofactoren als van atherosclerose. Tevens is het mogelijk dat pariëtale rCBF wordt beïnvloedt door zowel thrombogene als atherogene mechanismen, en dat lage temporo-pariëtale rCBF geassocieerd is met lage diastolische bloeddruk.

De ontwikkeling van effectieve behandelingsstrategieën is een van de belangrijke doelstellingen van dementie onderzoek. Symptomatische behandeling via neurotransmitter substitutie of metabole stimulatie heeft tot op heden teleurstellende resultaten opgeleverd (hoofdstuk 4). Methodologische aspecten betreffende onderzoeksopzet, dosis-titratie, en keuze van adequate uitkomstmaten zijn belangrijk voor de interpretatie van trial resultaten. Toekomstige interventies die de progressie van de ziekte vertragen of tegengaan kunnen worden gebaseerd op nieuwe inzichten uit diverse mechanismen die mogelijk een rol spelen in de neuronale degeneratie van de ziekte van Alzheimer, zoals het amyloïd metabolisme, excitatoire aminozuren, groeifactoren en oxidatieve stress (hoofdstuk 4).

De mogelijk verstorende rol die leereffecten bij herhaalde testafname kunnen

spelen in klinische trials werd onderzocht bij 17 vroege tot ernstige patiënten met de ziekte van Alzheimer en 16 controle personen (hoofdstuk 5.1). De gemiddelde testscores van Alzheimer patiënten vertoonden geen duidelijke verbetering, echter subtiele leereffecten werden gesuggereerd door de omgekeerde associatie van de ernst van de dementie en veranderingsscores van visueel en verbaal leren. Controle personen toonden leereffecten in 3 van de 9 testen. De resultaten suggereren dat de mate van cognitieve achteruitgang samenhangt met de leercapaciteit, hetgeen voorzichtigheid gebiedt voor leereffecten bij controle personen en mogelijk voor vroege Alzheimer patiënten.

Het effect van het nootropicum pramiracetam werd onderzocht bij 10 patiënten met de ziekte van Alzheimer (hoofdstuk 5.2). Er werd een twee-fasen onderzoeksopzet gebruikt met "verrijking", gebaseerd op de karakteristieke dosis-respons curve in de vorm van een omgekeerde-U bij preklinische modellen voor leren en geheugen. Acht patiënten hadden een "beste-dosis" (gedefinieerd als de grootste reductie op de ADAS schaal van tenminste 4 punten) bij dosis-titratie, echter in de navolgende replicatie fase verbeterden slechts 2 patiënten in dezelfde mate. Onderzoek met positron emission tomography bij 2 patiënten liet geen duidelijke veranderingen zien. Het is daarom onwaarschijnlijk dat pramiracetam in doseringen tot 4000 mg symptomatische verbetering geeft bij Alzheimer patiënten.

In hoofdstuk 5.3 werd de hypothese getest dat remming van serotonerge neurotransmissie met lisuride in lage doseringen een positief effect heeft op de symptomen van de ziekte van Alzheimer, gebaseerd op de hoge affiniteit van lisuride voor de 5-$HT_{1a}$ receptor. Zestien patiënten met de ziekte van Alzheimer namen deel aan een trial met een dubbel-blinde parallelle opzet met dosis-titratie van lisuride in doseringen tot 0.3 mg/dag. Uitkomstmaten waren globale klinische impressie, alsmede enkele testen van cognitieve en non-cognitieve functies. Hoewel een significante verbetering optrad in een test van verbaal geheugen bij patiënten behandeld met lisuride in vergelijking met de placebo groep, werden geen consistente verschillen tussen beide behandelingsgroepen gevonden in deze preliminaire studie. De globale klinische impressie was in overeenstemming met het uitblijven van een klinisch effect van lisuride. Het is derhalve onwaarschijnlijk dat modulatie van serotonerge activiteit bij patiënten met de ziekte van Alzheimer leidt tot klinische verbetering.

Enkele methodologische aspecten alsmede de interpretatie van in dit proefschrift gepresenteerde onderzoeksresultaten, worden besproken vanuit een epidemiologisch

gezichtspunt in hoofdstuk 6. Tevens worden de resultaten van deze studies besproken in relatie tot het huidige en toekomstige SPECT onderzoek bij degeneratieve dementie en in relatie tot therapeutische strategieën bij de ziekte van Alzheimer.

# Epilogue

Op deze plaats wil ik graag een ieder bedanken die een bijdrage heeft geleverd aan de totstandkoming van dit proefschrift. De patiënten die aan de SPECT studies en klinische trials hebben meegewerkt, ben ik allen zeer erkentelijk.

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# Curriculum vitae

The author was born on April 5, 1960 in Heerlen, the Netherlands. After his general education at the Gooi's Lyceum in Bussum (Gymnasium-$\beta$), he started to study psychology at the University of Utrecht in 1978. In 1980 he began his medical training at the University of Amsterdam, and obtained both his medical degree and doctoral psychology in 1988 at this University. From 1988 to 1990 he worked as a Visiting Fellow with a Fogarty Fellowship at the National Institutes of Health, Bethesda, U.S.A., in studies of dementing disorders (Dr T.N. Chase, Dr E. Mohr). From 1990 to 1992 he joined the Department of Epidemiology and Biostatistics, Erasmus University Medical School, Rotterdam, The Netherlands (Prof. Dr A. Hofman). Since September 1992 he works as a resident at the department of internal medicine at the Gooi-Noord Hospital in Blaricum (Dr D. Maingay), and June 1993 he will start his specialist training in neurology at the Academic Medical Center in Amsterdam (Prof. Dr H. van Crevel).