AKADEMISK AVHANDLING

som med vederbörligt tillstånd av Rektorsämbetet vid Umeå Universitet för avläggande av medicine doktorsexamen kommer att offentligen försvaras i Tandläkarhögskolans föreläsningssal B 9 tr, Regionsjukhuset Umeå, fredagen den 11 oktober 1985, kl 09.00

av

Irina Alafuzoff

Umeå 1985

A B S T R A C T

HISTOPATHOLOGICAL AND IMMUNOCYTOCHEMICAL STUDIES IN AGE-ASSOCIATED DEMENTIAS - The importance of rigorous histopathological criteria for classification of progressive dementia disorders.

Irina Alafuzoff, Department of Pathology, University of Umeå, Sweden.

Dementia is an age-associated organic brain disorder, recognizable by the essential features of psychological or behavioral abnormality associated with permanent dysfunction of the brain interfering with social and occupational functioning.
There are two clinical and three histopathological forms of dementia 1) primary degenerative dementia, (PDD), or Alzheimer's dementia/Senile dementia of Alzheimers type (AD/SDAT) which is associated with clinical features of uniform progression and insidious onset of symptoms and histopathologically identified by the occurrence of neurofibrillary tangles (NFT) and senile/neuritic plaques (SP/NP) in various cortical and subcortical regions; 2) vascular dementia, or multi-infarct dementia (MID), which is associated with clinical features of stepwise progress and patchy distribution of deficits, and histopathologically identified by the occurrence of multiple large and/or small haemorrhagic and/or ischaemic infarcts in various cortical and subcortical regions and 3) intermediate form of dementia or "mixed" ("combined") dementia (AD-MID), which is histopathologically associated with the coexistance of symptoms and lesions observed in AD/SDAT and MID, and clinically referred to the MID group. The DSM-III criteria separate the demented into two groups, AD/SDAT and MID, while there are no unique clinical criteria for the AD-MID patients. The clinical diagnosis of dementia according to the DSM-III criteria was shown to be insufficient. Histopathological diagnostic criteria were postulated by us for 1) pathological changes developing in mentally unimpaired ageing, 2) AD/ SDAT, 3) MID and 4) AD-MID.
These histopathological classes could be separated, by means of multivariate data analysis. The pathology in AD-MID was shown not to be merely a linear combination of the AD/SDAT and MID pathology.
Intrathecal synthesis of Ig, oligoclonal bands or other abnormal proteins in the CSF could not be demonstrated in aged non-demented and demented patients.
The blood-cerebrospinal barrier (B-CSF-B) or blood-brain barrier (BBB) function alters with age and this alteration was shown to be more pronounced in MID and AD-MID patients. In MID and AD-MID patients the BBB alteration involves primarily the grey matter while in AD/SDAT patients the alteration would appear to involve only the white matter. The BBB dysfunction and a possible complement activation, either through antibody-antigen activation or other complement activators, was visualized in MID and AD-MID patients as perivascular serum protein deposits in the grey matter, always with a capillary in the center. The occurrence of some serum proteins in plaques, and the previously descibed localization of plaques in close relationship to the capillaries, suggest that altered BBB function and serum factors may be involved in the etiology and maturation of plaques while the etiology and maturation of tangles may not be directly dependent on these factors, as they were never labelled with any of the antisera studied.

UMEÅ UNIVERSITY MEDICAL DISSERTATIONS
New series No 153 - ISSN 0346 - 6612

From the Department of Pathology and Geriatric Medicine, University of Umeå,
Umeå, Sweden

Histopathological and Immunocytochemical Studies in Age-associated Dementias

The importance of rigorous histopathological criteria for classification of progressive dementia disorders

by

IRINA ALAFUZOFF

Umeå University
Umeå 1985

CONTENTS

ORIGINAL PAPERS

I Albumin and immunoglobulin in plasma and cerebrospinal fluid, and blood-cerebrospinal fluid barrier function in patients with dementia of Alzheimer type and multi-infarct dementia.
Alafuzoff I., Adolfsson R., Bucht G. and Winblad B.
J Neurol Sci, 60:465-472, 1983.

II Isoelectric focusing and two-dimensional gel electrophoresis in plasma and cerebrospinal fluid from patients with dementia.
Alafuzoff I., Adolfsson R., Bucht G., Jellum E., Mehta P.D. and Winblad B.
Europ Neurol, in press, 1985.

III Histopathological validation of the DSM-III criteria in Alzheimer type dementia and multi-infarct dementia.
Adolfsson R., Alafuzoff I. and Winblad B.
Manuscript.

IV Perivascular deposits of serum proteins in cerebral cortex in vascular dementia.
Alafuzoff I., Adolfsson R., Grundke-Iqbal I. and Winblad B.
Acta Neuropathol (Berl), 66:292-298, 1985.

V Blood brain barrier (BBB) in Alzheimer dementia and non-demented elderly: an immunocytochemical study.
Alafuzoff I., Adolfsson R., Grundke-Iqbal I. and Winblad B.
Acta Neuropathol (Berl), in press, 1985.

VI Histopathologic criteria for progressive dementia disorders: clinical - pathological correlation and classification by multivariate data analysis.
Alafuzoff I., Iqbal K., Friden H., Adolfsson R. and Winblad B.
Acta Neuropathol (Berl), submitted, 1985.

In the thesis these publications are refered to by their Roman numerals.

A B B R E V I A T I O N S

AD	Alzheimer's disease
AD-MID	"mixed" or "combined" dementia, i.e. histopathological coexistance of degenerative and vascular changes to a greater exctent than is seen in non-demented patients
Alb	albumin
ANF	Alzheimer neurofibrillary tangles
APf/Rpf	absolute/rate number of plaques in frontal cortex
APh/RPh	absolute/rate number of plaques in hippocampus
ATf/RTf	absolute/rate number of tangles in frontal cortex
ATh/RTh	absolute/rate number of tangles in hippocampus
BBB	blood-brain barrier
B-CSF-B	blood-cerebrospinal fluid barrier
BP	Bodian silver staining with periodic acid Schiff (PAS)
BRA	brain reactive antibodies
BSA	bovine serum albumin
C1q, C3c	complement factors
CNS	central nervous system
CRP	C-reactive protein
CSF	cerebrospinal fluid
CVD	cerebrovascular disease
D	perivascular deposits of serum proteins
ECG	electrocardiogram
EEG	electroencephalogram
F	fibrinogen
GFAP	glial fibrillary acidic protein
HE	hematoxylin-eosin
IEF	isoelectric focusing
If	microscopic infarcts in frontal cortex
Ig	immunoglobulin
Ih	microscopic infarcts in hippocampus
ISO-DALT	technique for high resolution two-dimensional electrophoresis
MID	multi-infarct dementia
NFT	neurofibrillary tangle
NP	neuritic plaque or amorphous plaque or small plaque or plaque without amyloid core or "immature" plaque
P	plaque
PA	prealbumin
PAGE	polyacrylamide gel electrophoresis
PAP	peroxidase-antiperoxidase technique
PBS	phosphate buffered saline
PC	principal component
PDD	primary degenerative dementia
PHF	paired helical filaments
PLS	partial least squares modelling in latent variables
S	histopathological dementia score, i.e. the sum of rate number of tangles and plaques in frontal cortex and hippocampus
SDAT	senile dementia of Alzheimer's type
SIMCA	soft independent modelling of class analogies
SP	senile plaque or dense plaque or large plaque or amyloid plaque or classical plaque or "mature" plaque
T	tangle
vB	von Braunmühl silver staining

TABLES

FIGURES

A B S T R A C T

HISTOPATHOLOGICAL AND IMMUNOCYTOCHEMICAL STUDIES IN AGE-ASSOCIATED DEMENTIAS - The importance of rigorous histopathological criteria for classification of progressive dementia disorders.

Irina Alafuzoff, Department of Pathology, University of Umeå, Sweden.

Dementia is an age-associated organic brain disorder, recognizable by the essential features of psychological or behavioral abnormality associated with permanent dysfunction of the brain interfering with social and occupational functioning.
There are two clinical and three histopathological forms of dementia 1) primary degenerative dementia, (PDD), or Alzheimer's dementia/Senile dementia of Alzheimers type (AD/SDAT) which is associated with clinical features of uniform progression and insidious onset of symptoms and histopathologically identified by the occurrence of neurofibrillary tangles (NFT) and senile/neuritic plaques (SP/NP) in various cortical and subcortical regions; 2) vascular dementia, or multi-infarct dementia (MID), which is associated with clinical features of stepwise progress and patchy distribution of deficits, and histopathologically identified by the occurrence of multiple large and/or small haemorrhagic and/or ischaemic infarcts in various cortical and subcortical regions and 3) intermediate form of dementia or "mixed" ("combined") dementia (AD-MID), which is histopathologically associated with the coexistance of symptoms and lesions observed in AD/SDAT and MID, and clinically referred to the MID group. The DSM-III criteria separate the demented into two groups, AD/SDAT and MID, while there are no unique clinical criteria for the AD-MID patients. The clinical diagnosis of dementia according to the DSM-III criteria was shown to be insufficient. Histopathological diagnostic criteria were postulated by us for 1) pathological changes developing in mentally unimpaired ageing, 2) AD/ SDAT, 3) MID and 4) AD-MID.
These histopathological classes could be separated, by means of multivariate data analysis. The pathology in AD-MID was shown not to be merely a linear combination of the AD/SDAT and MID pathology.
Intrathecal synthesis of Ig, oligoclonal bands or other abnormal proteins in the CSF could not be demonstrated in aged non-demented and demented patients.
The blood-cerebrospinal barrier (B-CSF-B) or blood-brain barrier (BBB) function alters with age and this alteration was shown to be more pronounced in MID and AD-MID patients. In MID and AD-MID patients the BBB alteration involves primarily the grey matter while in AD/SDAT patients the alteration would appear to involve only the white matter. The BBB dysfunction and a possible complement activation, either through antibody-antigen activation or other complement activators, was visualized in MID and AD-MID patients as perivascular serum protein deposits in the grey matter, always with a capillary in the center. The occurrence of some serum proteins in plaques, and the previously descibed localization of plaques in close relationship to the capillaries, suggest that altered BBB function and serum factors may be involved in the etiology and maturation of plaques while the etiology and maturation of tangles may not be directly dependent on these factors, as they were never labelled with any of the antisera studied.

INTRODUCTION

Dementia is an age-associated organic brain disorder, recognizable by the essential feature of psychological or behavioral abnormality associated with permanent dysfunction of the brain and interfering with social and occupational function (* 1980).

CLINICAL STUDIES

The most common type of dementia is the primary degenerative dementia (PDD), commonly referred to as Alzheimer's disease/ Senile dementia of Alzheimer type (AD/SDAT), which accounts for approximately 50-70% of the cases in an autopsy material (Tomlinson 1980). Vascular dementia or multi-infarct dementia (MID) and the "mixed" or "combined" dementia (AD-MID) accounts for about 15% each of the demented cases in an autopsy material (Tomlinson 1980).

Essential features in AD/SDAT are the presence of dementia of insidious onset and gradual progression, involving multi-facetted loss of intellectual abilities, such as memory, judgement, abstract thinking and other advanced cortical functions, as well as change in personality and behaviour (Roth 1955, Slater and Roth 1970). With progression of the disease, the patient might be completely mute and inattentive. This late stage leads inevitabely to death. The prevalence is 2-4% at an age of 65 and increases with age particularly after 75 (* 1980, Adolfsson et al 1981). The disorder is more common in women than in men and first degree relatives of probands with AD/SDAT are four times more likely to develop the disease compared with the members of the general population (* 1980). The etiology of AD/SDAT is unknown, but various speculations are flourishing on involvement of possible endogenous (genetical, immunological, endocrinological) and precipitating (viruses, neurotoxic substances) factors (Oldstone and Dickson 1974, Crapper and De Boni 1978, Fudenberg et al 1984).

The dementia associated with vascular changes (MID) shows, contrary to AD/SDAT, an abrupt onset, a stepwise and fluctuating deterioration in intellectual functioning with rapid changes that, early in the course of the disease, leaves some of the intellectual function relatively intact (Hachinski et al 1974, 1975). Disturbances in memory, abstract thinking, judgement, impulse control and personality are common. Patterns of deficits depend upon which regions of the brain are affected (Fisher 1968, Hachinski 1979, de Reuck et al 1982). Focal neurological signs and symptoms are present, and cerebrovascular diseases (CVD) has been related to the etiology of the disorder (Hachinski 1975, Rosen et al 1980, Birkett and Raskin 1982).

Clinical diagnostic criteria

DSM-III criteria (* 1980), the most widely used clinical classification criteria for dementias, were introduced in 1980 by the American Psychiatric Association. According to these criteria there are two major forms of progressive dementia disorders, namely, AD/SDAT and MID. The major principles in these criteria are listed below:

criteria for AD/SDAT:
1) clinical signs of dementia,
2) insidious onset of symptoms,
3) uniform progression of the disease,
4) no signs of other specific causes of dementia;

criteria for MID:
1) clinical signs of dementia,
2) stepwise progression of the disease,
3) patchy distribution of deficits,
4) existance of focal neurological signs and symptoms,
5) evidences of cardio/cerebrovascular diseases.

To simplify the clinical differentiation between AD/SDAT and MID the "Hachinski score" (Hachinski et al 1975) was introduced. Symptoms and signs, such as abrupt onset, stepwise deterioration, fluctuating course, nocturnal confusion, depression, somatic complaints, emotional incontinence, hypertension stroke disturbances, associated arteriosclerosis, neurological signs or symptoms, were scored by this method. Patients with a score under four were classified as AD/SDAT patients and those with a score above seven were classified as MID patients.

Standardized operational criteria, e.g. the DSM-III (* 1980) criteria, described above, and the NIMH-criteria (McKhann et al 1984), have designated a group of progressive dementia disorders, either presumed AD/SDAT or MID. According to the NIMH-criteria for definition of definite AD/SDAT, a combination of specific clinical data and histopathological data are required. With the exception of a study investigating the reliability of the "Hachinski score" (Rosen et al 1980) and a study investigating the specificity of the clinical diagnoses of dementia with histopathological verification (Todorov et al 1975), little information is available on the validity of the different clinical criteria.

HISTOPATHOLOGICAL STUDIES

The diagnostic hallmarks for AD/SDAT are neurofibrillary tangles (NFT) and senile/neuritic plaques (SP/NP) and for MID macro-/microscopic infarcts in cortical and subcortical areas.

Among other structural changes of the brain in ageing and dementia are: brain atrophy, neuronal loss, dendritic atrophy, accumulation of lipofuscin, Hirano bodies and granulovacuolar degeneration (Tomlinson 1980, Brun 1983).

Neurofibrillary tangles (NFT)

Neurofibers in the mature neurons are of two principal classes: neurotubules (diameter 240 Å), identical to microtubules in other tissues; and neurofilaments (diameter 100 Å), members of the class of intermediate filaments (Terry and Davies 1980). The neurofibrillary tangle is an abnormal, intracytoplasmic argentophilic mass of neurofibers, passing through the perikaryon, around the nucleus towards the apical dendrite and often extending out into one or more neurites (Tomlinson 1980, Terry and Davies 1980). The neurofibers that form the tangle are different from the normal types in the sense that they are paired helical filaments (PHF), each member of the PHF measuring approximately 100 Å in diameter (Iqbal et al 1978, Wisniewski and Soifer 1979). PHF have been demonstrated also in non-demented individuals. They do not show similarities with normal neurofilaments, microtubules, actin or myosin fibers (Grundke-Iqbal et al 1982,1984). In some tangles there are an admixture of normally appearing filaments, normally appearing tubules and the PHF. Tangles, built up by these characteristic PHF, are also found in disorders other than AD/SDAT. They are found, for example, in the neocortex in dementia pugilistica, Guam-Parkinson's dementia complex, Down's syndrome, postencephalitic Parkinsonism, and in occasional cases of subacute sclerosing panencephalitis (SSPE) and Tuberous sclerosis (Wisniewski and Soifer 1979). The etiology of the tangles is unknown.

Senile/Neuritic plaques (SP/NP)

While the tangle is primarily within the perikaryon the senile/neuritic plaques (SP/NP) occupy the neurophil, where they are often adjacent to the capillaries (Miyakawa et al 1979, 1982). There are two morphological types of plaques: 1) the amorphous or neuritic plaques, small in size, consisting of a cluster of four or five distended neurites and 2) the discrete or senile plaques, large in size, with a uniform densely stained amyloid core around which there is frequently an unstained

halo surrounded by argentophilic rods and granulas (Tomlinson 1980, Gibson et al 1983). The amyloid core is made up largely of extracellular amyloid in the form of 70-90 Å filaments, quite different in texture and density from the normal neurofilaments and from the individual fibrils of PHF (Terry and Davies 1980). Astrocytic processes and occasional microglia are present in and around the plaques. Local neurons of different types contribute with their dendrites and axons to the plaques (Probst et al 1983). The two morphological types, senile and neuritic plaques and the "intermediate" form are most frequently found together in the same patient, although in many, one or the other predominates (Gibson et al 1983). As the degeneration progresses, the plaques seems to largely "disappear" (Brun and Englund 1981). The etiology of plaques is unknown.

In AD/SDAT patients, the tangles and plaques were observed in both cortical and subcortical areas with accentuation in the basal medial temporal limbic areas, the posterior cingulate gyrus and superior parietal lobe (Brun and Englund 1981, Brun 1983).
The number of these lesions were found to increase with the progress of the dementia (Blessed et al 1968, Ball 1976,1977, Perry et al 1978, Tomlinson et al 1970, 1980, Wilcock and Esiri 1982, Gibson et al 1983).

Macroscopic and microscopic infarcts

Macroscopic/microscopic infarcts (haemorrhagic or ischaemic) of a total volume greater than 100 ml (Tomlinson et al 1968, 1970,1980), infarcts localized in periventricular white matter or the thalamus (de Reuck et al 1982) or involving the hippocampus (Fisher 1968, Corsellis 1976) have been pointed out as a cause of some of the clinical symptoms of multi-infarct dementia. The results of the positive correlation between the clinical features in MID and the histopathological findings of multiple microscopic infarcts in cortical and subcortical regions (Rosen et al 1980) support the existance of

a separate class of vascular dementia in which various haemorragic or ischaemic lesions are of etiological importance. These lesions may be caused by various complications, such as thrombosis, embolism and haemorrhages.

Histopathological diagnostic criteria

The histopathological diagnoses of AD/SDAT and MID are based on the quantitation rather than occurrence of tangles, plaques and microscopic infarcts in the brain, as these "lesions" are also observed in non-demented individuals, although to a lesser degree. In some cases the correspondence between lesions and clinical symptoms might be disputable, especially in the borderline between normal aged patients on the one hand and patients with early stages of dementia on the other. Different investigators attach different significance to small infarcts and a small number of degenerative changes making the quantitations not fully comparable. In the AD-MID mixed dementia both AD/SDAT and MID lesions are seen in coexistance.

IMMUNOLOGICAL AND IMMUNOCYTOCHEMICAL STUDIES

The genetically controlled (Greenberg and Yunis 1968, Stuttman 1974, Yunis and Lane 1979) immunological response, both cellular and humoral, has been shown to decline with age (Yunis and Greenberg 1968, Nordin and Makinodan 1974, Walford et al 1974, Kay 1979), influencing the disease pattern (Makinodan 1979). The decline in function was shown to be more severe in demented patients compared with age-matched controls (Kalter and Kelly 1975, Jancovic' et al 1977, Tavolato and Argentiero 1980).

Plasma Ig

IgG, IgM and IgA concentrations have been shown to be dependent upon age, sex and race in healthy persons (Buckely and Dorsey 1971). A gradual upward trend in plasma IgG and IgA concentrations in surviving healthy individuals and a selecti-

ve mortality of elderly people with relative immunodeficiency has been described (Buckley et al 1974). In demented patients the concentrations of plasma IgG and IgA were both found to be lower (Tavolato and Argentiero 1980, Pentland et al 1982) and higher (Kalter and Kelly 1975, Cohen and Eisdorfer 1980a) than those found in age-matched mentally unimpaired controls. The more impaired patients were found to have lower plasma-IgG levels (Cohen et al 1976, Eisdorfer and Cohen 1980), suggesting a more severe impairment of immunological function in demented compared with non-demented individuals.

Cerebrospinal fluid Ig

No local or intrathecal production of plasma Ig in the CNS in demented patients has been observed (Jonker et al 1982). A slight elevation, of both albumin and IgG in the CSF with age (Tibbling et al 1977, Kobatake et al 1980) was observed and this elevation was found to be slightly higher in patients with clinical symptoms of dementia (Bock et al 1974, Böning 1979, Jonker et al 1982).

Auto-antibodies

The frequency of auto-antibodies (antibodies formed in response to, and reacting against, one of the individuals own normal antigenic endogenous body constituents) in plasma in elderly persons has been found to increase with age (Yunis and Greenberg 1968, Waltford et al 1974, Good and Yunis 1974, Blumenthal 1976, Mayer et al 1976, Beregi et al 1978). Auto-antibodies were observed more frequently in demented compared with non-demented individuals (Mayer et al 1976, Ikach and Hokama 1978, Cohen and Eisdorfer 1980b, Watts et al 1981) and in patients with cardiovascular disease or cerebrovascular lesions (stroke) compared with age-matched controls (Berry and Riches 1974, Beregi et al 1978). Brain reactive antibodies (BRA) (auto-antibodies, directed towards the brain tissue), have been demonstrated in aged mice (Nan-

dy 1972), in aged, non-demented humans (Mayer et al 1976, Watts et al 1981) and in demented patients (Mayer et al 1976, Ikach and Hokama 1978, Nandy 1978, Cohen and Eisdorfer 1980b, Watts et al 1981).

Oligoclonal and other abnormal Ig

Another immunological disturbance, the production of oligoclonal Ig (Ig produced as a rather limited reaction to a antigenic stimulation) was shown to be associated with diseases such as subacute sclerosing panencephalitis and diseases which are presumed to be infectious, such as multiple sclerosis (Iivanainen 1981, Mehta et al 1982 a,b,c). Oligoclonal Ig production has been observed in the CSF of demented patients (Williams et al 1980). Oligoclonal antibodies have also been observed in some patients with amyloid microangiopathy associated with clinical signs of dementia (Schuller et al 1982) Other abnormal CSF protein components have been demonstrated in various neurological diseases, including dementia (Siden 1980, Wikkelsö et al 1981, Wikkelsö and Blomstrand 1982).

Antigenicity of plaques and tangles

It has been shown that plaques are related to capillaries (Miyakawa et al 1982) and that they are built up by amyloid of unknown origin (Mandybur 1975, Cornwell and Westermark 1980, Glenner 1981, Mountjoy et al 1982). Plaque amyloid has been shown to contain different serum proteins, such as albumin, prealbumin and fibrinogen (Katenkamp et al 1970, Powers et al 1981, Shirahama et al 1982), different immunoglobulins and different complement factors (Katenkamp et al 1970, Ishii and Haga 1975,1976, Powers et al 1981, Torack and Lynch 1981, Eikelenboom and Stam 1982). These results have emphasized the probable importance of the serum factors and the immunological mechanism for the development of plaques. Tangles have been visualized with an antisera to prealbumin (Shirahama et al 1982).

Blood-cerebrospinal fluid barrier and blood-brain barrier function

The findings listed above of serum proteins in plaques and their relationship to vessel walls raises the question of the importance of the blood-cerebrospinal fluid barrier (B-CSF-B) or blood-brain barrier (BBB) function in the pathogenesis of dementia. The function of the barrier is modified by changes in blood pressure (Houthoff et al 1981), by epileptic seizures (Aarli 1983) and by drugs (Preskorn et al 1981).
The barrier function alter with age (Bock et al 1974, Tibbling et al 1977, Kobatake et al 1980) and this alteration was found to be more severe in AD/SDAT patients (Wisniewski and Kozlowski 1982).

THE AIMS OF THE PRESENT INVESTIGATIONS WERE

to evaluate the blood-cerebrospinal fluid barrier function in non-demented and demented patients (I) and to investigate the possibility of local Ig production in the CNS in demented and non-demented patients and to examine the occurrence of oligoclonal Ig and brain specific proteins in the plasma and CSF of aged non-demented and demented patients (II). Because of the variable results reported on the studies of plasma contents of proteins, among others, in clinically classified demented patients we considered it of importance to investigate the reliability and the validity of the DSM-III classification (III). Based on the findings of increased transudation through the B-CBF-B in MID patients an immunocytochemical technique was employed on brain tissue to visualize if possible the occurrence of serum proteins in the brain tissue (IV,V). Finally, because of the great discrepancy between the clinical and histopathological classifications, strict histopathological criteria for non-demented and demented patients were postulated. The "function" of the criteria was tested by means of multivariate data analysis (VI).

MATERIALS

PATIENTS (I-VI)

Two categories of patients were investigated: 1) ambulatory patients (I-II), who were investigated under standardized conditions during their stay at the Department of Internal Medicine, Umeå University hospital, for purposes of the research project, and 2) institutionalized patients (III-VI), who died at Umedalen hospital or at the Geriatric clinic, Umeå University hospital.

PLASMA AND CEREBROSPINAL FLUID (I,II)

Sample collection was performed under standardized conditions. Cerebrospinal fluid (CSF) samples were obtained by a routine lumbar puncture procedure and frozen in aliquots until analyzed. Blood samples were collected in heparinized tubes and plasma, after separation by centrifugation, was frozen until analyzed.

BRAIN TISSUE (III-VI)

Following a gross examination of the brain at autopsy, tissue was taken from two regions of the right hemisphere, the frontal cortex (Broadmann area 10) and the anterior part of the hippocampal formation. These regions, on account of the occurrence of degenerative lesions, represent the extremes (Brun 1983). The tissue pieces were fixed in 10% buffered neutral formalin (Romeis 1968) and bisected; one half was embedded in paraffin blocks and the other half was used to prepare frozen sections.

METHODS

CLINICAL INVESTIGATION (I-VI)

Most patients studied were investigated by means of a psychogeriatric and neurologic examination, EEG, ECG, and in some

cases, CT-scan. The demented patients had been followed clinically for several years and the clinical diagnoses of AD/SDAT and MID were based on the DSM-III classification (I:43, II:27, III:91, IV:13, V:8 and VI:55 demented patients) (* 1980) (Table 1).

Table 1. Schematic presentation of five operationally defined clinical classes of patients with progressive dementia (according to DSM-III classification, *1980)

Clinical diagnosis	Type of progress	Cerebrovascular risk factors	Neurological signs or symptoms
AD/SDAT	uniform	absent	absent
probable AD/SDAT	uniform	present	absent
MID	stepwize	present/absent	present
probable MID	stepwize	present/absent	absent
probable MID	uniform	present	present

Patients whose dementia was of non-progressive type or was of known etiology, such as that caused by alcohol abuse, infectious diseases, metabolic disorders, trauma and tumors, were excluded. Healthy age-matched volunteers without signs of dementia or immunologic disorders were included as controls.

ROCKET IMMUNOELECTROPHORESIS (I,II)

Rocket immunoelectrophoresis (Laurell 1972) represents a simple, quick and reproducible method for determination of a single protein in a protein mixture. The electrophoresis is performed in agarose gel containing a monospecific antiserum. The identification of the protein is revealed by the rocket-shaped precipitates, and the quantification is based upon mea-

suring the height of these precipitates.

The concentrations of albumin and the major classes of circulating immunoglobulins in plasma and CSF (43 demented and 16 non-demented patients) were determined using commercial human plasma sample as a standard and rabbit anti-human albumin, IgG, IgM, and IgA as antibodies.

Transudation (passage) of IgG through the blood-cerebrospinal fluid-barrier (B-CSF-B) and local synthesis of IgG in the CNS were calculated according to previously described formulas (Shuller and Sagar 1981). Local synthesis was also evaluated by means of the CSF-IgG index (Link and Tibbling 1977).
The B-CSF-B function was graphically demonstrated by an evaluation graph for the protein profile in the CNS built up by the Alb-, and IgG-ratios (Ganrot and Laurell 1974, Reiber 1980) (Figure 1).

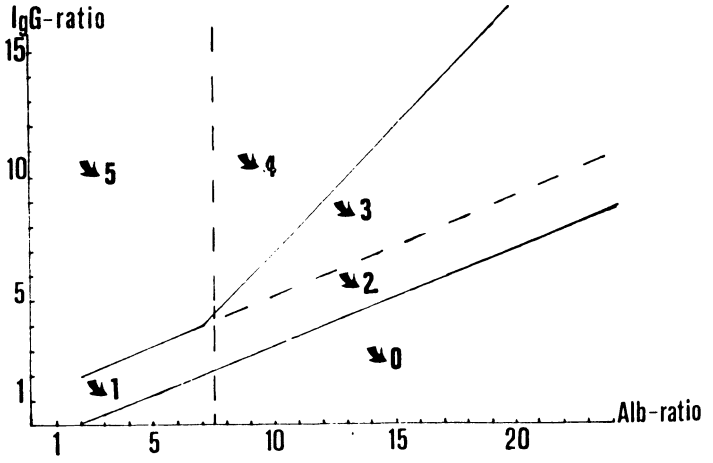

Figure 1. Evaluation graph for protein profile.
IgG ratio = CSF-IgG/plasma-IgG x 10, Alb ratio = CSF-Alb/plasma-Alb x 10. 1) normal range, 2) B-CSF-B dysfunction with proportionally increased IgG 3) B-CSF-B dysfunction with disproportionally increased IgG, 4) B-CSF-B dysfunction with additional increased IgG, locally produced in CNS, 5) range of values with IgG synthetized locally in the CNS and 0) biologically irrelevant range.

ISOELECTRIC FOCUSING WITH IMMUNOFIXATION (II)

Isoelectric focusing (IEF) is a method based on the variability of the protein charge with the pH of the surroundings. At a pH similar to the isoelectric point of the protein the protein charge is zero, which brings the protein to a standstill on the polyacrylamide gel in the electric field. Using this method, proteins in a mixture are separated from each other and then identified by means of immunofixation (Mehta et al 1982a,b).

Thin layer polyacrylamide gels were prepared, and concentrated CSF (27 demented and 5 non-demented) and diluted plasma (23 demented and 10 non-demented patients) were applied to the gel. After the focusing was completed the IgG bands were identified by immunofixation.

HIGH RESOLUTION TWO-DIMENSIONAL POLYACRYLAMIDE GEL ELECTROPHORESIS (II)

In high resolution two-dimensional polyacrylamide gel electrophoresis, a mixture of proteins are separated according to the isoelectric point in the first dimension and the molecular weight by sodium dodecyl sulfate electrophoresis in the second dimension to obtain a uniform distribution of protein "spots" across a two-dimensional gel (Anderson and Anderson 1977, 1979, Jellum and Thorsrud 1982).

The ISO-DALT system was used (Anderson et Anderson 1978 a,b), and the analysis was performed on diluted plasma samples (17 demented and 2 non-demented patients) and concentrated CSF samples (2 demented and one non-demented patients). After separation in the second dimension the gels were stained with Coomassie Brilliant Blue, and photographed.

IMMUNOCYTOCHEMISTRY (IV,V)

The peroxidase-antiperoxidase (PAP) method (Sternberger 1979) is an alternative to peroxidase conjugate and immunofluorescence procedures, with the advantage of enhanced sensitivity (estimated at 100 to 1000 times that of immunofluorescence). Immunoperoxidase procedures are used for detection and demonstration of cell surface antigens, intracellular antigens and antigens distributed in the extracellular space. The detection of the antigen in different localizations is dependent on the mode of exposure of the tissue to the immunoperoxidase system. Following fixation, contrary to the case with viable cells, there are varying degrees of penetration into the cells According to this technique, in the first (1) step (Figure 2), the sections were incubated with primary antisera (antisera to the antigen of interest). This step was followed (2) by a secondary antisera, such as the goat antirabbit IgG, applied in excess. Under these conditions one of the combining sites of the secondary antibody reacted with the primary antibody and the second binding site remained free. In the third step (3), a purified peroxidase-antiperoxidase (PAP) complex from rabbit was applied on the sections. The reaction was then developed (4) with hydrogen peroxide as substrate and 3'3 diaminobenzidine tetrachloride as electron donor, yielding a brown, insoluble product.

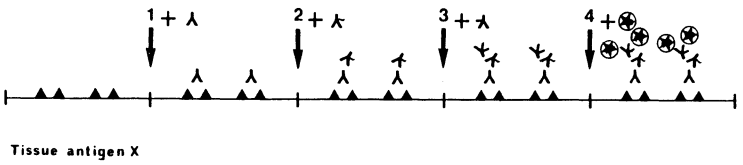

Figure 2. Principles of peroxidase-antiperoxidase technique.

The deparaffinized and rehydrated brain tissue sections (IV: 13 demented and 6 non-demented patients, V: 8 demented and 6 non-demented patients, VI: 55 demented and 19 non-demented patients) were examined for the presence of serum proteins using a somewhat modified version of the PAP technique.

HISTOPATHOLOGY (VI)

Six micron sections of the paraffin embedded material were stained by different methods:

1. hematoxylin-eosin stain (Figure 3c),
2. Bodian silver stain (Bodian 1936) with periodic acid Schiff (PAS) (Figure 3a) and
3. peroxidase-antiperoxidase staining method using anti-human IgG as the primary antibody (Figure 3d).

Twenty micron sections of the fixed frozen material were stained with:

4. von Braunmühl silver stain (Figure 3b) (Romeis 1968).

The absolute number of plaques and tangles was counted in five randomly selected fields at a magnification of 125x on both Bodian-PAS and von Braunmühl stained sections. The absolute number of the tangles (T) and plaques (P) on the Bodian-PAS stained sections (Figure 1a) were rated on a four-step scale (Table 2). Microscopic infarcts (I) were observed on the hematoxylin-eosin stained sections (Figure 3c) and were rated to three degrees of severity (Table 2). Perivascular serum protein deposits (D) were observed on immunocytochemically stained sections (Figure 3d, Table 2).

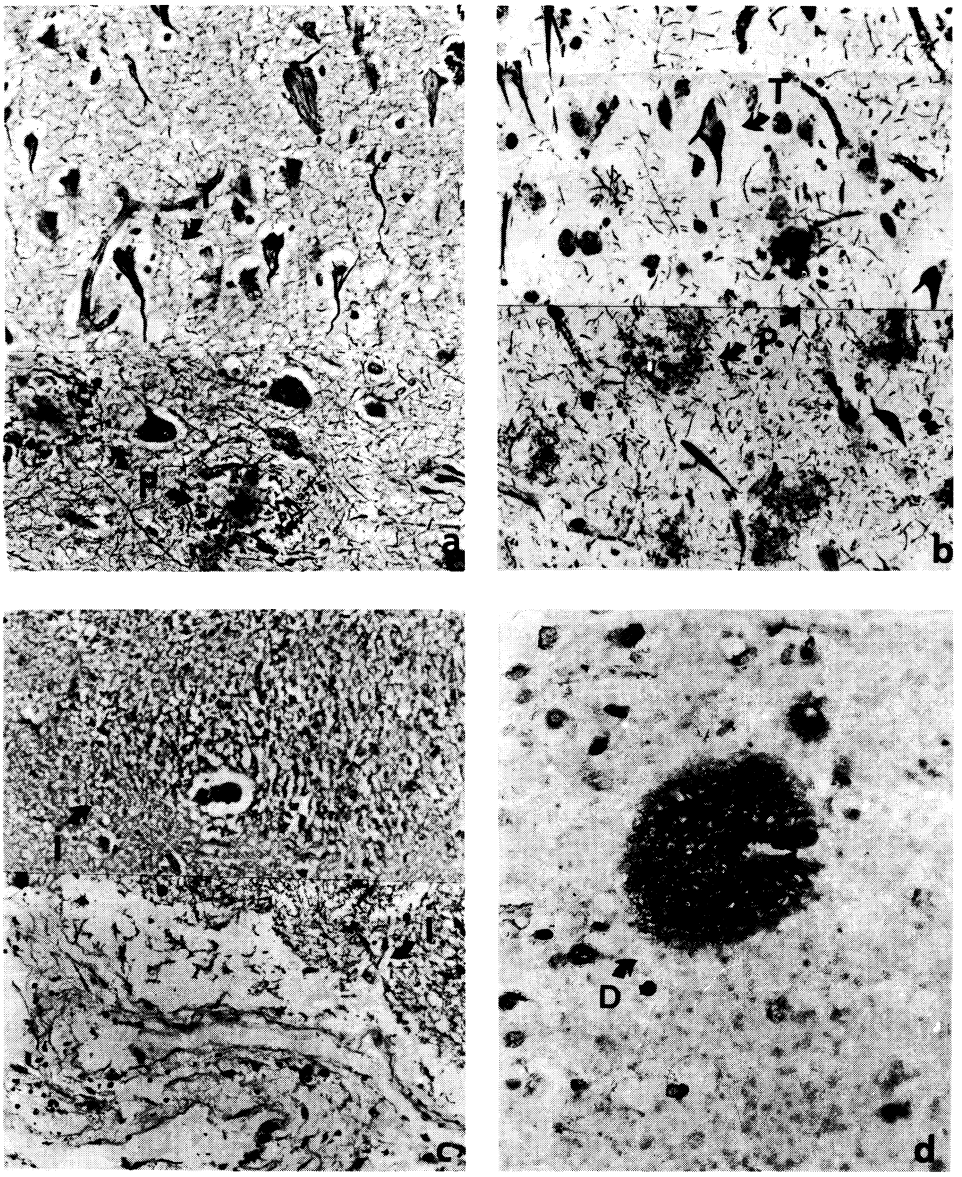

Figure 3. Light micrographs of AD/SDAT hippocampus. Tangles (T) and plaques (P) stained by a) Bodian-PAS and b) von Braunmühl silver stains. Light micrographs of MID hippocampus. Microscopic infarct (I) stained by c) hematoxylin-eosin stain and perivascular IgG deposits (D) stained by d) peroxidase-antiperoxidase methodology (PAP) in frontal cortex. Magnifications a,b,and d 290x and c 160x.

Table 2. Grading of the microscopic findings.

Observation		Absolute number	Rate number
plaques	*	not observed	0
		0.1 - 5.0	1
		5.1 - 10.0	2
		over 10.1	3
tangles	*	not observed	0
		0.1 - 10.0	1
		10.1 - 20.0	2
		over 20.1	3
histopathological dementia score	**		0 - 12
microscopic infarcts	***	not observed	0
		occasional	1
		widespread	2
perivascular serum protein deposits	****	not observed	-
		observed	+

* plaques and tangles counted in five randomly selected fields in magnification 125x on Bodian PAS stained sections: the absolute number is the mean of the five values

** the sum of rate number of plaques and tangles in the frontal cortex and the hippocampus,

*** noted on the hematoxylin-eosin stained sections

**** darkly stained deposits of IgG around the capillaries in the grey matter observed by the immunocytochemical technique (Alafuzoff et al 1985a, 1985b).

STATISTICS (I-VI)

Significant differences were determined using Student's t-test and the F-test. The correlations between different variables were calculated using linear regression analysis.

Multivariate data analysis (VI)

A method of pattern recognition, soft independent modelling of class analogies (SIMCA), including principal component modelling (PC) (Figure 4) and the partial least squares modelling in latent variables (PLS) (Figure 5) were used to define the different classes of dementia (Wold et al 1983, 1984a,b).

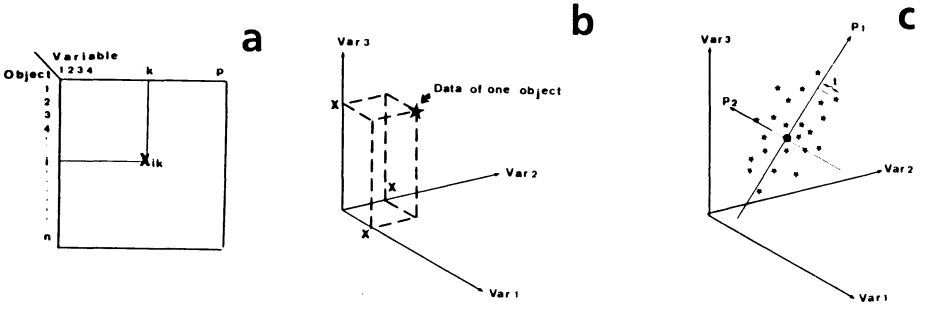

Figure 4. Drawing explaining the working of principal component (PC) analysis. a) Data matrix for principal component (PC) analysis where $\underline{x}$ is one measured value for one object. b) Multi(3)dimensional space, where all variable data for one object are represented by the position of a single point ✱. The variables used are autoscaled so that the variance is equal to one. This is done to give each variable equal influence on the modelling. c) The data are least squares $\underline{t}$ modelled by a line through the average. This line is the first principal component PC1. The equation of PC1 is defined by the loading vector $\underline{p1}$. Each additional component will reduce the residual variance. The number of significant components for each class model is determined by cross validation. The idea is to reduce the residual variance as far as possible without loosing predictability. The second principal component, PC2, is a line $\underline{p2}$ perpendicular to p1.

Representing the patients as points in a multi-dimensional space, the method projects the point swarm of the data set or parts of it down in a few-dimensional subspace which can be plotted.

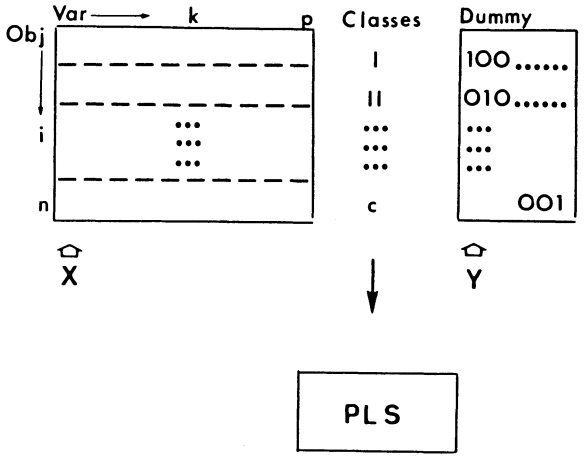

Figure 5. Principles of partial least squares modelling in latent variables (PLS). The information of first X and second Y data matrixes is combined to form the PLS plot. The first data matrix is formed by the independent variables, the second one is formed by the class dependent variables which "tilts" the planes for the first data matrix. This improves the relationship between the objects and the variables in the formed classes.

This provides an efficient way to convert the data table to a few informative figures showing the relationship between the patients and the variables.

RESULTS

IMMUNOLOGICAL STUDIES (I,II)

Plasma and cerebrospinal fluid concentrations of Ig

MID patients were found to have significantly higher concentrations of plasma-IgG and slightly higher plasma-IgA than both the AD/SDAT and the control patients. The plasma-IgM concentrations did not differ significantly from the controls. CSF-Alb was significantly higher in the MID group compared either with controls or with the AD/SDAT patients. CSF-Alb was higher in the AD/SDAT group than the controls. The IgG concentrations in CSF for the AD/SDAT patients were significantly lower than for the MID patients. The concentrations of Alb and IgG in plasma and CSF are presented as cumulative curves in Figure 6.

Blood-cerebrospinal fluid barrier function

Transudation (passage) of IgG through B-CSF-B was highest in the MID patients followed by the AD/SDAT patients and lowest in the control patients (Figure 7).

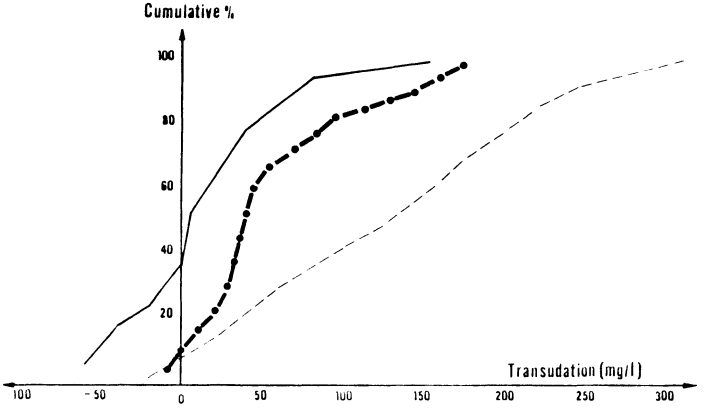

Figure 7. Serum protein transudation (passage) through blood-cerebrospinal fluid barrier (B-CSF-B) in non-demented (/), AD/SDAT (/) and MID (,) patients in cumulative concentrations.

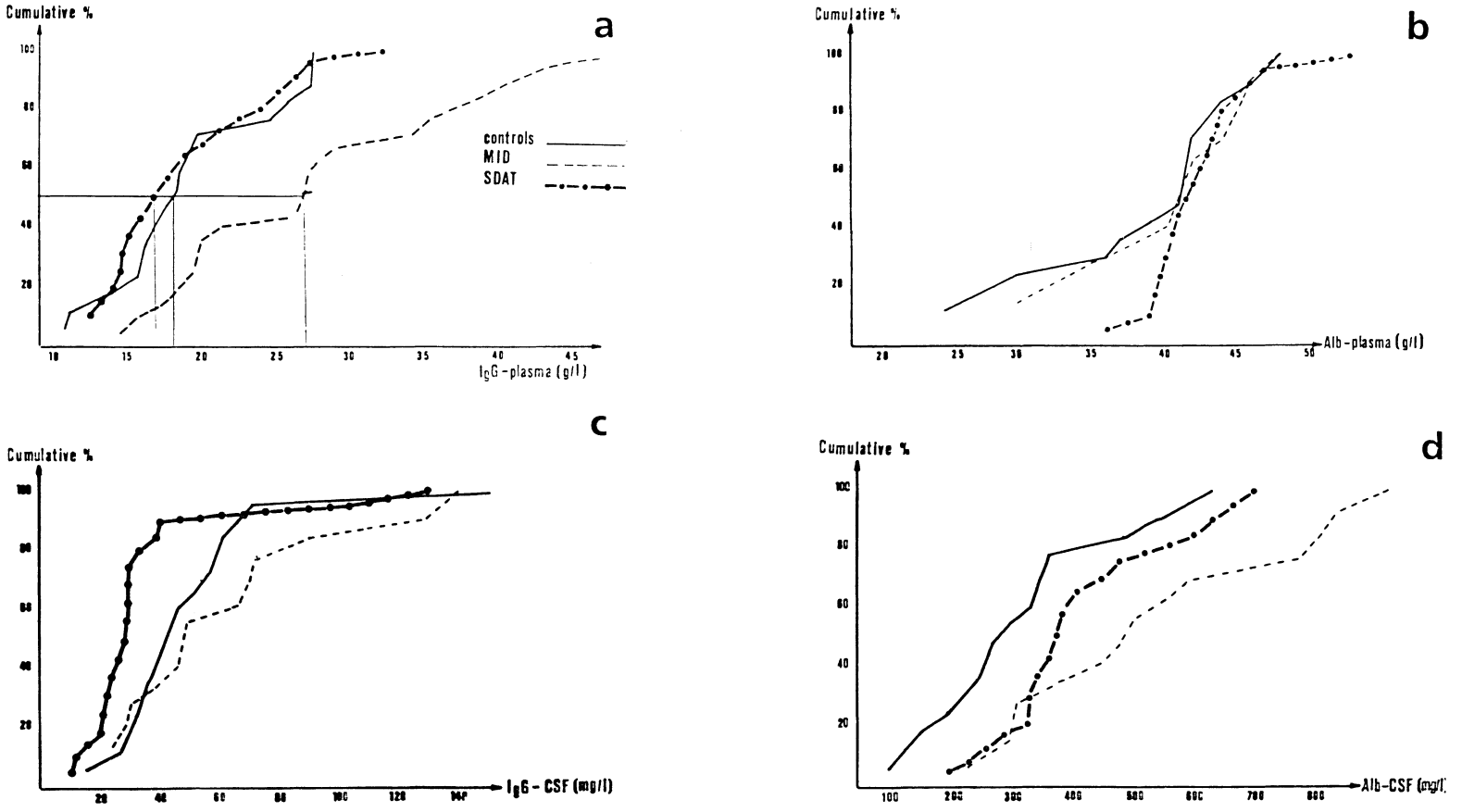

Figure 6. The IgG (a,c) and Alb (b,d) concentrations in plasma and CSF respectively, in cumulative concentrations.

The evaluation graph for the protein profile for CSF showed that both the AD/SDAT and the MID groups fell into an area which Reiber designated as biologically irrelevant (Figure 8).

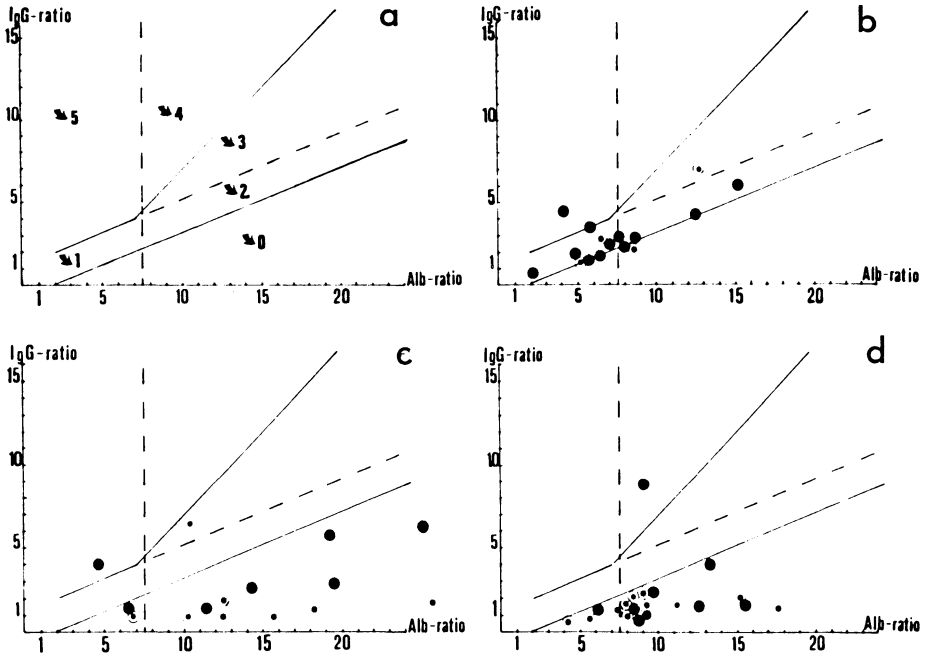

Figure 8. Protein profile by Reiber's graph in non-demented and demented patients. (For definition see Figure 1). a) explanation for priniples, b) non-demented, c) MID and d) AD/SDAT patients. Male (●) and female (◉) patients.

Local synthesis of Ig

According to the calculations based on the formulas for local synthesis described previously (Shuller and Sagar 1981, Link and Tibbling 1977) there were no signs of local synthesis of IgG in the CNS in either of the dementia groups.

Oligoclonal bands and other abnormal Ig

Matching pairs of CSF and plasma samples from patients with

MID and AD/SDAT showed an absence of oligoclonal IgG bands in immunofixation following iso-electric focusing (IEF) and were similar to those seen in the age-matched non-demented individuals.

No differences were seen in the protein pattern of plasma and CSF samples in two-dimensional PAGE between AD/SDAT patients, MID patients and non-demented individuals. No detectable amounts of brain specific proteins in the plasma and/or CSF were observed.

CLINICAL STUDIES (III)

The validity of the DSM-III criteria for AD/SDAT and MID were tested with the histopathological verification of the diagnosis (67 demented patients). A demented patient group (91 patients) with mean age of onset of 70 years and mean duration of the disease of 7 years was studied. Clinically these demented cases were classified as AD/SDAT 25%, probable SDAT 27%, MID 13% and probable MID 34%. Histopathologically the demented cases were classified in three different groups, namely, AD/SDAT, MID and AD-MID. Of the demented patients 45% fulfilled the histopathological criteria for AD/SDAT, 33% for MID and 21% of the demented cases were typed as AD-MID patients. Histopathological diagnosis with respect to clinical diagnosis in patients with AD/SDAT and MID are listed in Table 3.

Table 3. Comparison between clinical and histopathological diagnoses

Histopathological diagnosis (n)	Clinical diagnosis (DSM-III)				
	AD/SDAT	probable AD/SDAT	MID	probable MID	probable MID
AD/SDAT (30)	12 (57%)	9 (47%)	1	5	3
MID (22)	6	4	5 (71%)	7 (47%)	0
SDAT/MID (14)	3	5	1	3	2
NORMAL (1)	0	1	0	0	0

(%) confirmation rate

Clinical characteristics in patients with histopathologically verified dementia are listed in Table 4.

Table 4. Clinical characteristics of the histopathologically classified dementia patients

Histopathological diagnosis (n)	Age of onset	Duration of the disease	Hyper-tension	Diabetes mellitus	Heart diseases
AD/SDAT (30)	71.0±8.6	6.9±3.7	23.3%	10.0%	26.7%
MID (22)	70.1±8.3	6.0±2.8	45.5%	9.1%	50.0%
AD-MID (14)	69.4±7.7	7.0±2.9	42.9%	21.4%	63.44%
TOTAL (66)	70.5±8.3	6.6±3.2	34.3%	12.1%	42.4%

The sensitivity (the exactness of the inclusion criteria) and specificity (the exactness of the exclusion criteria), for the DSM-III criteria were calculated for the diagnosis SDAT/SDAT probable, and MID/MID probable (Table 5).

Table 5. Specificity / sensitivity of the DSM-III classification.

Clinical diagnosis	Histopathological diagnosis AD/SDAT (MID)	NON-AD/SDAT (NON-MID)
AD/SDAT	21	19
(MID)	(12)	(15)
NON-AD/SDAT	9	18
(NON-MID)	(10)	(30)
TOTAL	30	37
	(22)	(45)

Sensitivity AD/SDAT: 70 % (21/30), (MID): 54.5 % (12/22)

Specificity AD/SDAT: 51 % (19/37), (MID): 33.3 % (15/45)

IMMUNOCYTOCHEMICAL STUDIES (IV,V)

Antigenicity of tangles and plaques

In both demented and non-demented indivuduals, plaques could be visualized with anti-IgG, -C1q and -C3c (Figure 9).

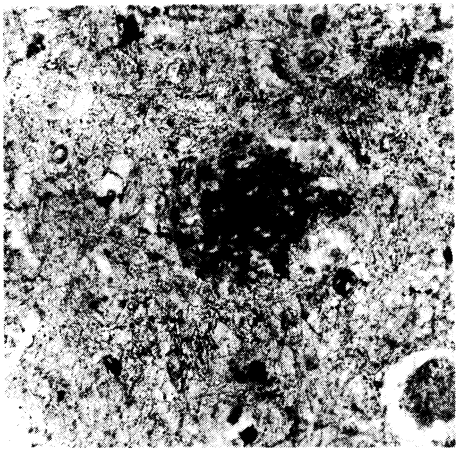

Figure 9. A section of frontal gyrus of a SDAT case stained with A-IgG serum (1:500). Note the staining of the senile plaque, predominantly the plaque core. Magnification 300x. The tissue was from a 82 year-old male patient with clinical and histopathological diagnosis of SDAT, duration of the disease six years.

Strikingly, no staining of plaques was observed with anti-Alb, anti-prealbumin or anti-fibrinogen sera despite the general staining of some areas of the section. Neurofibrillary tangles were not visualized with any of the antisera studied.

Blood-brain barrier function

In 61% of the MID cases, immunostaining with antisera to serum proteins revealed the presence of numerous dense deposits of serum proteins around capillaries in layers 1-4 in the grey matter of the frontal cortex (Figures 10a,b) and hippocampus.

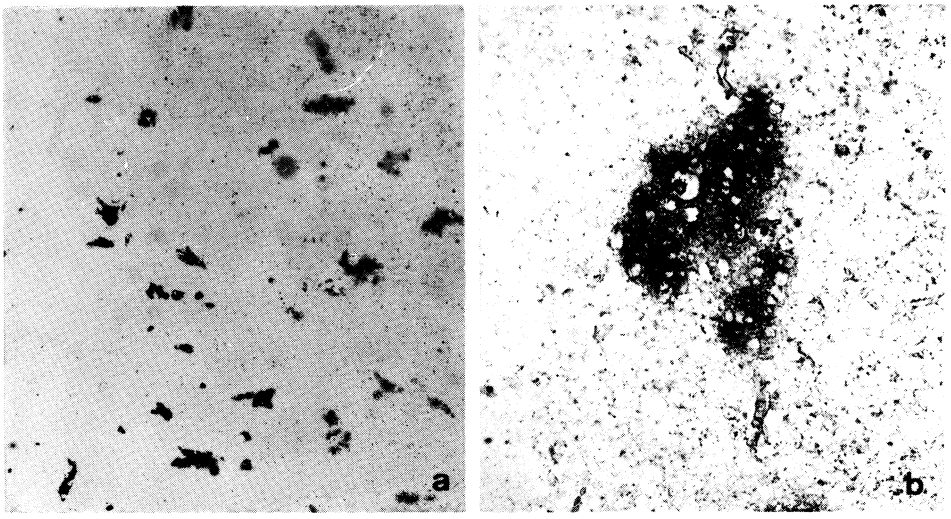

Figure 10. Seventy-six-year-old male patient with MID. Duration of the disease 3 years. Tissue taken from frontal cortex. A-IgG serum (1:500). Notice the localization of the perivascular IgG deposits in the gray matter. Magnifications a) 45x and b) 225x.

Both in demented and non-demented age-matched controls all serum proteins studied were shown to be present in the tissue surrounding most large vessels of the white matter (Figure 11).

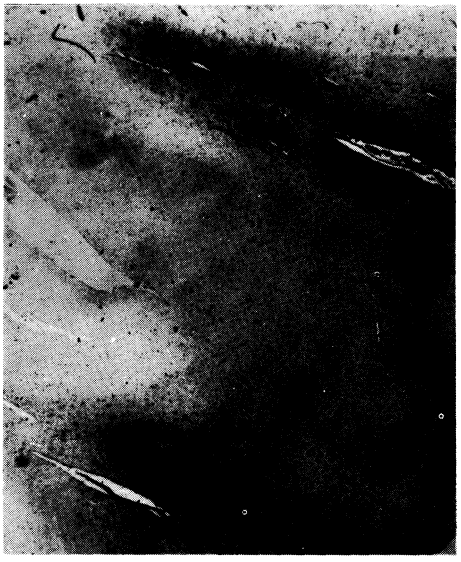

Figure 11. A section from frontal gyrus stained with A-IgG serum (1:500) showing the occurence of IgG in the surroundings of the vessels of the white matter. Magnification 25x. Same case as in figure 9.

The intensity of this staining gradually decreased with the distance from the vessel. No such immunostaining was observed in any of the normal age-matched controls. In demented cases and in normally aged controls staining of the neurons and glial cells within the diffusion area was observed with different antisera, except with the antisera to fibrinogen.

HISTOPATHOLOGICAL STUDIES (VI)

Description of the non-demented patients

Tangles were not observed in the frontal cortex of non-demented patients. The prevalence of plaques in the frontal cortex was 63%. In the hippocampus, the tangles were seen in 42% and plaques in 68% of the cases.

The range for the histopathological dementia score (the sum of the rate number of tangles and plaques in the frontal cortex and the hippocampus) was between 0 and 4 (For definition of the grading see Tab. 2). Prevalence of tangles and plaques together in both areas of the brain studied was 89%.

The severity of microscopic infarcts seen in the frontal cortex was estimated as 0-2 (prevalence 74%) and in the hippocampus as 0-1 (79%).

The perivascular deposits were not observed in non-demented cases (Table 6).

The number or severity of the lesion seen in the non-demented patient was used to define the "normal limits" for the normal ageing process. In AD/SDAT the primary lesions are plaques and tangles in MID vascular lesions. In AD-MID both types of lesions are coexistant. The histopathological criteria for the three different dementia types, namely, AD/SDAT, MID and AD--MID were postulated based on the number or degree of these lesions in non-demented patients (Table 7).

Table 6. Histopathological findings in non-demented aged patients

Case No	APf	RPf	ATf	RTf	APh	RPh	ATh	RTh	S	If/Ih	D
1	0.0	0	0.0	0	0.0	0	1.0	1	1	0 / 1	-
2	0.0	0	0.0	0	0.3	1	0.0	0	1	0 / 0	-
3	0.0	0	0.0	0	0.0	0	0.0	0	0	1 / 0	-
4	0.0	0	0.0	0	3.5	1	0.0	0	1	1 / 1	-
5	0.0	0	0.0	0	0.0	0	0.6	1	1	0 / 0	-
6	1.0	1	0.0	0	14.0	3	0.0	0	4	1 / 1	-
7	0.0	0	0.0	0	0.0	0	0.0	0	0	0 / 1	-
8	4.2	1	0.0	0	9.5	2	3.2	1	4	2 / 1	-
9	6.2	2	0.0	0	1.0	1	0.0	0	3	1 / 1	-
10	3.5	1	0.0	0	2.5	1	1.0	1	3	0 / 1	-
11	0.0	0	0.0	0	0.0	0	2.0	1	1	1 / 1	-
12	1.6	1	0.0	0	2.5	1	1.7	1	3	2 / 1	-
13	1.0	1	0.0	0	3.0	1	0.0	0	2	1 / 1	-
14	1.4	1	0.0	0	3.8	1	3.2	1	3	1 / 1	-
15	0.2	1	0.0	0	0.0	0	0.0	0	1	1 / 1	-
16	2.5	1	0.0	0	5.0	1	0.0	2	4	1 / 1	-
17	4.0	1	0.0	0	9.0	2	0.0	0	3	1 / 1	-
18	3.4	1	0.0	0	5.0	1	0.0	0	2	1 / 1	-
19	5.3	2	0.0	0	9.5	2	0.0	0	4	1 / 0	-
m±S.D.	1.8±2.0		0.0±0.0		3.6±4.1		1.7±4.6				
md (R)		1(0-2)		0(0)		1(1-2)		0(0-2)	2(0-4)		

A - absolute number of changes,
P - senile/neuritic plaques,
f - the frontal cortex,
R - rate number of changes (for definition see Table 2)
T - neurofibrillary tangles,
h - the hippocampus,
S - histopathological dementia score (for definition see Table 2)
I - microscopic infarcts,
D - perivascular serum protein deposits,
m - mean and S.D. - standard deviation,
md - median and (R) - range.
For definition of grading of microscopic findings see Table 2.

Table 7. The criteria for the histopathologic types of the dementias. The criteria listed are based on acceptable "normal limits" of dementia changes seen in non-demented aged individuals (for reference see Table 6).

Non-demented individual

no tangles in the frontal cortex;
variable number of plaques;
dementia score upp to 4;
microscopic infarcts in the frontal
cortex 0-2 and in the hippocampus 0-1;
no perivascular serum protein deposits.

Dementia with primary degenerative changes - AD/SDAT

either tangles in the frontal cortex, variable
number of plaques and dementia score of at least 4
or no tangles in the frontal cortex, variable number
of plaques and dementia score over 4;
microscopic infarcts in the frontal cortex
0-2, and the hippocampus 0-1;
no perivascular serum protein deposits.

Dementia with primary vascular changes - MID

no tangles in the frontal cortex;
variable number of plaques;
dementia score up to 4;
microscopic infarcts in the frontal cortex
0-2, and the hippocampus 2;
occurence of perivascular serum protein deposits.

Dementia with both degenerative and vascular changes - AD-MID

tangles in the frontal cortex;
variable number of plaques;
dementia score over 4;
microscopic infarcts in the frontal cortex
0-2, and the hippocampus 2;
occurence of perivascular serum protein deposits.

For definition of grading of microscopic findings see Table ?.

Description of the demented cases with definite diagnosis

The histopathological findings in demented patients are listed in Table 8.

Table 8. Histopathological findings in demented patients. Classification according to criteria listed in Table 7.

Case No	APf	RPf	ATf	RTf	APh	RPh	ATh	RTh	S	If/Ih	D	Clin. diagn
I	Histopathologically classified as AD/SDAT											
a)	In agreement with clinical diagnosis											
2	17.2	3	6.1	1	30.0	3	48.0	3	10	1 / 1	-	SDAT
4	10.6	3	12.5	2	5.0	1	2.4	1	7	1 / 1	-	SDAT
6	6.5	2	7.1	1	11.0	3	30.0	3	9	1 / 1	-	SDAT
8	3.0	1	1.0	1	3.0	1	2.0	1	4	1 / 0	-	SDAT
9	19.2	3	4.1	1	11.2	3	5.4	1	8	1 / 1	-	SDAT
10	9.8	2	6.6	1	6.1	2	9.2	1	6	2 / 1	-	SDAT
12	10.5	3	8.0	1	12.0	3	14.0	2	9	0 / 1	-	SDAT
13	7.1	2	11.4	2	10.0	2	27.7	3	9	0 / 1	-	SDAT
14	17.5	3	3.0	1	15.5	3	15.0	2	9	1 / 1	-	SDAT
15	5.0	1	2.4	1	20.0	3	10.0	1	6	1 / 1	-	SDAT
17	17.5	3	3.0	1	10.5	3	3.2	1	8	0 / 1	-	SDAT
21	9.3	2	21.7	3	10.2	3	32.9	3	11	0 / 1	-	SDAT
22	0.0	0	1.2	1	13.5	3	15.6	2	6	0 / 1	-	SDAT
24	4.1	1	2.1	1	3.6	1	8.6	2	5	0 / 0	-	SDAT
25	8.5	2	7.1	2	11.7	3	40.7	3	10	0 / 0	-	SDAT
31	4.6	1	2.4	1	7.1	2	15.1	2	6	0 / 1	-	SDAT
32	2.5	1	1.5	1	14.0	3	10.0	1	6	1 / 1	-	SDAT
b)	In disagreement with clinical diagnosis											
33	2.5	1	0.0	0	13.0	3	23.3	3	7	2 / 1	-	MID
37	2.2	1	0.2	1	4.0	1	0.3	1	4	1 / 0	-	MID
39	22.5	3	7.6	1	37.0	3	12.7	2	9	2 / 1	-	MID
41	1.5	1	0.0	0	14.0	3	16.7	2	6	0 / 1	-	MID
48	25.3	3	5.9	1	6.0	2	19.5	3	9	1 / 1	-	MID
50	11.9	3	3.4	1	11.3	3	38.3	3	10	1 / 0	-	MID
51	4.9	2	1.4	1	3.3	1	4.7	1	5	2 / 1	-	MID
52	7.0	1	4.0	1	12.0	3	15.0	2	7	2 / 1	-	MID
54	8.0	2	0.0	0	14.0	3	1.0	1	6	2 / 1	-	NUD
55	5.1	2	1.6	1	6.7	2	21.8	3	8	1 / 1	-	NUD
II	Histopathologically classified as MID											
a)	In agreement with clinical diagnosis											
34	0.0	0	0.0	0	0.3	1	3.0	1	2	1 / 2	-	MID
36	3.3	1	0.0	0	6.0	2	10.0	1	4	1 / 2	+	MID
38	3.3	1	0.0	0	0.7	1	0.0	0	2	1 / 0	+	MID
40	1.0	1	0.0	0	1.6	1	0.4	1	3	2 / 2	+	MID
44	0.0	0	0.0	0	0.0	0	0.0	0	0	2 / 2	-	MID
45	0.3	1	0.0	0	3.1	1	8.7	1	3	2 / 2	+	MID
49	2.4	1	0.0	0	3.0	1	8.0	1	3	2 / 2	+	MID
b)	In disagreement with clinical diagnosis											
5	0.0	0	0.0	0	0.0	0	0.0	0	0	2 / 2	-	SDAT
7	0.0	0	0.0	0	0.1	1	0.1	1	2	2 / 2	-	SDAT
27	0.0	0	0.0	0	0.0	0	0.0	0	0	1 / 2	+	SDAT
28	0.0	0	0.0	0	0.0	0	2.0	1	1	1 / 0	+	SDAT
29	1.0	1	0.0	0	1.0	1	1.0	1	3	2 / 2	-	SDAT
30	0.0	0	0.0	0	0.0	0	3.2	1	1	0 / 2	+	SDAT

III Histopathologically classified as AD-MID

1	12.7	3	2.7	1	15.2	3	18.2	2	9	2 / 2 +	SDAT
3	5.5	2	3.0	1	26.5	3	4.1	1	7	2 / 2 -	SDAT
11	10.2	3	4.7	1	3.0	1	4.1	1	6	2 / 1 +	SDAT
16	10.0	2	3.5	1	19.2	3	4.1	1	7	1 / 2 -	SDAT
18	6.3	2	1.9	1	10.7	3	27.7	3	9	1 / 0 +	SDAT
19	11.8	3	3.0	1	6.0	2	4.0	1	7	1 / 1 +	SDAT
20	4.5	1	0.5	1	4.2	1	6.6	1	4	2 / 2 -	SDAT
23	2.6	1	1.1	1	16.2	3	35.6	3	8	2 / 2 -	SDAT
26	7.2	2	21.1	3	13.4	3	29.0	3	11	1 / 2 +	SDAT
35	9.6	2	4.8	1	11.9	3	20.1	3	9	0 / 2 +	MID
42	5.7	2	7.0	1	10.7	3	21.7	3	9	2 / 2 +	MID
43	1.7	1	0.0	0	21.7	3	39.0	3	7	2 / 2 -	MID
46	9.5	2	2.2	1	15.7	3	12.9	2	8	1 / 2 -	MID
47	6.5	2	0.2	1	7.5	2	15.7	2	7	1 / 2 -	MID
53	6.7	2	0.0	0	4.7	1	20.8	3	6	1 / 2 +	MID
m±S.D.	6.6±6.0		3.3±4.7		9.4±7.8		13.6±12.3				
md (R)		2(0-3)		1(0-3)		2(0-3)		1(0-3)	7(4-11)		

A - absolute number of changes,
P - senile/neuritic plaques,
f - the frontal cortex,
R - rate number of changes (for definition see Table 2),
T - neurofibrillary tangles,
h - the hippocampus,
S - histopathologic dementia score (for definition see Table 2),
I - microscopic infarcts,
D - perivascular serum protein deposits,
m±S.D. - the mean and standard deviation of all demented cases,
md (R) - median and range of all demented cases.
For definition of grading of microscopic findings see Table 2.

There were seventeen cases with definite diagnosis of AD/SDAT (clinical and histopathological diagnoses in agreement) (Table 8,9). In these cases the prevalence of tangles was 100% in both the frontal cortex and the hippocampus. The plaques were seen in 94% of the cases in the frontal cortex and in every case in the hippocamus. The range of the histopathological dementia score was 4 - 11 and the prevalence of tangles and plaques together in both brain areas studied was 100%. The severity of microscopic infarcts seen in the frontal cortex was estimated as 0-2 (prevalence 53%) and in the hippocampus as 0-1 (prevalence 82%). The perivascular deposits were not observed in AD/SDAT cases.

Table 9. Description of the patients "with definite diagnosis"

Variables:	Non-demented n=19	Demented n=24 AD/SDAT	MID
Number M/F	13 / 6	11 / 6	7 / 0
Age	74.3 ± 10.5	77.7 ± 8.6	76.0 ± 6.6
Duration of disease	0	6.1 ± 3.2	3.9 ± 2.4
Brain weight	1293 ± 113	1225 ± 168	1393 ± 81
Arteriosclerosis	1 (0 - 3)	1 (0 - 2)	2 (1 - 3)
Macroscopic infarcts	0 (0 - 2)	0 (0 - 2)	2 (0 - 2)
The frontal cortex			
plaques: abs.number	1.8 ± 2.0	9.0 ± 5.9	1.5 ± 1.5
rate number	1 (0 - 2)	2 (0 - 3)	1 (0 - 1)
tangles: abs.number	0	6.0 ± 5.3	0
rate number	0	1 (1 - 3)	0
microscopic infarcts	1 (0 - 2)	1 (0 - 2)	2 (1 - 2)
The hippocampus			
plaques: abs.number	3.6 ± 4.1	11.4 ± 6.5	2.1 ± 2.1
rate number	1 (0 - 2)	3 (1 - 3)	1 (0 - 2)
tangles: abs.number	1.7 ± 4.6	17.1 ± 13.9	4.4 ± 4.5
rate number	0 (0 - 2)	2 (1 - 3)	1 (0 - 1)
microscopic infarcts	1 (0 - 1)	1 (0 - 1)	2 (0 - 2)
Histopathological dementia score	2 (0 - 4)	8 (4 -11)	3 (0 - 4)
Perivascular serum protein deposits (+)	0 %	0 %	72 %

xx ± xx = mean ± S.D.
x(x - x) = median (range)
For definition of grading of microscopic findings see Table 2.

There were seven cases with definite diagnosis of MID (Table 8,9). Tangles were not observed in the frontal cortex

and in the hippocampus they were seen in 71% of the cases. The prevalence of plaques was 71% in the frontal cortex and 86% in the hippocampus. The range of the histopathological dementia score was 0-4 and the prevalence of tangles and plaques together in both brain areas studied was 86%. The severity of microscopic infarcts seen in the frontal cortex was estimated as 1-2 and in the hippocampus as 2 (prevalence 86%). The perivascular deposits were seen in the grey matter in 71% of the MID cases.

Multivariate data analysis

Multivariate data analysis by principal component (PC) modelling (Wold et al 1983, 1984a,b) with age, sex, gross neuroanatomical and histopathological changes as the tested variables reveled that four histopathological groups (non-demented, AD/SDAT, MID and AD-MID) can be separated with some overlap (Figure 12a,b).

Analysis by the partial least squares modelling in latent variables (PLS), employing only those cases with definite diagnoses (SIMCA classes) clearly separated the non-demented, AD/SDAT and MID groups from one another (Figure 13a,b).

The remaining demented cases without definite diagnosis (31 patients) were for their visual classification into the constructed SIMCA classes projected into the calculated PLS model and ploted (Figure 14). The probabilities for membership of these cases to the PC models of the classes constructed on the patients with definite diagnosis and on patients histopathologically typed as AD-MID were determined (Table 10). The results of these analyses revealed that some of these 31 patients were indeed members of one of the SIMCA classes (AD/SDAT: case numbers 48,54; MID: case numbers 7,28,29,37 and AD-MID:case numbers 33,52 and 55). The AD-MID cases were separable in the two-dimensional space in the PLS plot (Figure 13). The results on the PC and PLS analyses suggest that the AD-MID cases might be considered as a separate dementia class.

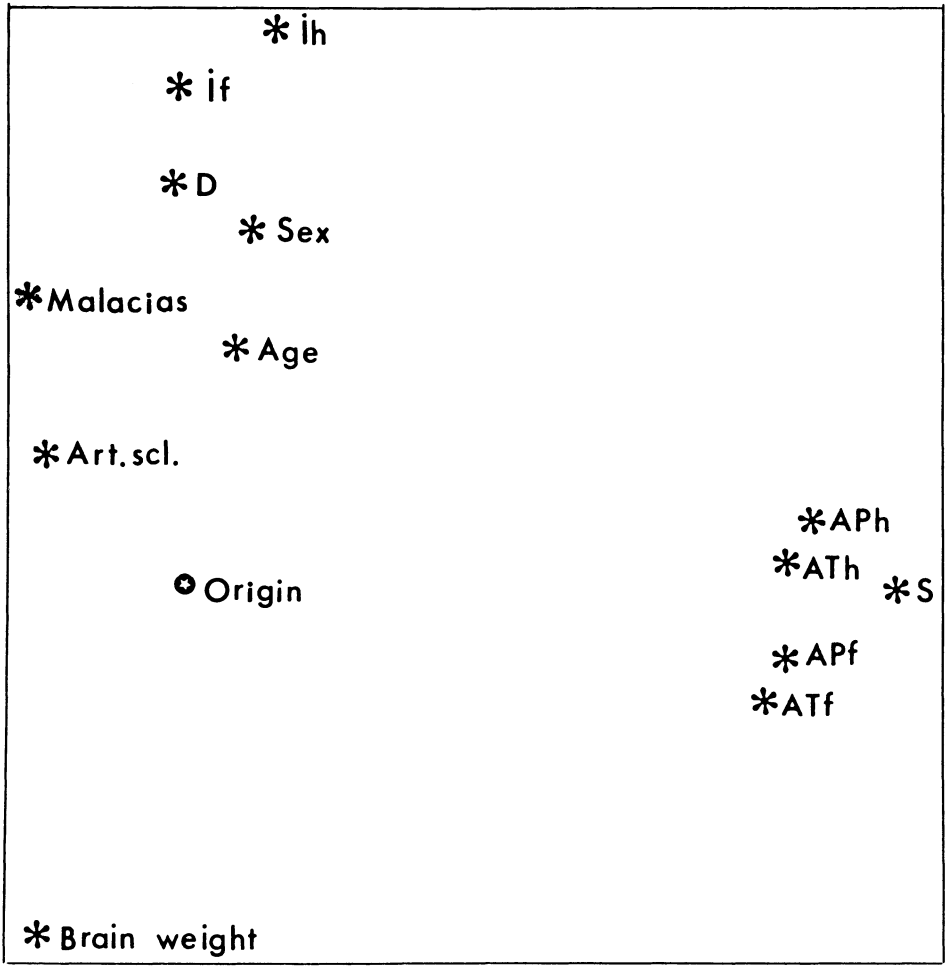

Figure 12a. Principal component analysis in non-demented and demented patients.
The variable loading plot employing age, sex, gross neuroanatomical and histopathological parameter. Variables furthest from the origin are those with the highest separation power.

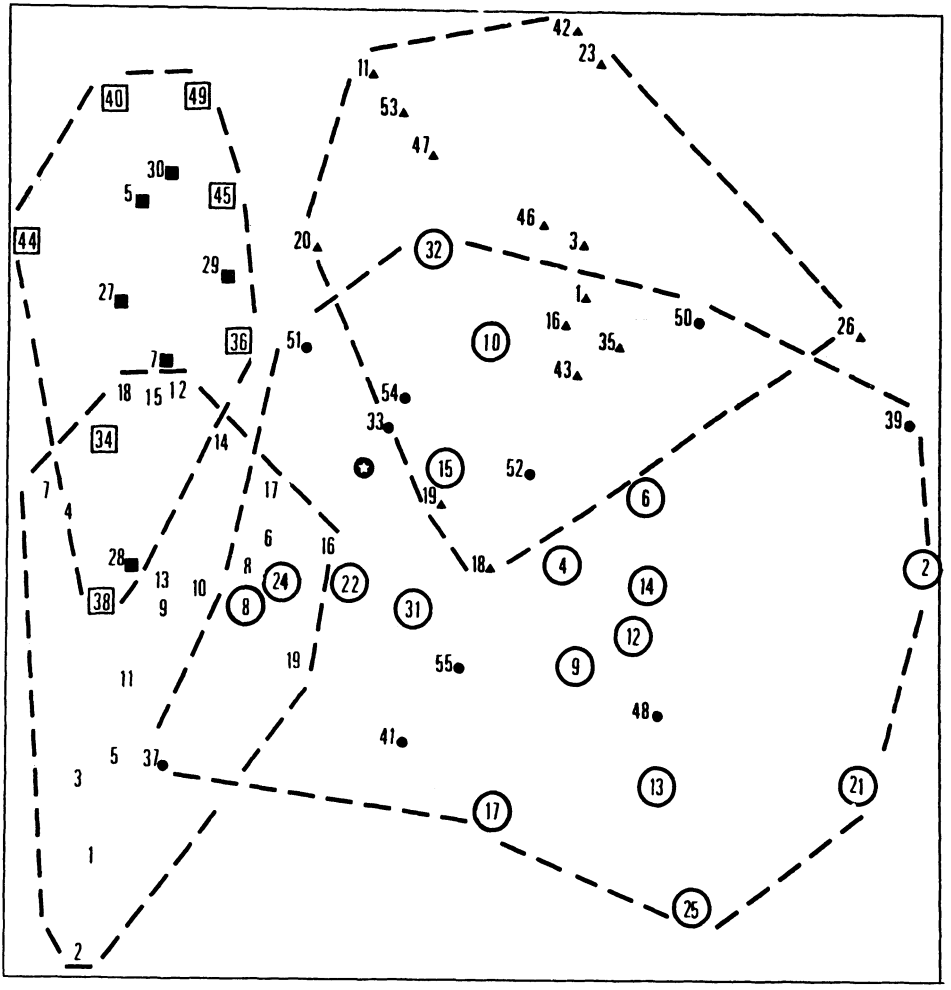

Figure 12b. Principal component (PC) analysis in non-demented and demented patients.
The PC pattern of all 19 non-demented and 55 demented patients in multi-dimensional variable space as seen through a two-dimensional window. Notice the separation into four different groups. Non-demented (n), histopathologically classified AD/SDAT (n) and MID (n) patients with definite diagnosis, AD/SDAT (n) and MID (n) patients without definite diagnosis and AD-MID (n) patients.

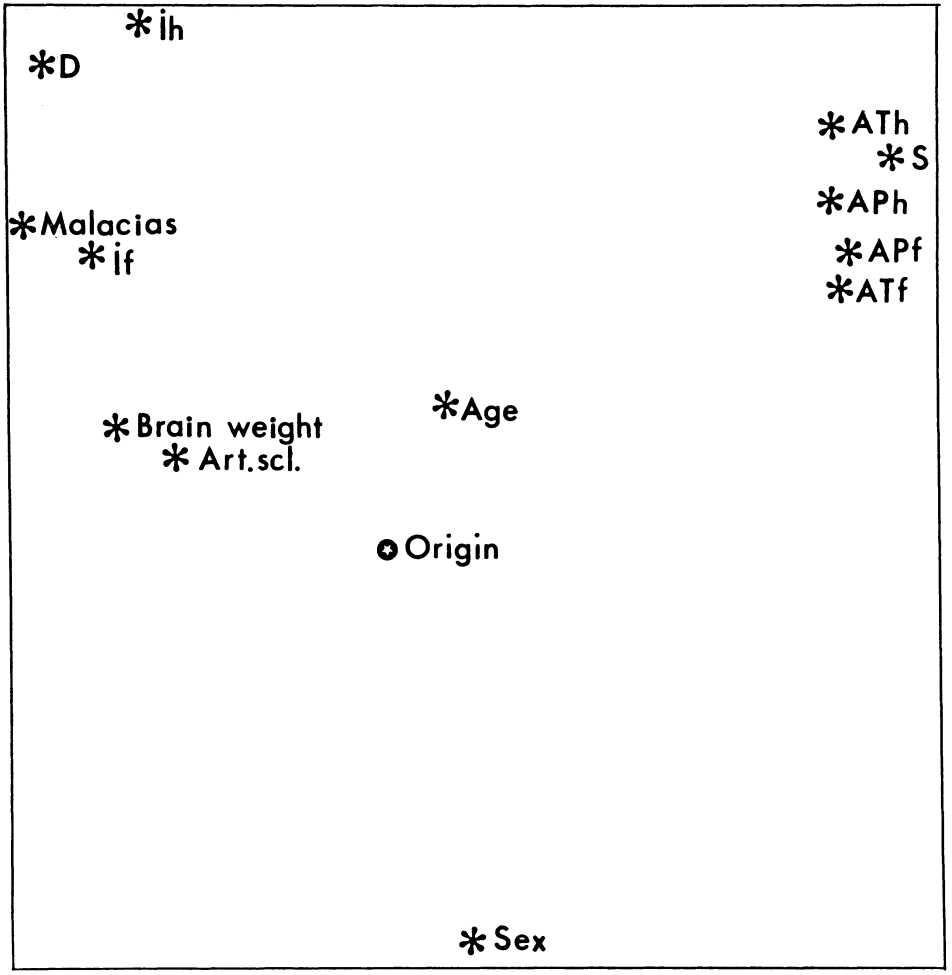

Figure 13a. Analysis of the non-demented and demented patients with definite diagnosis by the partial least squares modelling in latent variables (PLS).
The variable loading plot employing the same parameters as in Figure 12a but in the presence of the clinical diagnosis. The PLS classes were manually formed according to the rule that objects selected to a class are in some way supposed similar. The classes used were formed of patients with a definite diagnosis (clinical and histopathological diagnoses in agreement) and there were no "outliers" within each formed class.

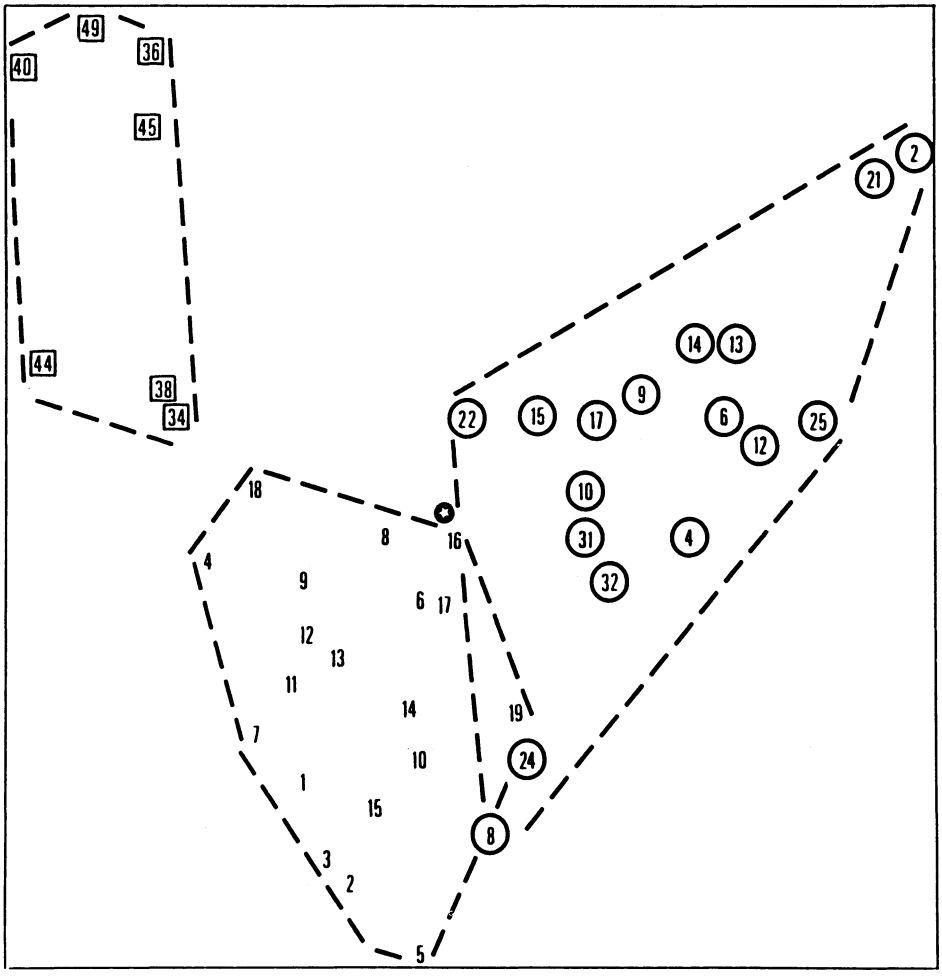

Figure 13b. Analysis of the non-demented and demented patients with definite diagnosis by PLS.
The PLS modelling patterns showing the three formed SIMCA classes, every point representing an individual case. Notice the clear separation of the classes. Non-demented (n), AD/SDAT (n) and MID (n) patients with definite diagnosis.

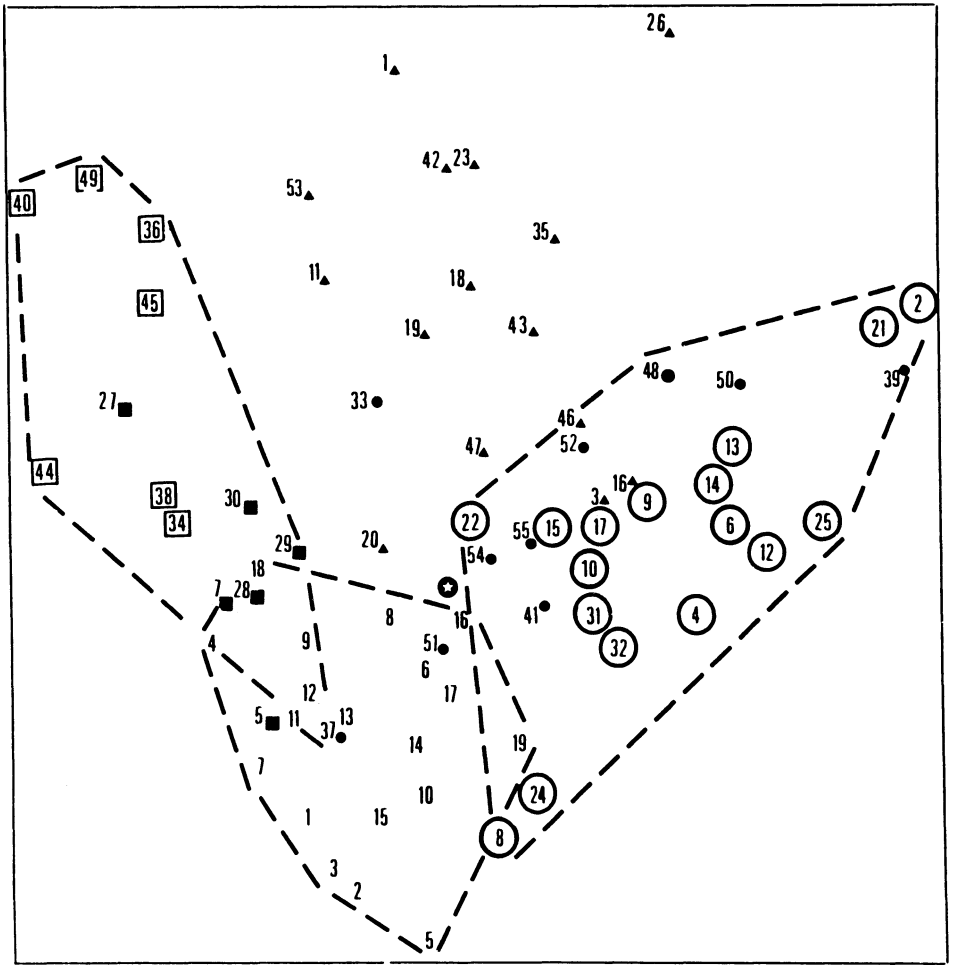

Figure 14. The PLS modelling patterns on non-demented and demented cases both with and without definite diagnoses. The variable loading plot same as in Figure 13a. Some of the unclassified cases were found to be members of AD/SDAT and MID classes. Notice the separate "group" of cases, histopathologically classified as AD-MID patients. Non-demented (n), AD/SDAT (ⓝ) and MID (ⓝ) with definite diagnosis AD/SDAT ($n_{\bullet}$) and MID ($n_{\blacksquare}$ without definite diagnosis and AD-MID ($n_{\blacktriangle}$) patients.

Table 10. Classification of the dementia cases without definite diagnosis.

Case No	Clin. diagn.	Histopath. diagn.	Probability of not membership. Nondemented	AD/SDAT	MID	AD-MID
1.	AD/SDAT	AD-MID	1.000 ***	1.000 ***	1.000 ***	0.827
3.	AD/SDAT	AD-MID	1.000 ***	1.000 ***	1.000 ***	0.306
5.	AD/SDAT	MID	0.998 **	1.000 ***	0.997 **	1.000 ***
7.	AD/SDAT	MID	0.882	0.999 ***	0.655	0.999 ***
11.	AD/SDAT	AD-MID	1.000 ***	1.000 ***	0.998 **	0.375
16.	AD/SDAT	AD-MID	1.000 ***	0.961 *	1.000 ***	0.808
18.	AD/SDAT	AD-MID	1.000 ***	1.000 ***	1.000 ***	0.613
19.	AD/SDAT	AD-MID	1.000 ***	1.000 ***	0.999 ***	0.783
20.	AD/SDAT	AD-MID	0.723	0.999 ***	0.935	0.669
23.	AD/SDAT	AD-MID	1.000 ***	1.000 ***	1.000 ***	0.651
26.	AD/SDAT	AD-MID	1.000 ***	1.000 ***	1.000 ***	0.650
27.	AD/SDAT	MID	1.000 ***	1.000 ***	0.964 *	1.000 ***
28.	AD/SDAT	MID	1.000 ***	1.000 ***	0.935	1.000 ***
29.	AD/SDAT	MID	0.939	0.999 ***	0.774	0.999 ***
30.	AD/SDAT	MID	1.000 ***	1.000 ***	0.998 **	0.999 ***
33.	MID	AD/SDAT	1.000 ***	0.999 ***	0.999 ***	0.819
35.	MID	AD-MID	1.000 ***	1.000 ***	0.999 ***	0.392
37.	MID	AD/SDAT	0.982 **	0.978 *	0.732	0.999 ***
39.	MID	AD/SDAT	1.000 ***	0.999 ***	1.000 ***	1.000 ***
41.	MID	AD/SDAT	0.998 **	0.997 **	0.999 ***	0.999 ***
42.	MID	AD-MID	1.000 ***	1.000 ***	0.999 ***	0.307
43.	MID	AD-MID	1.000 ***	0.999 ***	1.000 ***	0.056
46.	MID	AD-MID	1.000 ***	0.936	0.999 ***	0.708
47.	MID	AD-MID	1.000 ***	0.995 **	0.999 ***	0.471
48.	MID	AD/SDAT	1.000 ***	0.938	1.000 ***	0.999 ***
50.	MID	AD/SDAT	1.000 ***	0.998 **	1.000 ***	0.995 **
51.	MID	AD/SDAT	0.630	0.855	0.960 *	0.702
52.	MID	AD/SDAT	1.000 ***	0.964 *	0.999 ***	0.826
53.	MID	AD-MID	1.000 ***	1.000 ***	1.000 ***	0.191
54.	NUD	AD/SDAT	1.000 ***	0.821	0.999 ***	0.829
55.	NUD	AD/SDAT	1.000 ***	0.897	0.998 **	0.778

A principal component (PC) modelling of each of the formed classes (non-demented, demented "with definite diagnosis" i.e. AD/SDAT and MID. and for histopathologically classified AD-MID cases) was performed. Using the distance of each case to the models i.e. classes in space, the probability of class membership was calculated by F-test for patients "without definite diagnosis". This was done to find both the members of the classes and the "outliers" (not a member of the formed classes).

F-test: * $p < 0.05$, ** $p < 0.01$ and *** $p < 0.001$.

Case numbers 7, 29, 46, 54 and 55 showed probability of being members of two classes and case numbers 20 and 51 showed probability of being members of three classes. In these cases note the lowest probability for membership.

In order to verify that the pathology in these AD-MID cases were not merely a linear combination of the pathology in AD/SDAT and MID, these cases were tested on a PLS model calculated for the lumped definite dementia cases. The AD-MID group remained separate from the lumped cases (F-test on the 2.5% level). The lack of additivity might roule out the notion that the AD-MID cases were merely a reflection of cases which, for example, started with AD/SDAT and then developed MID, or vice versa, since the use of post-mortem tissue always reflects the end stage of the disease, unless secondary illness can obfuscate the interpretation.
Six of the remaining unclassified cases were "outliers" without any membership to the non-demented, AD/SDAT, MID or AD-MID (SIMCA) classes and one case showed high probability of being a member of the non-demented class.

Summary on classification of demented cases by multivariate data analysis

A total of nineteen cases (17 with and 2 without definite diagnosis) were found to belong to the AD/SDAT, eleven cases (7 with and 4 without definite diagnosis) to the MID and eighteen (15 with and 3 without histopathological diagnosis of AD-MID) to the AD-MID classes.
Remaining seven cases were unclassified by means of the multivariate data analysis.
A schematic presentation of the relationships between the a) clinical DSM-III classification, b) histopathological classification according to the criteria listed in Table 7 and c) SIMCA classifications is given in Table 15.

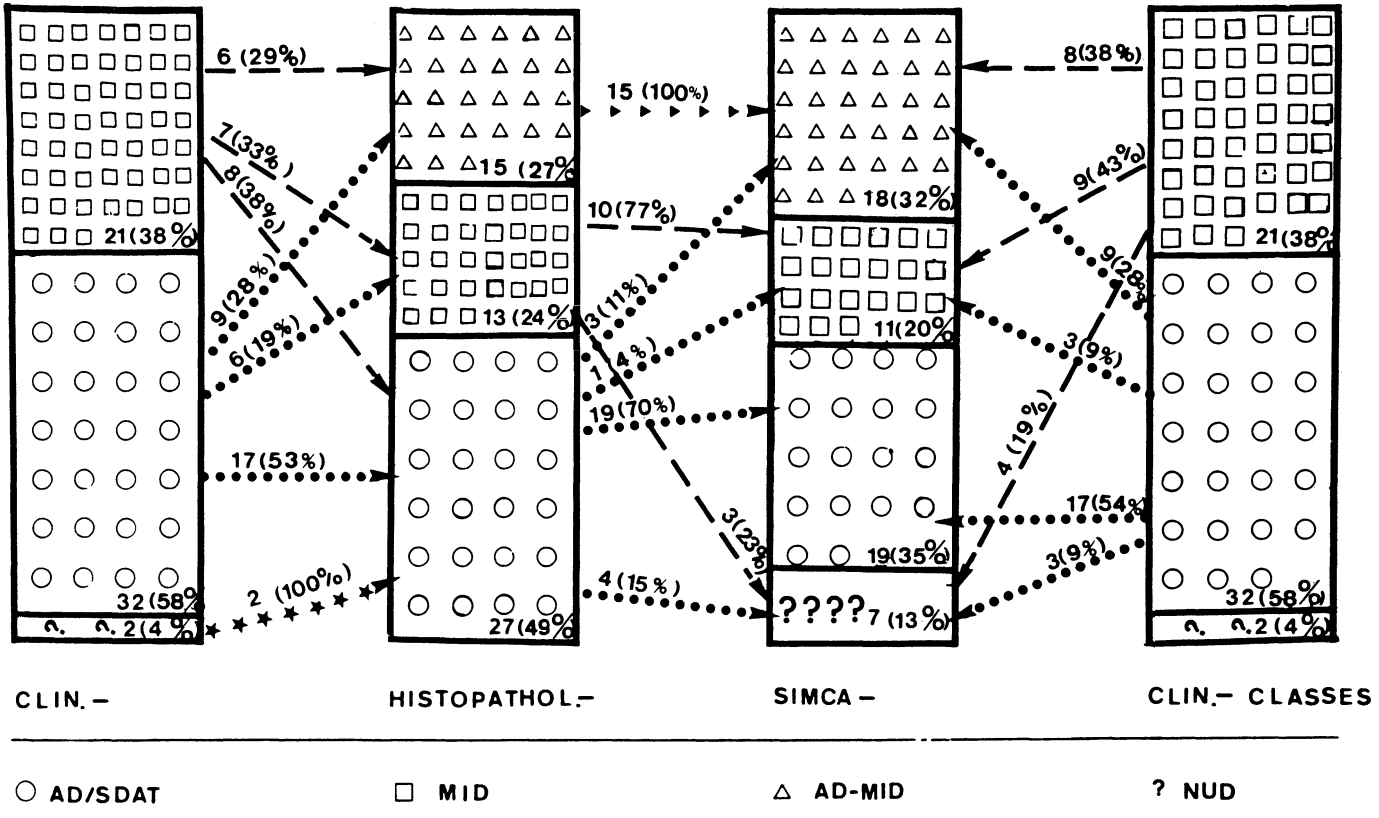

Figure 15. Schematic presentation of the relationship between a) clinical DSM-III classification of dementia patients, b) histopathological classification of demented patients according to the criteria listed in Table 7 and c) SIMCA classification of demented patients.

Correlations between different variables in the non-demented and demented cases with definite diagnosis

In the non-demented aged individuals, there was a positive correlation between age and both the number of plaques in the frontal cortex and with the histopathological dementia score. The number of plaques in the frontal cortex showed positive correlation with the number of plaques in the hippocampus. A negative correlation was observed between age and brain weight.

In the AD/SDAT patients with definite diagnosis, in contrast to the non-demented patients, the age of the patient was negatively correlated with the number of plaques in the frontal cortex. In the hippocampus numerous plaques were seen in combination with a high number of tangles.

In the MID patients with definite diagnosis, the degree of microscopic infarcts seen in the frontal cortex was most severe in the older patients. The number of plaques in the hippocampus correlated with the number of tangles in the MID patients.

Comparison of counts of tangles and plaques by two silver staining methods

Overall, the counts of plaques and tangles were slightly higher with von Braunmühl silver staining method than found with the Bodian-PAS silver staining method (Table 11). However, the absolute number of plaques and tangles visualized with these two staining methods are not fully comparable, as the thickness of the sections differed (Bodian PAS: 6 micron, von Braunmühl: 20 micron) and, though neighbouring and taken from same tissue block, the sections were not adjacent.

Table 11. Comparison of the counts of tangles and plaques visualized by von Braunmühl (vB) and Bodian PAS (BP) silver stains.

		Mean	Standard Deviation	Range
Control cases	APfvB	2.1	3.3	0 - 7.7
n = 17	BP	0.5	0.7	0 - 1.8
	ATfvB	0.0	0.0	0 - 0.0
	BP	0.0	0.0	0 - 0.0
	APhvB	3.0	4.0	0 - 10.0
	BP	2.2	3.3	0 - 9.0
	AThvB	1.5	0.9	0 - 6.4
	BP	1.2	2.4	0 - 2.0
Demented	APfvB	11.8	11.6	0 - 40.0 *
n = 27	BP	4.5	4.4	0 - 15.0 *
	ATfvB	4.9	7.1	0 - 31.7
	BP	3.1	5.0	0 - 22.0
	APhvB	12.7	12.6	0 - 49.0
	BP	7.7	7.8	0 - 34.0
	AThvB	16.7	16.4	0 - 63.0
	BP	12.0	14.5	0 - 60.0

AP - absolute number of plaques,
f - the frontal cortex,
AT - absolute number of tangles,
h - the hippocampus.
All numbers are avarages of five counts carried out at magnification 125x.
* Significant difference in counts ($p<0.005$) carried out on vB and BP stained sections.
stained sections in the dementia class were compared.

D I S C U S S I O N

IMMUNOLOGICAL FINDINGS

People over 65 years without cognitive impairment had higher plasma-IgG levels (I) compared to reference values for 30-year-old individuals (Buckley et al 1974). Similar elevation in plasma-IgG have been reported earlier (Buckley and Dorsey 1971), and might be explained by an increased synthesis of IgG in elderly people (Makinodan 1979). Higher IgG concentrations might indicate a well-functioning immune system necessary for survival (Walford 1974).

Plasma-IgG levels in the MID group were higher compared to age-matched controls (I), suggesting a more active immune responce. The elevation in plasma-IgG may indicate an increase in auto-antibody concentration, found both in patients with cardiovascular diseases (Beregi et al 1978) and in patients with cerebrovascular lesions (stroke) (Berry and Riches 1974).

The MID patients had higher CSF-IgG concentrations than the AD/SDAT patients (I) but were not significantly different than those found in non-demented controls. These findings, in addition to low CSF-IgG index (I) and the low value of local synthesis of IgG (I), speak against an intrathecal IgG production in the CNS in demented patients.

When compared with healthy individuals between 21 and 30 years of age (Eeg-Olofsson et al 1981), unimpaired aged patients showed higher values in CSF-Alb (I), indicating an alteration in B-CSF-B function with age, in agreement with a study by Tibbling et al (1977) and Kobatake et al (1980).

The MID and the AD/SDAT patients had elevated CSF-Alb concentrations (I), suggesting either a less "dense" barrier function or a lower CSF turnover in the CNS. The increased Alb ratio in the MID group is consistent with both suggestions.

Contrasting results for the B-CSF-B function in demented individuals and, especially, for the clinical group of AD/SDAT, have been reported (Bock et al 1974, Jonker et al 1982

Leonardi et al 1985, Elovaara et al 1985a).
The findings of lower IgG ratio and CSF-IgG index in the AD/SDAT group along with the moderately higher transudation value compared to the controls and the observation that both MID and AD/SDAT groups fell into the so-called biologically irrelevant area (I) has not been reproduced by others (Leonardi et al 1984). This difference is primarily based on the proportionally lower CSF and plasma concentrations of IgG than Alb in our study compared with the results of Leonardi et al (1984).

Some of the possible explanations for these different results are: a) differences in clinical classification, b) differences in collection procedure of the investigated material, c) differences in the storage time of the material (the stability of the investigated proteins in -70 C is not sufficiently established and c) differences in the methodology used.

Both infectious diseases and diseases which are presumed to be infectious have been associated with the presence of oligoclonal antibodies in the CSF (Iivanainen et al 1981, Mehta et al 1982b,c). Protein bands, probably representing oligoclonal Ig production, have both been and not been demonstrated in CSF samples from demented patients (Williams et al 1980, Elovaara et al 1985b, Wikkelsö et al 1981). No such "bands" however were demonstrated by us (II). Oligoclonal Ig production by plasma cells in CSF might be initiated by an unspecific reaction to the tissue damage found in the late stages of degenerative dementia and early in the course of vascular dementia.

The lack of precise criteria for the clinical classification of AD-MID may in combination with the etiology of oligoclonal antibodies provide a possible explanation for the contrasting results.

The two-dimensional PAGE method is a reliable procedure for analysis of a complex mixture of proteins. CNS specific proteins and also abnormal CSF-Ig components have been found to

be present (Siden 1980, Wikkelsö et al 1981) and absent in various neurological diseases, including dementia (Harrington et al 1984). No enrichment of unique proteins in either plasma or CSF could be identified in the demented patients (II) as visualized by two-dimensional PAGE. This does not exclude the possible occurrence of CNS specific proteins in plasma, as the concentrations of plasma proteins greatly exceed the levels of proteins in the CSF, thereby making the detection of brain specific proteins difficult.

CLINICAL FINDINGS

In clinical use the DSM-III criteria were found to be insufficient for the classification of progressive dementia disorders (III). The clinical characteristics of AD/SDAT and MID were heterogenous (III). Only a subgroup of the investigated patients fulfilled the DSM-III criteria for definite diagnosis (III,VI). The diagnosis of dementia is based on clinical signs caused by rather extensive damages to the CNS, making it difficult to distinguish between different patterns or pathograms in behavioral alterations. The increased frequency of cardio-/cerebrovascular diseases with age, leading to ischaemic and/or haemorrhagic damage of the brain parenchyma, also makes it difficult to observe the specific "symptoms" caused by the alteration in function of a specific neuronal "group".
There is clearly a need for more precise clinical criteria for AD/SDAT, MID and AD-MID patients.

IMMUNOCYTOCHEMISTRY

Blood-brain barrier function in MID and AD-MID

The current histopathological criteria for vascular dementia are the destruction of 100 ml or more of brain tissue (Tomlinson 1980), tissue destruction localized in the thalamus

and periventricular space of white matter (de Reuck et al 1982), or bilateral destruction of the hippocampus (Corsellis 1976).
Using immunocytochemical methodology, lesions were visualized in vascular dementia which have not been shown previously by conventional histopathological procedures (IV). These lesions consist of deposits of serum proteins in sharply demarcated areas in the grey matter, always with a capillary in the center. The presence of fibrinogen besides other serum proteins such as Alb, PA, and IgG in these lesions indicate severe changes in the permeability of the capillary walls in the area of the cerebral cortex investigated. Blood cells were not observed in the neurophil, and no thrombo-embolies were observed in any of the cases, indicating that these lesions do not represent haemorrhagic and/or ischaemic infarcts. That these lesions have been mostly found in association with cortical capillaries but not with the vessels of the white matter may point out a defect in the cortical capillary system and thus be pathogenetically different from macro/microscopic infarcts.

The perivascular serum protein deposits observed (IV) may explain, 1) the higher transudation of serum proteins through the B-CSF-B observed in MID patients (II), 2) the "consumption" of IgG by CNS (II) and the decrease of regional blood flow shown in MID patients (Hachinski et al 1975, Lavy et al 1978).
Although the concentration of C3 in normal serum is known to be about 17-fold higher than C1q (Brown et al 1984), the size of the perivascular deposits stained with antisera to human C1q was greater than that labelled with antisera to C3c (IV). These findings may indicate the presence of antigen-antibody complexes in the perivascular serum protein deposits since C1 is known to bind to antigen-antibody complexes through the subunit C1q. It is tempting to suggest that perivascular deposits might be due to the binding of antibody to brain tissue. This may be considered as a support to the hypothesis that the elevation in plasma-IgG in MID (II) is caused by auto-antibody production, with possible reactivity

towards brain tissue. However, the presence of C1q in the perivascular deposits might be due to factors other than antibody-antigen complexes since a variety of other substances, such as, the lipid A component of lipopolysaccarides (Cooper et al 1976), RNA viruses (Cooper and Morrison 1978) and CRP complexes (Kaplan and Volanakis 1974, Siegel et al 1974), also activate complement by binding C1q in the absence of antibody-antigen complexes. On the other hand, it is possible that the presence of C1q in the lesions might be due to catabolic differences between the serum proteins. The specific changes in the capillary function of the grey matter in the vascular dementia might be an etiopathologic factor involved in the disease.

Blood-brain barrier function in AD/SDAT

In both non-demented and demented patients transudation of serum proteins through the vessel walls was visualized as occurence of serum proteins in the surroundings of the vessels in the white matter. There were no obvious differences in this diffusion, visualized with different antisera to serum proteins, between the demented and the non-demented, aged individuals. These findings indicate that the BBB function was compromised equally in AD/SDAT and in non-demented, aged individuals (V), contrary to another study where alteration in BBB function in AD/SDAT was observed (Wisniewski and Kozlowski 1982). In both demented and normal aged individuals a broad spectrum of variation of the intensity of the protein staining surrounding the vessels was observed without any correlation to age, cause of death or time between death and autopsy. These differences may be due to the fact that BBB function is labile, influenced by drugs (Preskorn et al 1981), hypertension (Houthoff et al 1981), epileptic seizures (Aarli 1983) and, possibly, by adrenergic activity (Mann et al 1982).

Antigenicity of plaque and tangles

The immunostaining of plaques with antisera to IgG and to the complement components C1q and C3c and with no reaction with antisera to other serum proteins studied suggest the presence of antigen-antibody complexes in plaques (V). This conclusion is supported by previous studies, which showed immunoglobulins (Katenkamp et al 1970, Powers et al 1981, Ishii and Haga 1975,1976) and complement factors in plaques in AD/SDAT (Powers et al 1981, Eikelenboom and Stam 1982). The occurrence of Ig and complement in the plaques (V) and the localization of plaques in close relationship to vessel walls (Miyakawa et al 1979,1982) suggest the possible implication of serum factors in development and/or maturation of plaques. This hypothesis partly supports the conclusion of "consumption" of Ig by the CNS (I). Non-detectable concentrations of brain reactive antibodies in the CSF (I) and the lack of intrathecal production of antibodies in the CSF of AD/SDAT patients (I) support the contention that the serum proteins might be the source of IgG in the plaques. Thus, it seems possible that, the etiology and maturation of plaques in normal aged and demented subjects may be a consequence of an altered B-CSF-B (I) / BBB (I,IV,V) function and serum factors. The etiology and maturation of tangles may not, on the other hand, be directly dependent on these factors, because they were never labelled with any of the antisera used in our study, although this finding is contrary to the report of Shirahama et al (1981), who found that tangles were labelled with antisera to prealbumin.

HISTOPATHOLOGY

AD/SDAT and MID together constitute about 90% of all dementia cases. As yet, the selection criteria for the diagnoses of these diseases are not completely established. Non-demented aged cases can be separated from the demented patients not only clinically but also histopathologically. Although even by both clinical and histopathological work up a number of cases are difficult to diagnose. Because of the difficulty to diag-

nose such borderline cases the terms "possible" and "probable" for diagnoses were introduced. To avoid all doubt in the histopathological differentiation of the demented patients, operational histopathological criteria for AD/SDAT, MID and AD--MID were postulated and used. The use of the histopathological dementia score, i.e. the sum of the degenerative changes seen in the frontal cortex and the hippocampus, simplified the assessment of the histopathological diagnoses. In 53% of the clinically classified AD/SDAT cases and in 33% of the clinically classified MID cases, the clinical diagnosis according to the DSM-III classification corresponded to the histopathological diagnosis according to our postulated criteria. The low clinical to histopathological correlation in AD/SDAT and especially in MID demonstrates that the diagnosis of these two major progressive dementias is not made with certainty with current clinical criteria.

The demented classes, namely AD/SDAT and MID (including only patients with definite diagnosis i.e. SIMCA classes), were shown to be homogenous when tested by multivariate data analysis. These classes were separable by means of the PLS method, and the pattern of class formation was observed also in the PC plot in which the information from the clinical diagnoses was not used.

Using the multivariate data analysis 35% of the demented patients were found to be members of the AD/SDAT (SIMCA) class, 20% were found to belong to the MID (SIMCA) class and 32% cases formed a separate class of AD-MID dementia. The clinical diagnosis did not provide us with any "clues" for the classification of AD-MID cases, as half of the cases were classified as AD/SDAT cases and the other half as MID cases. The AD-MID cases were clearly separable in the PLS plot strongly indicating that AD-MID cases represent a separate and independent dementia class. One patient showed high probability of being a member of the class of non-demented patients, suggesting that this patient might represent a borderline dementia case. Six cases did not fit into any of the formed SIMCA classes and were considered as "outliers". The information available was not satisfactory for the classification of these outlier" cases.

Histopathology in aged non-demented individuals

The prevalence of tangles and plaques (the histopathological dementia score greater than zero) in the class of non-demented patients was as high as 89%. Other investigators have reported similar values 79% (Tomlinson 1980), 83% (Matsuyama and Nakumara 1978), 84-97% (Blessed et al 1968), although lower values have been reported by Ulrich (1982) 25% and Dayan (1970) 21.4%. The different prevalences of the degenerative changes seen in different studies are probably due to the different age ranges of the patients and different areas of the brain studied. In the present study in non-demented patients, the number of plaques in both the frontal cortex and the hippocampus and, consequently, the numerical value of the histopathological dementia score, increased with age (VI).

Tangles were not observed in the frontal cortex and the rate number of tangles in the hippocampus was 0-1 and exceeded only in a single 85 years old patient (VI). The number of tangles in the hippocampus increased slightly with age (VI), a result comparable with other reports (Dayan 1970, Ball 1976). It has been reported that the number of tangles increase with the the number of plaques (Tomlinson et al 1968) in non-demented, aged individuals. No such significant relationship could be demonstrated in our study (VI), supporting the possibility of a separate pathology involved in the formation of these two lesions.

Based on the findings above, there seems to be no doubt that degenerative changes and specifically plaques are common in aged non-demented individuals and that the severity of these degenerative changes and consequently the histopathological dementia score increases with advancing age. The histopathological dementia score never exceeded a value of four in non-demented individuals, indicating a "limitation" in the degeneration process related to ageing.

The prevalence of macroscopic infarcts was 16% and of microscopic infarcts in the frontal cortex and hippocampus 74% and 79%, respectively (VI), comparable with the frequency of softenings reported previously (Tomlinson et al 1968). The se-

verity of vascular changes observed in non-demented patients was small. Widespread infarcts in the frontal cortex were seen only in two investigated cases and never in the hippocampus.

Histopathology in AD/SDAT with definite diagnosis

In the AD/SDAT class, the number of plaques, both in the frontal cortex and the hippocampus, correlated negatively with age (VI), in agreement with previous results (Gibson et al 1983) Similar, though weaker correlation, was also observed between age and number of tangles in the frontal cortex and the hippocampus (VI). Consequently, the histopathological dementia score showed negative correlation with age in AD/SDAT patients. The controversy over the plaque/age correlation in non-demented and AD/SDAT patients might be explained by the predominancy of different forms of plaques in "health" and "disease". It has been shown that, in AD/SDAT patients, the majority of plaques are of an amorphous form (neuritic plaques or plaques without amyloid core). In non-demented patients the discrete form (senile plaques or plaque with amyloid core) dominate (Gibson et al 1983). The amorphous plaques were shown to occur in increasing numbers up to 60 years of age and, after that, in decreasing numbers. The discrete plaques on the contrary showed a tendency to slowly increase, starting at about 65 years of age (Gibson et al 1983).

The correlation between age and the number of tangles was low, making it impractical to analyse these correlations. Contrary to the negative correlation age/plaques with the course of the disease, both the number of plaques and tangles increased slightly both in the frontal cortex and the hippocampus. In this study, older patients had a shorter duration of the disease than younger ones. This finding is probably more related to the greater cardio-/cerebrovascular and infectious risk factors, as a cause of death at high age, than indicating a difference in the progress of the dementia.
The degree of microscopic infarcts in the frontal cortex and the hippocampus showed a slight negative correlation with the

age of the patient, contrary to the positive correlation seen in the non-demented, aged individuals, supporting the notion that AD/SDAT patients have a less damaged microvasculature in the CNS compared with the non-demented aged (Fisher 1968).
Findings discussed above would suggest that AD/SDAT is not merely an accelerated type of normal ageing process but a specific reaction to a pathological event which leads to a histopathological picture of degeneration qualitatively if not quantitatively seen in non-demented aged individuals.
The similar variability of plaque and tangle counts do not provide us with more information as to whether the plaque and tangle formations are separate independent "responses" to an unknown mutual/non-mutual mechanism or a combined dependent response to an unknown mutual mechanism.

Histopathology in MID with definite diagnosis

A slight negative correlation between the plaque count and the age and the duration of the disease was found in the MID class. These partly similar (age) and partly controversial (duration of the disease) correlations to those seen in the AD/SDAT cases are probably due to the destruction of the brain tissue by infarcts. These findings neither support nor dispute the notion that MID is in some way related to AD/SDAT.
The age of the patient and the degree of microscopic infarcts in the frontal cortex showed a significant positive correlation, contrary to the correlation seen in the AD/SDAT patients and similar to the correlation seen in the non-demented, aged patients. Widespread infarcts were commonly seen in MID patients, and the hippocampal formation was always involved. The number of patients in the MID class was low (seven cases) and more detailed analysis of the relationships of the investigated variables was therefore not possible.
It is nevertheless seen specifically with the PC plot of the whole material that the pattern of the variability of the parameters studied is in MID cases similar to that seen in the non-demented, aged individuals and that there do not seem to be any major similarities between MID and AD/SDAT cases.

Histopathology in AD-MID

Patients, histopathologically classified as AD-MID cases, were clearly separable from the major dementia forms in the two-dimensional space by multivariate data analysis.
The pathology in these cases was shown not to be merely a linear combination of the pathology in AD/SDAT and MID, pleading for a specific dementia type. Retrospective investigations of the case histories, clinical symptoms and signs are needed, as no unique clinical criteria for AD-MID dementia type is described earlier.

C O N C L U S I O N S

In summary, the publications included in this thesis have studied 1) the occurrence of some "lesions" typical in dementias, 2) the relationship between these lesions and the clinical classification commonly in use and 3) the possible occurrence of some pathological signs typical for dementia in plasma and CSF.

When investigating the plasma and CSF, the patients studied were clinically classified according to the classification which further on was shown to have both low sensitivity and specificity when the clinical diagnosis was verified by histopathological verification, making, of course, the results published, disputable.
The histopathological study revealed the existance of a separate histopathological dementia type, namely, AD-MID.
To avoid similar "unpleasant" surprises in the future the author stresses the importance of using unvarying findings rather than classifications which probably will change with time when different findings are later presented.

The DSM-III criteria are insufficient to be used as a guide for precise clinical classification of dementias. This is probably due to a wide heterogeneity in the disease group commonly referred to as dementia disorders.

Using the traditional histopathological methodology, estimating the severity of plaques, tangles and microscopic infarcts in two cortical regions, histopathological criteria for three dementia types namely AD/SDAT, MID and AD-MID were postulated These histopathological classes were clearly separable by means of the multivariate data analysis. The AD-MID pathology was shown to be not merely a linear combination of that of AD/SDAT and MID but a dementia form of its own.

The BBB or B-CSF-B function was shown to be altered in unimpaired aged, AD/SDAT, MID and AD-MID patients. In cases of MID and AD-MID patients, vessels of both grey and white matter were involved while in AD/SDAT patients only white matter seems to be affected.

No intrathecal synthesis of Ig, oligoclonal Ig or other abnormal proteins could be demonstrated in aged or demented patients. The perivascular serum protein deposits in the grey matter were visualized as densely stained, sharply demarcated areas around the capillaries while in the transudation of the serum proteins trough the BBB, mainly in the white matter, the intensity of the staining gradually decreased away from the involved vessel. The perivascular deposits of IgG, C1q and C3c around vessels may involve complement activation by antigen-antibody binding.

The localization of plaques in the surroundings of the vessels and the visualization of IgG, C1q and C3c as some of the constitutients of plaques, point to the possibility that the etiology and maturation of plaques in the normal aged and the demented may involve 1) compromised BBB function, 2) serum factors and 3) the immunological system, while the tangles may not be directly dependent upon these factors, as they were never labelled in our study with any of the antisera.

ACKNOWLEDGEMENTS

I wish to express my gratidude to following persons:

Professor Bengt Winblad, my friend and my supervisor who introduced me to this research field, for his ability to care and for his everlasting tolerance.

Professores Inge Grundke-Iqbal and Khalid Iqbal my supervisors in N.Y., for their knowledge in their fields that has guided me through several difficulties and for their true support.

Rolf Adolfsson, Gösta Bucht, Håkan Friden, Egil Jellum and Pankaj Mehta, my co-workers/advisors from Umeå, Oslo and N.Y., for teaching me "everything" about their respective field.

Professor Frank Bergman, for his kind and supporting attitude to my work at the Department of Pathology.

Birger Brändström, autopsy technician, for being a good friend and for always being there when needed.

Gertrud Nilsson and Ann Britt Lundström, laboratory technicians, for their excellent assistance.

Bengt Carfors, photographer, for his professional work.

Birgitta Forsgren, Karin Gladh and Patricia Calimano, secretaries, for assistance in typing and for introducing me to the secrets of ABC-800 computer system.

Robert Elston for his excellent linguistic revision of this thesis.

Members of Umeå Dementia Research Group, for their open-minded attitudes in discussions.

The personnel at the Departments of Pathology and Geriatric Medicine for their supporting attitudes.

Marie Bixo, Irina Elovaara, Eva Dehlin, Jan Marcusson and Per Nyberg for being real friends, for their honest critisism and always supporting attitude and for having good time together during all these years.

My family in Helsinki for love, support and understanding.

These investigations were supported by grants from Swedish Medical Research Council (12X-5664), King Gustaf V:s and Queen Victorias Foundations, the Medical Faculty in Umeå, Umeå University, Hansson's, Mångberg's, Osterman's, Pfannenstill's and Thuring's foundations and NIH grants NS 18105 & NS 17487.

REFERENCES

*DSM-III (1980) Organic dementia disorders. In: American Psychiatric Association, Diagnostic and statistical Manual of Mental Disorders. 3rd ed. Washington CD, APA. 101-161.

Aarli J.A. (1983) The immune system and nervous system. J Neurol 229:137-154.

Adolfsson R., Gottfries C.G., Nyström L., Winblad B. (1981) Prevalence of dementia disorders in institutionalized Swedish people - The work load imposed by caring for these patients. Acta Psychiat Scand 63:225-244.

Anderson L., Anderson N.G. (1977) High resolution two-dimensional electrophoresis of human plasma proteins. Proc Nat Acad Sci USA 74:12:5421-5425.

Anderson N.G., Anderson N.L. (1978a) Analytical techniques for cell fractions XXI. Two-dimensional analysis of serum and tissue proteins: Multiple isoelectric focusing. Anal Biochem 85:331-340.

Anderson N.L., Anderson N.G. (1978b) Analytical techniques for cell fractions XXII. Two-dimensional analysis of serum and tissue proteins: Multiple gradient-slab gel electrophoresis. Anal Biochem 85:341-345.

Anderson N.G. Anderson N.L. (1979) Molecular anatomy. Behring Inst Mitt 63:169-210.

Ball M.J. (1976) Neurofibrillary tangles and the pathogenesis of dementia: a quantitative study. Neuropathol App Neurobiol 2:395-410.

Ball M.J. (1977) Neuronal loss, neurofibrillary tangles and granulovacuolar degeneration in the hippocampus within ageing and dementia: a quantitative study. Acta neuropathol (Berl) 37, 111-118.

Beregi E., Lengyel E., Biro S. (1978) Auto-antibodies in aged individuals. Akt Gerontol 8:77-80.

Berry M., Riches A.C. (1974) An immunological approach to degeneration in the central nervous tissue. Brit Med Bull 30:135-140.

Birkett D.B., Raskin A. (1982) Arteriosclerosis, infarcts and dementia. J Am Geriatr Soc 30:4, 261-266.

Blessed B., Tomlinson B.E., Roth M. (1968) The association between quantitative measures of dementia and of senile change in the cerebral grey matter of elderly subjects. Brit J Psychiat 114:797-811.

Blumenthal H.T. (1976) Immunological aspects of the ageing brain. In: Neurobiology of Ageing. Ed. Terry R.D., Gershon S. Raven Press, N.Y., 313-334.

Bock E., Kristensen V., Rafaelsen D.J. (1974) Proteins in serum and cerebrospinal fluid in demented patients. Acta Neurol Scand 50:91-102.

Bodian D.A. (1936) New method for staining nerve fibers and nerve endings in mounted paraffin sections. Anat Rec 65:89-97.

Brown E.J., Joiner K.A., Frank M.M. (1984) Complement In: Fundamental immunology. Ed. Paul W.E., Raven Press, N.Y., 24:645-668.

Brun A., Englund E. (1981) Regional pattern of degeneration in Alzheimer's disease: neuronal loss and histopathological grading. Histopathol 5:549-564.

Brun A. (1983) An overview of light and electron microscopic changes. In: Alzheimer's disease: the standard reference. Ed. Reisberg B., MacMillan Inc, N.Y., 4:37-47.

Buckley III E.E., Dorsey F.C. (1971) Serum immunoglobulin levels throughout the lifespan of healthy man. Ann Int Med 75:673-682.

Buckley III C.E., Buckley E.G., Dorsey F.C. (1974) Longitudinal changes in serum immunoglobulin levels in older humans. Fed Proc 33:9:2036-2039.

Böning J. (1979) Immunologische liquordiagnostik bei cerebral alterprozessen. Nervenartzt 50:237-240.

Cohen D., Matsuyama S.S., Jarvik L.F. (1976) Short communication. Immunoglobulin levels and intellectual functioning in the aged. Exp Aging Res 2:345-348.

Cohen D. Eisdorfer C. (1980a) Serum immunoglobulins and cognitive status in the elderly - A population study. Brit J Psychat 136:33-39.

Cohen D., Eisdorfer C. (1980b) Brief communication: Antinuclear antibodies in the cognitively impaired elderly. J Nerv Ment Dis 168:3:179-180.

Cooper N.R., Jensen F.C., Welsh R.M. Jr., Oldstone M.B.A. (1976) Lysis of RNA tumor viruses by human serum: direct antibody-independent triggering of the classical complement pathway. J Exp Med 144:970-982.

Cooper N.R., Morrison D.C. (1978) Bindning and activation of the first component of human complement by the lipid A region of lipopolysaccharides. J Immunol 120:1862-1868.

Cornwell III G.G., Westermark P. (1980) Senile amyloidosis: a protein manifestation of the ageing process. J Clin Pathol 33:1146-1152.

Corsellis J.A.N. (1976) Ageing and dementia. In: Greenfield's neuropathology. Eds. Blackwood W., Corsellis J.A.N. Edward Arnold Publ., London, 798.

Crapper D.R., De Boni U. (1978) Brain ageing and Alzheimer's disease. Can Psychiatr Assoc J 23:229-233.

Dayan A.D. (1970) Quantitative histological studies on the aged human brains. I. Senile plaques and neurofibrillary tangles in "normal" patients. Acta Neuropathol (Berl) 16:85-94.

Eeg-Olofsson O., Link H., Wigertz A (1981) Concentrations of CSF proteins as a measure of blood-brain barrier function and synthesis of IgG within the CNS in normal subjects from the age of 6 months to 30 years. Acta Paediatr Scand 70:167-170.

Eikelenboom P. Stam F.C. (1982) Immunoglobulins and complement factors in plaques. Acta Neuropathol 57:239-242.

Eisdorfer C., Cohen D. (1980) Serum immunoglobulins and cognitive status in the elderly. 2. An immunological behavioral relationship. Brit J Psychiat 136:40-45.

Elovaara I., Ice'n A., Palo J., Erkinjuntti T. (1985a) CSF in Alzheimer's disease: studies on blood brain barrier function and intrathecal protein synthesis. J Neurol Sci (in press).

Elovaara I. Palo J., Erkinjuntti T., Sulkava R. (1985b) Blood brain barrier, CSF protein levels and oligoclonal IgG in Alzheimer's disease and multi-infarct dementia. (Subm.)

Fisher C.M. (1968) Dementia and cerebral vascular disease. In: Cerebral vascular disease. Eds Toole J.E., Sickert R.G., Vishnant V.P., 6th Printington Conference, N.Y., 232-236.

Fudenberg H.H., Whitten H.D., Arnaud B., Khansari N. (1984) Hypothesis: Is Alzheimer's disease an immunological disorder, observations and speculations. Clin Immunol Immunopathol 32:127-131.

Ganrot K., Laurell C.B. (1974) Measurement of IgG and albumin content of CSF and its interpretation. Clin Chem 20:571-573.

Gibson P.H. (1983) Form and distribution of senile plaques seen in silver impregnated sections in the brains of intellectually normal elderly people and people with Alzheimer--type dementia. Neuropathol App Neurobiol 9:379-389.

Glenner G.G., (1981) Current knowledge of amyloid deposits as applied to senile plaques and congophilic angiopathy. In. Alzheimer's disease: Senile dementia and related disorders (Ageing vol7) Ed.s Katzman R., Terry R.D., Bick K.L. Raven press, N.Y. 493-502.

Good R.A., Yunis E. (1974) Association of autoimmunity, immunodeficiency and ageing in man, rabbit and mice. Fed Proc 33:9:2040-2050.

Greenberg L.J., Yunis E.J. (1978) Genetic control of autoimmune disease and immune responsibility and the relationship to ageing. In: Birth defects: Original article series 14:1:249-260.

Grundke-Iqbal I. Iqbal K., Wisniewski H.M. (1982) Immunochemical studies of neurofibrillary changes. Exp Brain Res suppl 5:20-25.

Grundke Iqbal I., Iqbal K., Tung Y.C., Wiesniewski H.M. (1984) Alzheimer paired helical filaments: immunochemical identification of polypeptides. Acta Neuropathol (Berl) 62:259-267,

Hachinski V.C., Lassen N.H., Marshall J. (1974) Multi-infarct dementia, a cause of mental deterioration in the elderly. Lancet 2:207-209.

Hachinski V.C., Iliff L.D., Zilhka E., DuBoulay G.H., McAllister V.L., Marshall J., Russell R.W., Symon L. (1975) Cerebral blood flow in dementia. Arch Neurol 32:632-637.

Hachinski V.C. (1979) Relevance of cerebrovascular changes to mental function. Mech Ageing Dev 9:173-183.

Harrington M.G., Merril C.R., Goldman D., Xu X., McFarlin D.E. (1984) Two dimensional electrophoresis of cerebrospinal fluid proteins in multiple sclerosis and various neurological diseases. Electrohphoresis 5:236-245.

Houthoff H.J., Go K.G. Molenaar I. (1981) The permiability of the blood-brain barrier in acute hypertension, comparison of an endogenous and exogenous protein tracer. Acta Neuropathol (Berl) suppl 7:13-16.

Iivanainen M. (1981) The significance of abnormal immun responce in patients with multiple sclerosis. J Neuroimmunol. 1:141-172.

Ishii T., Haga S. (1975) Immunofixation of components of immunoglobulins in senile plaques by means of fluorescent antibody technique. Acta Neuropathol (Berl) 32:157-162.

Ishii T., Haga S. (1976) Immuno-electron microscopic localization of immunoglobulins in amyloid fibrils of senile plaques. Acta Neuropathol (Berl) 36:243-249.

Iqbal K., Grundke-Iqbal I., Wisniewski H.M. Terry R.D. (1978) Chemical relationship of the paired helical filaments of Alzheimer dementia to neurofilaments and neurotubules. Brain Research 142:321-332.

Jankovic' B.D., Jaculic' S., Horvat J. (1977) Cerebral atrophy: an immunological disorder. Lancet 219-220.

Jellum E , Thorsrud A.K. (1982) Clinical application of two-dimensional electrophoresis. Clin Chem 28:4:876-883.

Jonker C., Eikelenboom P. Tavenier P. (1982) Immunological indices in the cerebrospinal fluid of patients with presenile dementia of Alzheimer type. Brit J Psychiat 140:44-49.

Kalter S., Kelly S. (1975) Alzheimer's disease evaluation of immunological indices. N.Y. State J Med 75:8:1222-1225.

Kaplan M.H., Volanakis J.E. (1974) Interaction of C-reactive protein complex with the complement system. J Immunol 112:2135-2147.

Katenkamp D., Stiller D., Thoss K. (1970) Untersuchungen zum immunohistochemischen Verhalten der senile Plaques des menschlichen Gehirns. Virchows Arch Abt A (Path Anat) 351:333-339.

Kay M.M.B. (1979) An overview of immune ageing. Mech Ageing Dev 9:39-59.

Kobatake K., Shinohara Y. Yoshimura S. (1980) Immunoglobulins in cerebrospinal fluid. J Neurol Sci 47:273-283.

Laurell C.B. (1972) Electroimmunoassay. Scand J Clin Lab Invest 29:21-37.

Lavy S., Melamed E., Bentin S., Cooper G., Rinot Y. (1978) Bihemispheric decreases of regional cerebral blood flow in dementia: Correlation with age-matched normal controls. Ann Neurol 4:445-450.

Leonardi A, Gandolfo C., Caponetto C., Arata L. Vecchia L. (1985) The integrity of the blood brain barrier in Alzheimer type and multi-infarct dementia. Evaluated by the study of albumin and IgG in serum and cerebrospinal fluid. J Neurol Sci 67:253-261.

Link H., Tibbling G. (1977) Principles of albumin and IgG analysis in neurological disorders. 2. Relations of the concentrations of the proteins in serum and cerebrospinal fluid. Scand J Clin Lab Invest 37:391-396.

Makinodan T. (1979) Control of immunologic abnormalities associated with ageing. Mech Ageing Dev 9:7-17.

Mann D.M.A., Yates P.O., Hawkes J. (1982) Plaques and tangles and transmitter deficiencies in dementia. J Neurol Neurochir Psychiat 45:563-564.

Mandybur T.I. (1975) The incidence of cerebral amyloid angiopathy in Alzheimer's disease. Neurology 25:120-126.

Matsuyama A., Nakumara S. (1978) Senile changes in the brain in the Japanese. In: Alzheimer's disease. Ed. Katzman R., Terry R.D., Bick K.L. Raven Press, N.Y. 287-297.

Mayer P.P., Chugtai M.A., Cape R.T.D. (1976) An immunological approach to dementia in the elderly. Age Ageing 5:164-179.

McKhann., Drachman D., Folstein R., Katzman R., Price D., Stadham E.M. (1984) Clinical diagnosis of Alzheimer's disease: Report of the NINCDS-AFRDA work group under the auspices of department of health and human service task force of Alzheimers' disease. Neurology 34:939-944.

Mehta O.D., Mehta S.R., Patrick B.A. (1982a) Identification of light chain type bands in CSF and serum oligoclonal IgG from patients with multiple sclerosis. J Neuroimmunol 2:119-129

Mehta P.D. Patrick R.A., Thorman H., Wisniewski H.M. (1984b) Oligoclonal IgG bands with and without measles antibody activity in sera of patients with subacute sclerosing panencephalitis (SSPE) J Immunol 129:5:1983-1985.

Mehta P.D., Miller J.A., Tourtelotte W.W. (1982c) Oligoclonal IgG bands in plaques from multiple sclerosis brain. Neurology 32:4:372-376.

Miyakawa T., Shikai I., Shimoji A., Kuramoto R., Nagatoshi K. (1979) Electron microscopical study on senile plaques in Alzheimer's disease. Folia Psychiat Neurol Jap 2:198-203.

Miyakawa T., Shimoji A., Kuramoto R. (1982) The relationship between senile plaques and cerebral blood vessels in Alzheimer's disease and senile dementia. Virchows Arch B (Cell Pathol) 40:121-129.

Mountjoy C.Q., Tomlinson B.E., Gibson P.H. (1982) Amyloid and senile plaque and cerebral blood vessels. J Neurol 57:89-103.

Nandy K. (1972) Brain-reactive antibodies in mouse serum as a function of age. J Gerontol 27:2:173-177.

Nandy K. (1978) Brain-reactive antibodies in ageing and senile dementia: In: Alzheimer's disease, senile dementia and related disorders (Ageing vol 7) Eds. Katzman R., Terry R.D., Bick K.L. Raven Press, N.Y. 503-514.

Nordin A.A., Makinodan T. (1974) Humoral immunity in ageing. Fed Proc 33:9:2033-2035.

Oldstone M.B.A., Dickson F.J. (1974) Ageing and chronic virus infection: Is there a relationship in immunopathology of ageing. Fed Proc 33:2057-2059.

Pentland E., Christie J.E., Watson K.C., Yap P. (1982) Immunological parameters in Alzheimer's presenile dementia. Acta Psychiat Scand 65:325-379.

Perry E.K., Tomlinson B.E., Blessed G., Bergman K., Gibson P.H., Perry K.H. (1978) Correlation of cholinergic abnormalities with senile plaques and mental test scores in senile dementia. Brit Med J 2:1457-1459.

Powers J.M., Schlaepfer W.W., Willingham M.C., Hall B.J., (1981) An immunoperoxidase study of senile cerebral amyloidosis with pathogenetic considerations. J Neuropathol Exp Neurol 40:6:592-612.

Preskorn S.H., Irwin G.H., Simpson S., Friesen D., Rinne J., Jerkovich G. (1981) Medical therapies for mood disorders alter the blood-brain-barrier. Science 213:469-471.

Probst A., Basler W., Bron B. Ulrich I. (1983) Neuritic plaques in senile dementia of Alzheimer type: Golgi analysis in the hippocampal region. Brain Res 268:249-254.

Reiber H. (1980) The discrimination between different blood-CSF barrier dysfunctions and inflammatory reactions of the CNS by a recent evaluation graph for the protein profile of CSF. J Neurol 224:89-99.

de Reuck J., Sieben G., de Coster W, vander Eecken H. (1982) Dementia and confusional state in patients with cerebral infarcts. A clinicopathological study. Eur Neurol 21:94-97.

Romeis B. (1968) Mikroskopische Technik. R. Oldenbourg verlag, München Wien, 65.

Rosen W.C., Terry R.D., Fuld P-A., Katzman R., Peck A. (1980) Pathological verification of ischaemic score in differentiation of dementias. Ann Neurol 7:486-488.

Roth M. (1955) The natural history of mental disorder in old age. J Ment Sci 101:281-301.

Schuller E., Sagar H.J. (1981) Local synthesis of CSF immunoglobulins - A neuroimmunological classification. J Neurol Sci 51:361-370.

Schuller E., Escourelle R., Gray F., Sagar H.J., Hauw J.J. (1982) CSF oligoclonal pattern in two cases of cellular amyloid angiopathy. In: Immunology and ageing. Ed Fabris N., Martinus Nijhoff Publ, 204-210.

Shirahama T., Skinner M., Westermark P., Rubinow A., Cohen A.S., Brun A., Kemper T.L. (1982) Senile cerebral amyloid. Prealbumin as a common constituent in the neuretic plaque in the neurofibrillary tangle and in the microangiopathic lesion. Am J Pathol 107:41-50.

Siden Å. (1980) Abnormal CSF immunoglobulin components detected by isoelectric focusing J Neurol 224:133-144.

Siegel J., Rent R., Gewurz H.J. (1974) Interaction of Creactive protein with the complement system. J Exp Med 140:631-644.

Slater E., Roth M. (1970) Ageing and the mental diseases of the aged. In: Clinical Psychiatry. Eds. Mayer-Gross W., Slater E., Roth M. Bailliere, Tindall and Cassell Inc., London 3:8 533-630.

Sternberger L.A. (1979) The unlabelled antibody peroxidase-antiperoxidase method. In: Immunocytochemistry. Ed. Sternberger L.A. John Wiley & sons Inc., N.Y. 2:5:104-168.

Stuttman Å. (1974) Cell mediated immunity and ageing. Fed proc 33:2028-2032.

Tavolato B., Argentiero V. (1980) Immunological indices in presenile Alzheimer's disease. J Neurol Sci 46:325.331.

Terry R.D., Davies P. (1980) Dementia of the Alzheimer type. Ann Rev Neurosci 3:77-95.

Tibbling G., Link H., Öhman S. (1977) Principles of albumin and IgG analyses in neurological disorders. Part 1, Scand J Clin Lab Invest 37:385-390.

Tkach J.R., Hokama, Y. (1978) Autoimmunity in chronic brain syndrome. Arch Gen Psychiat 2361-64.

Todorov A.B., Go R.C.P., Constantinidis J., Elston R.C. (1975) Specificity of the clinical diagnosis of dementia. J Neurol Sci 26:81-98.

Tomlinson B.E., Blessed G., Roth M. (1968) Observation on the brains of non-demented old people. J Neurol Sci 7:331-356.

Tomlinson B.E., Blessed G., Roth M. (1970) Observation on the brains of demented old people. J Neurol Sci 11:205-242.

Tomlinson B.E. (1980) The structural and quantitative aspects of the dementias In: Biochemistry of dementia. Ed. Roberts P.J. John Wiley 6 sons Inc., N.Y. 15-51.

Torack R.M., Lynch R.G. (1981) Cytochemistry of brain amyloid in adult dementia. Acta Neuropathol (Berl) 53:189-199.
Ulrich J. (1982) Senile plaques and neurofibirllary tangles of the Alzheimer type in non-demented individuals at presenile age. Gerontol 28:86-90.
Walford R.L. (1974) Immunologic theory of ageing - Current status. Fed Proc 33:2020-2027.
Watts H., Kennedy P.G.E., Myfanwy T. (1981) The significance of anti-neuronal antibodies in Alzheimer's disease. J Neuroimmunol 1:107-116.
Wikkelsö C., Blomstrand C., Nordqvist P. (1981) Cerebrospinal fluid investigations in multi-infarct dementia and senile dementia. Acta Neurol Scand 64:1-11.
Wikkelsö C., Blomstrand C. (1982) Cerebrospinal fluid specific poteins in various degenerative neurological diseases. Acta Neurol Scand 66:199-208.
Wilcock G.K., Esiri M.M (1982) Plaques, tangles and dementia. J Neurol Sci 56:343-356.
Williams A. Papadopoulos N., Chase T.N. (1980) Demonstration of CSF-gamma globulin banding in presenile dementia. Neurology 30:882-884.

Wisniewski H.M., Soifer D. (1979) Neurofibrillary pathology: Current status and research perspectives. Mech Ageing Dev 9:119-142.
Wisniewski H.M., Kozlowski O.B. (1982) Evidence for blood-brain barrier changes in senile dementia of Alzheimer's type. In: Alzheimer's disease, Down's syndrome and Ageing. Eds Sinex M.F., Merill F.M., N.Y. Acad Sci Publ 386:119-129.
Wold S., Albana C., Dunn III W.J., Esbensen K., Hellberg S., Johansson E., Sjöström M. (1983) Pattern Recognition: finding and using regularities in multivariate data. In: Food research and data analysis. Eds. Martens H., Russwurm Jr. H. App Sci Publ, London N.Y. 1-36.
Wold S., Albano C., Dunhill W.J., Edlund U., Esbensen K., Geladi P., Hellberg S., Johansson E., Lindberg W., Sjöström M. (1984a) Multivariate data analysis in chemistry. In: Proceedings NATO Adv Study Inst on Chemometrics, Cosenza, Italy. Ed. Kowalski B.R., Reidel Publ., Holland 1-79.
Wold S., Dunn III W.J., Hellberg S. (1984b) Pattern recognition as a tool for drug design. In: Drug Design: fact or fantasy. Academic Press. London. 6:93-117.
Yunis E.J., Greenberg L.J. (1968) Immunopathology of ageing. Fed Proc 33:2017-2019.
Yunis E.J. Lane M.A. (1979) Cellular immunity in ageing. J Invest Dermatol 73:24-28.