From
Institutions of KARO and NEUROTEC
Karolinska Institutet,
Department of Diagnostic Radiology,
Huddinge University Hospital, Stockholm, Sweden

MAGNETIC RESONANCE IMAGING IN DEMENTIA;

A STUDY OF BRAIN WHITE MATTER CHANGES

Lena Bronge

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ABSTRACT

Non-specific white matter changes (WMC) in the brain are common findings in the elderly population. Although they are frequently seen in non-demented persons, WMC seem to be more common in demented patients. The significance of these changes, as well as their pathophysiological background is incompletely understood. The aim of this thesis was to study different aspects of WMC using magnetic resonance imaging (MRI) and to investigate the clinical significance of such changes in subjects with mild cognitive impairment or dementia.

In *study I* post-mortem MRI of the brain was compared to corresponding neuropathology slices. White matter changes were quantified and found to be more extensive on neuropathology. The areas that appeared normal on MRI but not on histopathology represented only minor changes with increased distance between the myelinated fibres but with preserved axonal network and glial cell density.

Study II evaluated the blood-brain barrier (BBB) integrity to investigate if an increased permeability could be shown in WMC. A contrast enhanced MRI technique was used to detect small degrees of enhancement. No general increase in BBB could be detected in the WMC areas.

In *study III* the relation between WMC and Apolipoprotein E (APOE) genotype was explored in patients with Alzheimer's disease (AD). Results showed that AD patients who were homozygous for the APOE ε4 allele had more WMC than patients with other genotypes. This was most significant for changes in the deep white matter. Results also indicate that in AD patients carrying the ε4 allele, WMC are not age related phenomena, but might be related to the aetiology of the disease.

Study IV aimed to investigate if WMC in a specific brain region affect cognitive functions related to that area. Periventricular WMC in the left frontal lobe predicted a decrease in initial word fluency, a test thought to reflect left frontal lobe functioning. This indicates that WMC might have specific effects in different brain regions.

In *study V* we evaluated the prognostic significance of WMC in patients with memory impairment, regarding the rate of further global cognitive decline. There was no difference in outcome between patients having extensive WMC and a matched control group, during 2-4 years of follow up, and assessed by the "Mini Mental State Examination".

In conclusion, this work has shown and characterised pathological changes in the white matter not visible on conventional MRI. We have also shown that there is no major general increase in BBB permeability in areas of WMC. In addition, homozygosity with regard to the APOE ε4 gene allele implies an increased extent of WMC in Alzheimer's disease patients. In AD patients carrying this gene allele WMC are not merely age related phenomena, but might be related to the aetiology of the disease. We also claim that WMC in a specific location might impair cognitive functions that rely on those specific pathways. In contrast, WMC do not seem to have any prognostic value in predicting the rate of global cognitive decline in patients at a memory clinic.

LIST OF PUBLICATIONS

This thesis is based on the following five papers, referred to in the text by their roman numerals.

    I.   **Post mortem MRI and histopathology of white matter changes in Alzheimer brains; a quantitative, comparative study**
Lena Bronge, Nenad Bogdanovic, Lars-Olof Wahlund
Accepted for publication in Dement Geriatr Cogn Disord

    II.   **White matter lesions in dementia; an MRI study on Blood-brain barrier dysfunction**
Lena Bronge, Lars-Olof Wahlund
Dement Geriatr Cogn Disord 2000 Sep-Oct;11(5):263-7

    III.   **White matter lesions in Alzheimer patients are influenced by Apolipoprotein E genotype**
Lena Bronge, Sven-Erik Fernaeus, Mari Blomberg, Martin Ingelson, Lars Lannfelt, Bengt Isberg, Lars-Olof Wahlund
Dement Geriatr Cogn Disord 1999 Mar-Apr;10(2):89-96

    IV.   **White matter lesions impair initiation of FAS flow**
Sven-Erik Fernaeus, Ove Almkvist, Lena Bronge, Per Östberg, Åke Hellström, Bengt Winblad, Lars-Olof Wahlund
Dement Geriatr Cogn Disord 2001 Jan-Feb;12(1):52-6.

    V.   **The prognostic significance of age related white matter changes, in a memory clinic population**
Lena Bronge, Lars-Olof Wahlund
Manuscript submitted (2001)

CONTENTS

LIST OF ABBREVIATIONS

AD	Alzheimer's Disease
APOE	Apolipoprotein E (gene)
ApoE	Apolipoprotein E (protein)
ARWMC	Age Related White Matter Changes
AT1	Area Type 1
AT2	Area Type 2
AT3	Area Type 3
BBB	Blood Brain Barrier
BGH	Basal Ganglia Hyperintensities
CAA	Cerebral Amyloid Angiopathy
CSF	CerebroSpinal Fluid
CT	Computed Tomography
DWMH	Deep White Matter Hyperintensities
EEG	ElectroEncephaloGraphy
FA	Flip Angle
FOV	Field of View
FLAIR	Fluid Attenuated Inversion Recovery
FSE	Fast Spin Echo
GE	Gradient Echo
Gd	Gadolinium
LFB	Luxol Fast Blue
MCI	Mild Cognitive Impairment
MMSE	Mini Mental State Examination
MPRAGE	Magnetisation Prepared Rapid Gradient Echo
MR	Magnetic Resonance
MRI	Magnetic Resonance Imaging
MRS	Magnetic Resonance Spectroscopy
MTI	Magnetisation Transfer Imaging
NAA	N-AcetylAspartate
NPH	Normal Pressure Hydrocephalus
PD	Proton Density
PVH	PeriVentricular Hyperintensities
RF	Radio Frequency
ROI	Region Of Interest
SE	Spin Echo
SMD	Subjective Memory Disorder
SPECT	Single Photon Emission Computed Tomography
T	Tesla
TR	Repetition Time
TE	Echo Time
VaD	Vascular Dementia
WMC	Non-specific White Matter Changes
y	years

INTRODUCTION

BACKGROUND

The introduction of modern neuroimaging techniques in the 1970's and 1980's, such as computed tomography (CT) and magnetic resonance imaging (MRI) made it possible to obtain detailed images of the living human brain. These images revealed unexpected changes in the white matter that were of high frequency in elderly individuals. The changes were different from other well-recognised white matter diseases, with aetiologies such as infection, inflammation, neoplasm or metabolic disorders, and their causes and consequences were unknown. They seemed to be common in demented patients and were initially often considered as being part of a dementing disorder, but it was soon evident that white matter changes were also frequent among non-demented elderly subjects. Many individuals with such "non-specific" or "age-related" white matter changes (WMC or ARWMC) lack obvious neurological or cognitive symptoms and therefore these WMC have often been considered a mere radiological aspect with uncertain clinical significance. In the last decade, however, the number of publications dealing with these non-specific WMC has constantly increased and mounting evidence now indicates that such changes are clinically important, although their significance, pathogenesis, pathological correlates and radiological aspects remain to be fully elucidated.

This thesis is an attempt to further characterise the non-specific WMC and to investigate their significance in patients with memory disturbances, such as mild cognitive impairment or dementia. The basis for all the studies is the use of MRI, and the major approach is from a radiological viewpoint. All patients included were referred from the memory clinic at Huddinge University Hospital.

MAGNETIC RESONANCE IMAGING

History and background

Magnetic resonance (MR) is a phenomenon that was first described in the 1940s, and used for many years after that as an analytical technique. The method uses the magnetic properties of certain atomic nuclei. When placed in a magnetic field these nuclei can absorb energy in the form of radio frequent waves and thereafter emit this energy when returning to their original energy level. A specific frequency of the radio signal, the Larmour frequency, matches each specific magnetic field strength. Two scientists (Bloch and Purcell) were awarded the Nobel Prize for physics in 1952 for this discovery and in 1991 a chemist (Ernst) was also awarded the Nobel Prize for his contributions to the development of this field.

The MR signal carries information about the physical and chemical environment of the nuclei and in the early 1970's methods for making images based on this principle were presented. To be able to calculate images a weak magnetic field, called a gradient, was

added to the strong magnetic field. This created slightly different magnetic field strengths in different parts of the sample and the radio signal emitted thus had variable frequency depending on from where in the sample the signal was emitted.

Basic principles of MRI

In the human body hydrogen makes up about 80% of all atoms. The hydrogen nucleus, *the proton*, has magnetic properties, which can be used in making MR images. Only eight years after the original publication presenting a method to make MR images the first medical MRI equipment was in clinical use. Since the energy used in MRI is very low (compared to X-ray and radioisotopes) no harmful side effects of this method have yet been identified. The magnets used are very large and form a tunnel in which the patient is positioned. The typical field strength used in the high field systems has been 1.0-1.5 tesla, but higher field strengths are now being introduced into clinical practice.

MRI of the human body has superior soft-tissue contrast but is also a very complex and sophisticated method with a lot of new applications continuously being introduced. When put in the strong magnetic field all protons in the body are aligned parallel to the field. Slightly more than 50 % of them are aligned along the magnetic field while the rest have an opposite direction. Because of this small "net magnetisation" it is possible to measure the net magnetic vector. To produce images, different MRI sequences are used which contain a number of successive radio frequency (RF) pulses together with different magnetic gradients.

The excitation is the initial RF pulse in the MRI sequence. When exposed to the RF pulse the net magnetic vector is flipped away from its original direction along the external magnetic field (B_0). The flip angle (FA) (often 90° or 180°) is proportional to the amount of energy applied. After excitation the RF pulse is switched off and the magnetic vector returns to its original direction, thereby emitting energy in the form of a radio signal. This phenomenon is called relaxation and the time required differs according to the chemical and physical surrounding of each nucleus and is thus different in different tissues in the body. There are two major types of relaxation, T1 and T2, and images can be either T1, T2 or proton density (PD) weighted. The MRI sequence determines the repetition time (TR), i.e. the time between each excitation, and the echo time (TE), i.e. the time after which the emitted radio signal, the echo, is collected. These parameters determine the image weighting (T1, T2 or PD) which greatly influences the contrast in the final image. The magnetic field gradients are used to define the spatial encoding, slice thickness and orientation of the images, and the final image is calculated by a Fourier transformation.

The original basic imaging sequence was the spin echo (SE), formed by a 90º RF pulse followed by a 180º pulse, but there has been a rapid development of new techniques mainly aimed at more rapid imaging. Also the SE sequences, still widely used in clinical routine, are now faster due to the use of "turbo" or fast SE techniques. In the SE situation

the contrast between different tissues, in the final image, is determined by the TR and TE. The TR primarily determines the influence of the T1 relaxation in the image, and the TE determines the influence of T2 relaxation. To get a T1 weighted image the TR is chosen so as to achieve a significant difference in signal between different types of tissue. If the TR is too long all tissues will have a high grade of T1 relaxation and the signal differences between tissues, depending on T1, will be minimal and the contrast in the image will be more related to the proton density than to T1 relaxation. To achieve a T2 weighted image the TE has to be chosen so as to achieve a large signal difference depending on T2, between different tissues. With short TE the differences are minimal while with longer TE they grow. At the same time the TR has to be long enough to minimise the influence of the T1 relaxation in the image. Similarly, the TE in a T1 weighted image has to be kept short in order to reduce the influence of T2 weighting. The typical T1 weighted spin echo sequence has a short TR and a short TE, and the typical T2 weighted sequence has a long TR and a long TE.

THE BRAIN WHITE MATTER

General features

The normal white matter of the brain contains nerve fibres (axons), neuroglial cells (astrocytes and oligodendrocytes), vascular structures and interstitial space. The nerve structures in the white matter are mainly axons, surrounded by a myelin sheath, while the nerve cell bodies are located in the cerebral cortex. The myelinated axons of the white matter make up tracts leading to and from the brain as well as connections between different areas in the brain, both between and within the cerebral hemispheres. The frontal lobes for example, have extensive subcortical connections to cortical areas in more posterior parts of the same hemisphere, as well as to the subcortical nuclei, building up a complex association network.

The myelin that surrounds the axons is produced and maintained by the oligodendrocytes, and is metabolised and replenished rather slowly. As long as the oligodendrocytes are preserved, regions of destroyed myelin can be remyelinated, although often incompletely. Myelin, a lipid-rich tissue containing large macromolecules that possess high electrical resistance and low capacitance, acts as an insulator around the axons. The myelin is arranged in segments separated by the nodes of Ranvier, where sodium channels are clustered in high density in the axonal membrane so that they can produce action potentials. Myelin covers and masks the internodal parts of the axon, which contain fewer sodium channels, and a higher density of potassium channels, which tend to oppose the generation of action potentials. Myelination increases the speed of conduction and improves its efficiency. Damage to the myelin is accompanied by a decrease in conduction velocity and, when severe, by conduction block. A loss of myelin is also associated with destabilising changes in the molecular structure of the axonal cytoskeleton [1].

Damage to the white matter can be seen as a range of changes from mild degradation of myelin, to more pronounced demyelination. There can also be a destruction of axons, sometimes with a concomitant increase in the number of glial cells, so called gliosis. In many cases there is instead reduction of glial cell numbers, and finally cavitation and infarction.

Blood supply

The long medullary branches, issued from the three major cerebral arteries via the pial network, supply the major part of the deep white matter of the cerebral hemispheres. They run from the brain surface to the centre and converge in the direction of the lateral ventricles. The long medullary branches are end arteries with considerable length and do not form a collateral system. These anatomic features are considered to underlie the observed vulnerability to ischemic events. The vascular supply of the long medullary branches is clearly separated from the cortical-subcortical ones that consist of cortical and short medullary branches, ending in the subcortical U-fibres (arcuate fibres). Between these two territories there exists an arterial borderzone that seems to have an increased susceptibility to ischemia [2]. This borderzone is located in the subcortical area, deep to the subcortical U-fibres, mainly at the level of the sulci where bending long medullary branches generally are absent. A second arterial borderzone is thought to be present in the periventricular white matter in the borderzone between the long medullary branches from the cortex and the branches derived from central arteries supplying the white matter closest to the ventricular walls.

Although blood flow is significantly lower in the white matter than in the cerebral cortex the oxygen demands are relatively high and are achieved by an increased oxygen extraction from arterial blood. This also indicates a higher susceptibility to ischemia for these areas than for the better supplied cortex and subcortical fibres. Normally the arteries and arterioles of the brain can adjust their calibre in response to changes in blood pressure to maintain optimal blood perfusion. Impairments in this autoregulation of the brain appear with increasing age particularly in patients with hypertension and diabetes. An impaired autoregulation makes the brain, and thus predominantly the white matter, more susceptible to episodes of hypoxia or hypotension.

The blood brain barrier

The integrity of the Blood-brain barrier (BBB) is of great importance for maintaining an optimal environment in the CNS. The endothelial cells of the brain capillaries are tightly fused together forming "tight junctions". Together with a relative absence of pinocytosis in the brain capillaries these "tight junctions" constitute the BBB, which restricts

movements of ions, water-soluble non-electrolytes and proteins. Passage through the BBB occurs by free diffusion and is only possible for small, highly lipophilic molecules that are not bound to plasma proteins. Other substances including certain sugars and amino acids [3] can pass only by active or facilitated carrier mediated transport systems. Proteins such as albumin cannot normally pass the BBB. The small amount of these proteins found in the normal cerebrospinal fluid (CSF) emanates from the blood as a probable result of passage through the fenestrated capillaries in the choroid plexus where most of the CSF is produced.

An abnormally high level of albumin in the CSF is considered a sign of a general BBB disruption due to defects in the endothelial lining causing an increased permeability. Subtle changes in the BBB might occur due to ageing but these changes seems to be aggravated by vascular conditions such as hypertension and diabetes [4].

MRI features of the white matter

Differences in physical, chemical and biological properties affect the relaxation times and thus the appearance of the actual tissue on MR images. The relaxation times, T1 and T2, are long for water whereas they are short for lipids. Healthy white matter is heavily myelinated with a high content of long-chain fatty acids and 12 % less water than grey matter. However, the relaxation times in white matter are even shorter than would be predicted by the lower water content alone. This is due to the presence of macromolecular lipids such as cholesterol, sphingomyelin and galactocerebroside that increases relaxation rates.

Any process, such as metabolic or ischemic injury, that changes the chemical composition of myelin will make the structure less stable and more susceptible to injury. With degradation of myelin, as seen for example in age related WMC, the tissue will, due to a higher water content as well as to degeneration of macromolecular structures, dramatically change its relaxation rates. This will be seen as a higher MR signal in T2 or PD weighted MR images. The increased water content can be due to either a formation of large, fluid filled, extracellular spaces, vacuoles, but also to an increase in the amount of glial components of the tissue, i.e. gliosis.

Pathology in the white matter is well depicted with conventional PD or T2 weighted SE or fast SE (FSE) sequences but is even more conspicuous on fluid attenuated inversion recovery (FLAIR) images. Fast FLAIR sequences are available today on all modern MRI equipments and have the advantage of making free fluid, such as the CSF, look dark while the white matter pathology still appears bright. This improves the conspicuousness of lesions, especially in areas close to the CSF spaces.

AGE RELATED WHITE MATTER CHANGES

Nomenclature and rating scales

Non-specific changes in the white matter appear in high frequency on CT and MRI in elderly patients presenting with either stroke or cognitive impairment. They are also commonly seen in normal elderly individuals. The prevalence differs considerably among studies and varies from 10% up to 100% [5] in different elderly populations. On CT they appear as ill-defined areas of slightly reduced density and the descriptive term "leukoaraiosis" was first proposed by Hachinski et al [6]. On MRI the changes appear with high signal intensity that were initially often referred to as "UBO's" ("unidentified bright objects"). Over the years the nomenclature but also the system for grading these changes have varied considerably between different studies, causing confusion when comparing the results. The terms WMH (white matter hyperintensities) WMC (white matter changes), WML (white matter lesions), SIWI (selective incomplete white matter infarction), DWML (deep white matter lesions), PVL (periventricular lesions) and SCL (subcortical lesions) have, among many others, often been used with different definitions in different studies. Opinion as to the anatomic equivalents of terms like subcortical, deep, central and periventricular also seems to vary between radiologists and pathologists [7]. Recently the term "age related white matter changes", ARWMC, was proposed by the " European Task Force on Age Related White Matter Changes" as a general term considering that these changes are most clearly related to advancing age [8].

A great number of scales have been constructed for visual rating of the extent and location of WMC, on both CT and MRI [9]. The scales have different properties and may serve various purposes. The agreement between scales is often poor, and when Mäntylä et al [10] compared 13 different scales they concluded that some of the inconsistencies in previous studies were due to differences in the scales. There is thus a need for international harmonisation of WMC rating, to allow comparison of results from different studies.

White matter changes on MRI

Magnetic resonance imaging is highly sensitive to lesions affecting the white matter since pathological processes usually prolong T2 relaxation by increasing the tissue water content and degrading the macromolecular structure of myelin. The prolonged T2 relaxation results in an increased signal on T2 weighted images in this normally dark tissue.

In subjects over 60 years of age it is common to find non-specific hyperintensities in the white matter as seen on MRI images of the brain. The changes are non-specific in the sense that they are not obviously connected to any disease, or cause any apparent symptoms. MRI shows these changes with high sensitivity and delineates them with great

detail, but more pronounced changes are equally well depicted on CT scans [8]. It has been claimed that MRI exaggerates non-pathologic changes [11] and that there is a poor correlation between findings on MRI and histopathology [12]. Others have however reported good correlation [13], but that the lesions seem to be more extensive on histopathologic examination than on MRI [12, 14-16]. WMC with a number of different features can be recognised on the MR images.

Small, sharply demarcated, punctuate foci or more linear structures can be seen in all ages in the white matter. They increase in number and size with advancing age. These fluid filled Virchow-Robin **perivascular spaces** are subarachnoid spaces that follow the pia around the penetrating vessels through the white matter. They are usually considered a normal finding but they might become enlarged as an effect of atrophy.

Well demarcated **caps** (Fig 1; image 1) immediately adjacent to the frontal horns of the lateral ventricles is a common finding which is also considered to be normal when well defined and limited in size. These caps relate to a looser structure of the tissue with less myelin and higher water content.

Periventricular hyperintensities (PVH) are seen as caps around the frontal and/or posterior horns (Fig 1; image 2), and as bands along the bodies of the lateral ventricles (Fig 1; image 3). They can range from small, well demarcated caps and well defined thin bands along the ventricles to extensive periventricular hyperintensities that extend deep into the white matter in which case they might be confluent with changes in these areas (Fig 1; image 4).

Deep white matter hyperintensities (DWMH) often have a **punctuate** (Fig 1; image 5) or **patchy** (Fig 1; images 6 and 7) appearance. The majority of changes are located in the centrum semiovale, often in the subcortical region. They usually spare the subcortical U-fibres, but might appear anywhere in the white matter. With more extensive changes the areas become **confluent** (Fig 1; image 8) and might involve the entire white matter, predominantly in the frontal and parietal lobes. Changes in the temporal and occipital lobes are uncommon on MRI and involvement of the corpus callosum is rarely seen.

Diffuse, irregular hyperintensities in the brainstem, primarily in the pons, can be seen in a number of patients most often coexisting with supratentorial WMC. Changes of this type can occasionally also be seen in the cerebellum.

Figure 1. MRI brain images, *obtained in the axial plane with a "FLAIR" sequence. Images showing different types of white matter lesions: 1) Small "caps" adjacent to the frontal horns, 2) More pronounced "caps" next to the frontal and posterior horns, 3) Periventricular bands, 4) Pronounced periventricular bands extending into the deep white matter, 5) Punctuate WMC in the deep white matter, 6 and 7) Punctuate and patchy WMC in the deep white matter, with sparing of the subcortical U-fibres, 8) Confluent WMC engaging most of the white matter in the frontal and parietal lobes, with sparing of the subcortical U-fibres.*
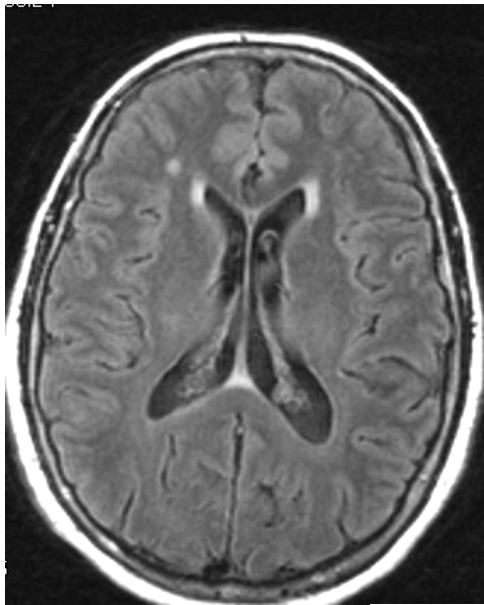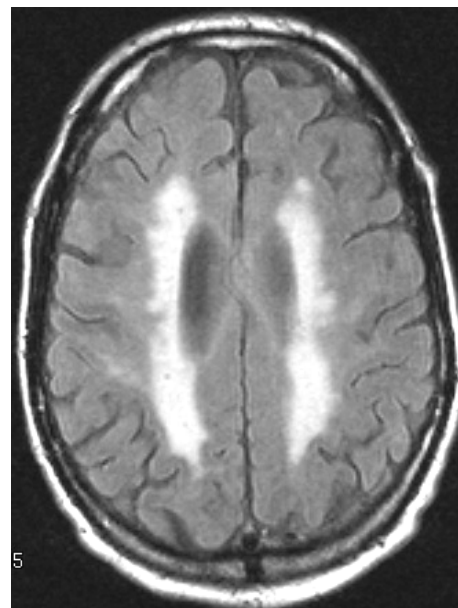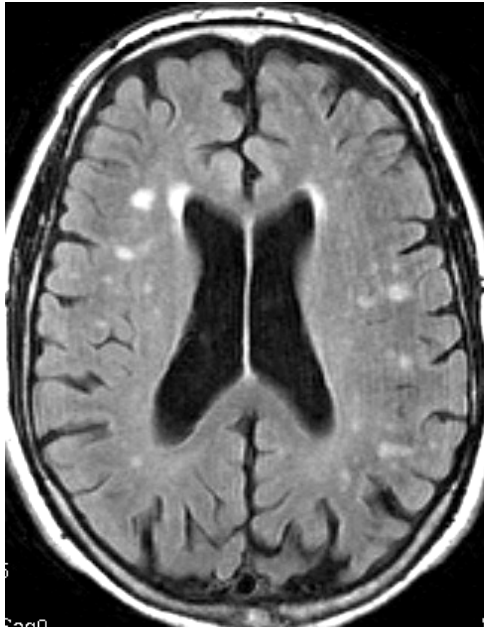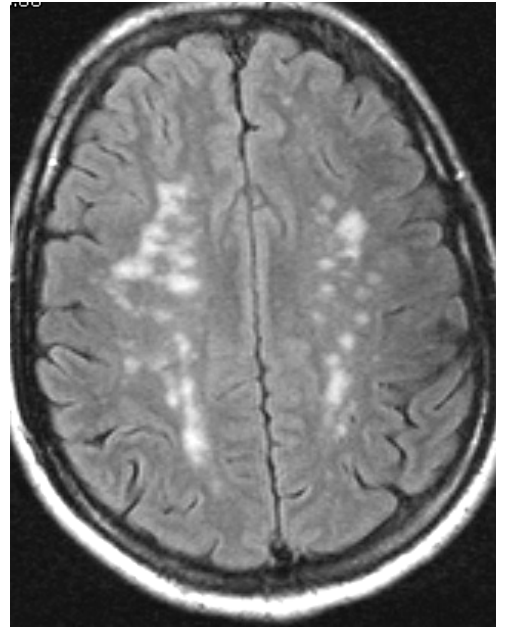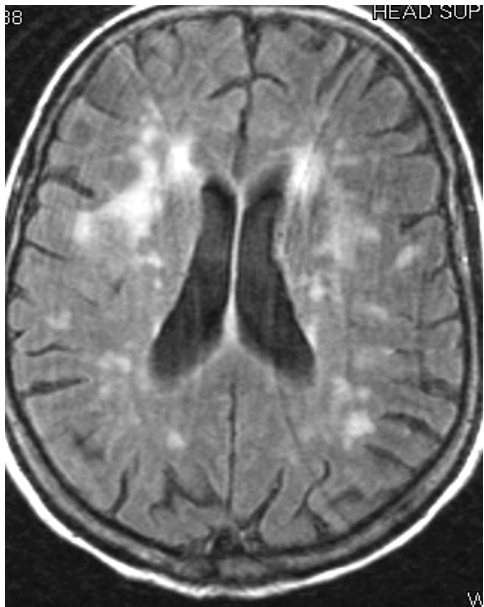

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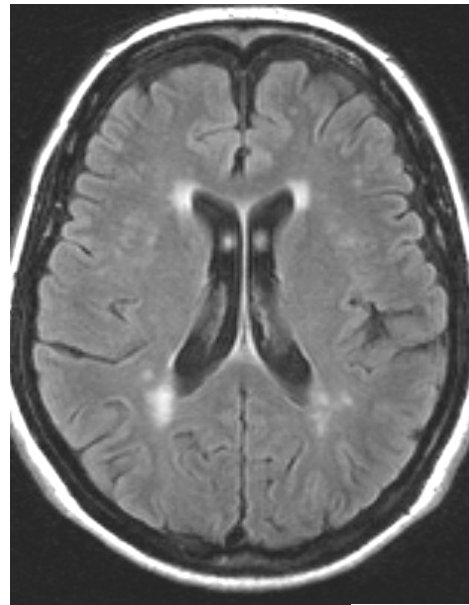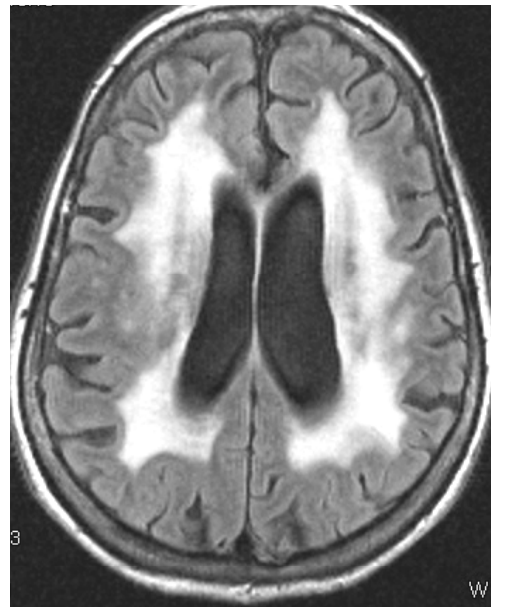

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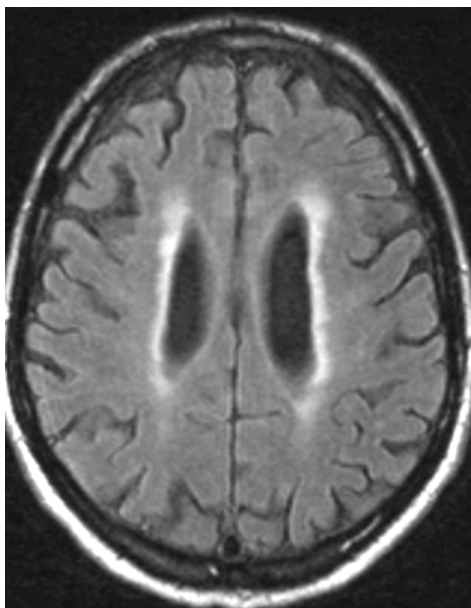
Image 3

Image 4

Image 5

Image 6

Image 7

Image 8

Pathology

MRI has proved to be a very sensitive method to detect all forms of white matter abnormalities but at the same time it is unspecific and unable to distinguish between different types or grades of damage. Most pathological processes in the white matter will appear as hyperintensities in the T2 weighted MR images. Because of this, various neuropathologic findings have been reported in different studies as histopathological correlates to WMC. However, since the white matter is a morphological homogeneous tissue there are a limited number of structural reactions to a wide variety of different pathological conditions. The basic components of white matter pathology are; loss of parenchymal elements (myelin, axons and glial cells), increase of glial cells (gliosis), vascular changes, atrophy, and necrosis with scarring or cavitation [7].

Most areas of **patchy or confluent WMC** in the deep white matter are clearly accompanied by vascular changes in the arterioli, i.e. fibrohyaline arteriolosclerosis, with narrowing of the small vessels [7, 16-18] supporting a presumed ischemic origin. The parenchymal changes in such areas have been described as a form of incomplete infarction since they are histologically similar to changes seen in the periphery of white matter infarcts [19, 20]. There is subtotal tissue destruction with partial loss of myelin, oligodendroglial cells and axons together with a mild reactive astrocytic gliosis [19]. These features are also often referred to as vacuolation, and described as an increase in intra- or extracellular water forming a kind of oedema. This oedema might have different causes, and is not necessarily ischemic in nature [18]. The tissue destruction can be of different grades ranging from pronounced demyelination together with loss of vital tissue components to mild degradation of myelin with slight vacuolation that gradually blends out with normal white matter [18, 19]. Lacunar infarction with cavitation or glial scarring is only found in a minority of cases. Electron microscopy studies have showed that in WMC in humans there are no demyelinated, naked axons but rather a general loss of fibres [18].

Punctuate WMC have, besides those explained only by dilated perivascular spaces, been correlated to minor perivascular damage that is histologically similar to the changes seen in more extensive WMC. Areas of reduced myelination and atrophy around fibrohyalinotic arteries as well as perivenous damage [21] and dilated perivascular spaces [13] are described. The changes range from spongiform transformation, vacuolisation, and scattered foci of demyelination to large perivenous areas with marked degradation of myelinated fibres [21].

The smooth and well defined thin **periventricular caps and bands** have a distinctive different histologic appearance and are characterised by disruption of the ependymal lining of the ventricle, associated with subependymal gliosis and a larger area of reduced myelin content [13, 15]. The tissue here has a looser structure with higher water content. These changes are usually not accompanied by arteriosclerotic vessel changes [15] and based on the pathological appearance are often considered not to be of pathological significance [12, 16, 22].

Irregular and extensive periventricular changes on the other hand, show the same pattern of tissue changes as the patchy or confluent WMC in the deep white matter and are often continuous with such changes. Many of the vessels in these areas show fibro- or lipohyalinosis.

Since WMC are found to be over represented in Alzheimer's disease (AD) [11, 23-25] and vessel changes due to cerebral amyloid angiopathy (CAA) are common in AD, many studies have looked for CAA in the WMC areas. Most pathological studies have not found any connection between CAA and WMC [11, 13, 26].

✓ In summary, the pathological studies have shown that non-specific WMC in the deep white matter represent a panorama of different grades of destructive changes with loss of vital tissue components. The picture is similar to what is seen in the periphery of old infarcts, and is most often accompanied by small vessel pathology. Periventricular changes, when well defined and limited in size relate to a disrupted ependymal lining of the ventricle, with a loose tissue structure that has higher water and lower myelin content. Extensive periventricular changes share pathology with deep white matter changes and are often continuous with those.

Aetiology

Although numerous studies have been published on the subject, the possible mechanisms underlying WMC are still matters of discussion. Since the histological responses to different pathogenetic mechanisms in the white matter are limited and non-specific, the neuropathological examination gives few clues as to what the underlying pathogenic mechanisms might be. Several models for explaining the pathogenesis have been proposed and several mechanisms, as well as combinations of them, can probably play different roles in different patient groups or even within the same patient. In addition, there is no agreement on the hypothesis of a continuum between small, punctuate or patchy lesions and extensive WMC. Data about the longitudinal evolution of WMC are still lacking although there is evidence for some progression over time [27]. The pathogenesis of WMC might thus be different for tiny punctuate or patchy lesions and extensive changes. Also with respect to the possible different vascular supply, the aetiology of changes in the deep white matter might not be the same as for those in the periventricular area.

Risk factors

A great majority of studies have pointed out that increasing age is the major independent risk factor for developing WMC. Secondly, hypertension is established as an important factor [28-30] especially if long standing or poorly controlled [29]. In addition, there is

some evidence for association with other cerebrovascular risk factors, such as atherosclerosis [31] diabetes [32], myocardial infarction [28], smoking [29, 30], hypotension [30, 33], cardiac arrhythmia [32] and bradykardia [34], although some studies also show contradictory results without any connections between WMC and various cerebrovascular risk factors [35, 36]. WMC are more frequently seen in patients with a history of stroke [28, 30], particularly of the lacunar type, and risk factors for these two conditions largely overlap. A couple of studies have shown females to have more WMC [30, 37, 38], and one study showed differences in risk factors for different age groups and for different types of WMC, i.e. deep versus periventricular changes [32].

Pathogenesis

Ischemia

Strong evidence suggests that the more extensive WMC, often have an ischemic origin [39, 40]. Animal studies have demonstrated that the white matter is very vulnerable during brain ischemia [41] and that ischemia can cause non specific changes like WMC [42, 43]. This vulnerability might be related to the suggested arterial borderzones located in this area [2] and thus caused by a small vessel disease. Aging and hypertension, the major risk factors for WMC, both induce changes in the small penetrating arteries and arterioles of the white matter. Such changes include replacement of the smooth muscle cells by fibro-hyaline material with thickening of the wall and narrowing of the vessel lumen (arteriolosclerosis), a finding almost always detected in areas of age related WMC. Hypertensive patients with WMC also more often suffer from an impaired cerebral autoregulation due to the inability of the vessels to dilate in situations of reduced perfusion pressure [44]. This mechanism is considered a major contributor to the type of chronic, repeated ischemic events suspected to underlie WMC [39]. In fact WMC might be a marker of both too high and too low blood pressure [34] or rather of a disturbed cerebral blood flow regulation [45, 46]. Decreased perfusion has been described in areas of WMC [47, 48] but whether this decreased blood flow is the primary event or rather an effect of the lowered metabolism secondary to parenchymal destruction is unclear. On the other hand observations of an increased oxygen extraction fraction [49] indicate an ischemic state that to a certain extent might be compensated.

Since the periventricular area has a different arterial supply than the deep white matter, the risk factors and the aetiology of lesions in this area might be different from those in the deep white matter. Since most studies did not investigate these changes as separate phenomena data are sparse. However, studies that do have this approach report that the risk factors differ between the two types of changes [32, 50, 51].

There are indications that the mechanisms behind WMC might be different in AD than in other conditions, like stroke or vascular dementia [52], or in healthy elderly. Since WMC are common findings in AD [19] and 90% of AD patients show cerebral amyloid angiopathy (CAA) a small vessel disease, at autopsy [53], it has been hypothesised that white matter changes in AD are linked to CAA. However, the white matter areas are mostly uninvolved by CAA, a condition primarily affecting the cortical or meningeal

vessels, and most pathoanatomical studies do not show CAA in WMC areas [11, 13, 26]. It is possible however, that white matter damage reflects the effects on the terminal fields of loss of regulation resulting from changes in the meningeal or cortical arteries [18]. CAA in cortical and meningeal vessels is reported to almost always accompany WMC in AD patients [18], and might be an overlooked cause of WMC in AD [39]. Another proposed cause for WMC in AD is the so called Wallerian degeneration, which is a secondary white matter degeneration due to overlying cortical neuronal pathology.

Blood brain barrier disturbances and cerebral oedema of other causes

Mechanisms alternative to ischemia have also been proposed to explain the origin of non-specific WMC. It has been hypothesised [54] that the changes could be caused by local blood-brain barrier (BBB) damage. Plasma extravasation into the brain resulting in an oedema, can give extensive white matter changes including gliosis, demyelination, cystic changes and lacunes [55]. Cerebrovascular conditions such as chronic hypertension and an impaired autoregulation of cerebral perfusion can open up the tight junctions of the BBB [3]. Acute hypertensive episodes might induce a rapid and transient BBB dysfunction exposing the brain to plasma constituents. Chronic hypertension can result in persistent or intermittent leakage. Even if the primary leakage area is not located in the white matter there is a rapid spread of the extravasated plasma constituents along the fibre tracts into the white matter where they might remain for a prolonged period of time [18]. This spreading is related to blood pressure and the higher the pressure the larger the spread [55]. A number of authors have reported that patients with WMC have a higher CSF/serum albumin ratio than controls. Such a finding is considered indicative of BBB dysfunction, and is seen in both AD [56, 57], VaD [56, 58], and in non-demented patients with WMC [59]. The increased albumin ratio in these patients is unrelated to cerebral infarcts, which suggests that the BBB impairment occurs as a consequence of a diffuse small vessel disease. In vascular dementia WMC have been linked to BBB dysfunction in pathological studies [60]. Since the pathogenesis of WMC might be different in different patient groups it has been hypothesised that in younger individuals the BBB damage might be the primary event, whereas among elderly a chronic ischemia may be a more common cause of WMC [55]. However, a BBB dysfunction can be secondary not only to arterial hypertension but also to chronic cerebral ischemia.

Normal pressure hydrocephalus (NPH) is another condition that is claimed to be accompanied by white matter changes. However, since severe WMC result in atrophy of the white matter the ventricles will become dilated as a secondary effect. The question of what is the cause and what is the effect is unresolved. It is hypothesised however, that increased ventricular pressure may cause ischemic changes in the white matter. This is supported by observations that after shunting such patients the cerebral blood flow returned to normal and the white matter changes decreased [61]. Another explanation consistent with this observation is that the increased intraventricular pressure in NPH causes a white matter oedema. Hypertension is also common in NPH [62] a fact that might explain the observed relation to WMC.

Impaired venous return in the deep white matter is another proposed cause of white matter oedema [63] giving a BBB disruption at the venular level as well as by increasing the perfusion pressure on the arterial side of the capillary bed.

✓ In summary, different processes might be active in the pathogenesis of non-specific WMC in different individuals. Strong evidence suggest that chronic low grade ischemia due to several different mechanisms is an important factor and that damage to the blood-brain barrier may also be a primary cause of WMC in certain groups of individuals.

Clinical consequences

The functional status in subjects with WMC is highly variable, ranging from normal to severe disability, either from cognitive or physical conditions. The association of WMC to different deficits is currently a matter of great research interest since it has been claimed that WMC is one of the processes involved in the transition to disability in the elderly population [64].

A large number of studies have addressed the possible clinical consequences of WMC. Regarding cognitive dysfunction, large cross sectional population based studies as well as studies of smaller samples of healthy individuals or demented patients have been performed. Subtle effects of white matter dysfunction are probably easier to show in healthy individuals, while in demented patients subtle symptoms would be expected to be obscured by symptoms related to the massive cortical dysfunction of dementia. The instruments for assessing cognitive dysfunction vary greatly between studies. Simple comprehensive tests are mostly used in large population based samples, whereas extensive neuropsychological examinations are sometimes performed in smaller studies.

In non-demented elderly

Many studies of healthy elderly have reported a relation between WMC and global as well as selective cognitive deficits [30, 32, 65-69], although the symptoms often appear to be subtle. Most studies have demonstrated that the speed of mental processing is most affected [30, 32, 38, 65-68], together with deficits in attention [65-67] and executive functions [66, 70]. More specific symptoms like decline in frontal functions, such as verbal fluency tasks, have in some studies been attributed to WMC [66]. In addition, a poorer performance on some memory tests [32, 38, 65-68] as well as mood disturbances, i.e. depression [30, 71] and motor symptoms, e.g. gait disturbances [72, 73] are frequently observed. A number of studies reported that subjects with WMC performed worse on global cognitive tests [30, 38, 68, 70, 74], such as the Mini Mental State examination (MMSE) [75]. There is however no evidence for a connection between WMC and measures of general intelligence.

Few studies have analysed the periventricular changes separate from subcortical or deep white matter changes. A couple of studies have however reported that the periventricular changes (including those extending into the deep white matter) are more closely related to different cognitive deficits and to motor disturbances [38, 73] than are changes located in the subcortical area.

The *prognostic relevance* of WMC in terms of predicting cognitive disability is not completely elucidated. Some studies have addressed the question of whether WMC are a predictor of cognitive decline among healthy elderly individuals. Such studies have pointed towards a greater decline over time in subjects with WMC, as compared to those without [76-78]. However, there is no evidence for an increased risk of developing dementia among healthy elderly with WMC. On the other hand, a number of studies on stroke have reported an increased risk of developing post-stroke dementia. [79, 80] Severe WMC are also accompanied by an increased risk to develop stroke and myocardial infarction [81-85] and indicate an increased risk of vascular death together with a shorter survival time [83].

In demented patients

Non-specific WMC have in a number of studies been reported to be more common in demented than in non-demented individuals, not only in patients considered to have vascular dementia but also in AD [24, 40, 86, 87]. However, it is not known whether this is an effect of a causal relationship between the WMC and dementia or if WMC only coexists and adds to the cognitive deficits.

The majority of studies looking at global cognitive decline or memory functions in demented subjects failed to find a relation with WMC [88-90]. It seems probable that once dementia is present it is difficult to detect the more subtle influence of WMC on cognition. There are however some studies that report findings of cognitive disturbances similar to those described in non-demented individuals [65, 91, 92]. A remaining question is the influence of WMC on disease progression in already demented or cognitively impaired patients. One recent study implicated a higher rate of dementia development among mild cognitively impaired patients with WMC, as compared to those without such changes [93]. In already demented patients studies of disease progression rate have failed to show a predictive value of WMC [90, 94, 95]. However, in all these studies there was either a very short follow up time, [90], or a limited number of patients with WMC [94, 95]. The question therefore remains as to whether the pathology causing WMC also affects the progression rate of the dementia syndrome.

✓ In summary, the symptoms attributable to WMC indicate that these changes may slow down certain basic information processing, the typical findings being suggestive of fronto-subcortical brain dysfunction [96]. The mechanisms by which WMC cause cognitive impairment remain hypothetical. Individuals with similar degree of changes can have very

different clinical manifestations. It is only possible to determine the extension, and not the degree of pathology, on conventional neuroimaging. The possibility of a threshold effect has been suggested, in the sense that the lesions may have to be of a certain severity or size before symptoms occur. Location of lesions is another important factor together with other potentially confounding factors such as age and concomitant diseases or risk factors.

THE DEMENTIA SYNDROME

The dementia disorders are devastating brain diseases of middle-aged and elderly humans. The vast emotional and economic costs of these diseases will continue to grow, as the population gets older [97]. Although much knowledge about the dementia diseases has been gained during the last decade many of the aspects of aetiology and pathogenesis are not yet understood.

Dementia is defined as an acquired clinical syndrome characterised by deterioration of mental functions with respect to cognition, emotion, and conation. Cognition refers to intellectual functions such as memory, linguistic, analytic and constructive abilities. Conation refers to the ability to plan and control the behaviour. Several functions must be affected simultaneously, to a certain degree and under a period of at least six months before the diagnosis is met. Dementia is a general term that includes multiple clinical profiles and disease progressions. The dementia diagnosis does not need to be linked to a certain aetiology and can be in a pre-clinical state at the time of diagnosis.

There are several different dementia syndromes with a variety of post-mortem pathological changes [98]. The two major groups are the **primary degenerative dementias**, of which Alzheimer's disease is the most important, and **the vascular dementias**. The vascular dementias include such entities as multi-infarct dementia and subcortical white matter dementia. The dividing line between vascular and non-vascular dementia is, however, vague [98] and mixed forms are common.

Alzheimer's disease (AD)

AD, the most common cause of dementia in the western world, is a primary neurodegenerative disorder often divided into a familiar and a sporadic type, and also into early- or late-onset disease. The onset is often insidious with signs of mild cognitive impairment which progresses into dementia. AD is neuropathologically characterised by the *neuritic plaques* and *neurofibrillary tangles* together with atrophy in the cortical structures. The relationship between plaques and tangles as well as the mechanisms causing AD are unknown and controversial subjects and both plaques and tangles can be seen, to a lesser degree, also in healthy elderly. The disease process probably starts in the

temporal-limbic structures and progresses to the temporal-parietal cortex. There is strong evidence that disturbances in amyloid metabolism are important for the development of AD, and beta-amyloid is a major component of the neuritic plaques. Tau-protein, the most important constituent of the neurofibrillary tangles, is also abnormal in AD. This leads to defective microtubuli and thereby to impaired axonal function. Changes of ischemic type in the white matter, i.e. WMC, are also common [99] and there is a growing interest in the possible role of vascular changes in the pathogenesis of AD [87, 100, 101]. There are indications that cerebral ischemia may promote Alzheimer type changes in the aging brain [87, 102].

There is currently no "in vivo" biological marker for AD and by the time the disease has progressed to dementia damage to the brain is widespread and irreversible. The diagnosis is built upon the fulfilment of certain diagnostic criteria [103] as well as the exclusion of other possible causes of dementia. A definitive diagnose, however, is not possible without post-mortem pathologic examination.

APOE

Apolipoprotein E (ApoE) is active in the transportation of lipids in the blood but it also plays a complex role in the central nervous system. Apolipoprotein E seems to participate in the formation of both the neuritic (amyloid) plaques and the neurofibrillary tangles in AD as well as in healthy individuals [104-106]. The apolipoprotein E (APOE) gene polymorphism is an important genetic factor that has been shown to influence the risk for late-onset AD. There are three common APOE alleles; $\varepsilon2$, $\varepsilon3$ and $\varepsilon4$, resulting in the six genotypes $\varepsilon2/2$, $\varepsilon2/3$, $\varepsilon2/4$, $\varepsilon3/3$, $\varepsilon3/4$ and $\varepsilon4/4$. The frequencies vary somewhat between different populations but $\varepsilon3$ is the most common (in Caucasians around 78%) followed by $\varepsilon4$ (around 15%) and $\varepsilon2$ (around 7%). There are numerous studies confirming the increased risk for AD in individuals carrying the APOE $\varepsilon4$ gene allele [107], and the risk increases with the number of $\varepsilon4$ alleles [108]. APOE $\varepsilon4$ has also been associated with an increased risk for cardiovascular disease [109, 110] as well as for dementia of presumed vascular origin [111]. However no relation between APOE $\varepsilon4$ and atherosclerosis of the cerebral vessels has been confirmed [112] and the $\varepsilon4$ allele was not related to an increased stroke risk in a large community study [113]. Furthermore, no connection between WMC and the APOE $\varepsilon4$ allele could be seen in a healthy elderly population [77].

Cerebral amyloid angiopathy

The relation between parenchymal and vascular deposits of β-amyloid is still unknown. Observations suggest that vascular amyloid derives from a different source than the plaque amyloid and might be produced by the smooth muscle cells in the vessel walls as a result of their degeneration [101]. Cerebral amyloid angiopathy (CAA) is age related and found in 10-50% of all elderly individuals but is even more frequent in AD [53] in which case it approaches 90% in most autopsy studies [114]. The vessel changes of CAA increase the risk for cerebral haemorrhage and micro infarcts. Individuals carrying the APOE $\varepsilon4$ gene allele more frequently have CAA, and this was shown in AD [115, 116] as well as in non demented subjects [112]. A neuropathological study [101] also indicated

that CAA in AD patients might be correlated to atherosclerosis in the cerebral vessels as well as with hypertension.

Vascular dementia (VaD)

VaD is a complex disorder characterised by cognitive impairment resulting from ischemic or haemorrhagic stroke or from hypoxic-ischemic brain lesions [117]. VaD can thus be associated with different underlying vascular pathology such as multiple or strategically located infarcts, haemorrhages or subcortical small-vessel i.e. white matter disease with or without lacunar infarcts.

Although a VaD diagnosis cannot be made in the absence of relevant vascular changes on brain imaging, there are no pathognomonic MR images and a definite VaD diagnose can only be made by fulfilment of clinical criteria together with histopathologic examination. Guidelines for the "in vivo" diagnosis of VaD, for clinical and research purposes, have been proposed [117] but the dividing line between vascular and non-vascular dementia is vague [98]. In a considerable number of VaD cases the post-mortem study finally shows a mixed dementia or even pure AD [100].

Mild cognitive impairment (MCI)

MCI is a recently characterized clinical entity that appears to represent a boundary or transitional state between normal aging and AD. Patients with MCI have subjective memory complaints that can be verified in memory tests as a slight decrease compared to age normality. These individuals progress into AD disease at a rate of 10%-15% per year.

✓ In recent years vascular factors have received increasing attention in the discussion of dementia, not only of the vascular type but also for the aetiology of AD. Observations of a high stroke incidence in AD [87] as well as frequent findings of WMC support this hypothesis. Moreover, there is an overrepresentation of CAA in AD, a small vessel disease that is linked to APOE ε4. This gene allele is, besides being a strong risk factor for AD, also connected to cardiovascular disease. The relationships between these different factors are however still unknown and seem complex. For example, it has been suggested that neither the APOE ε4 allele nor white matter lesions are sufficient risk factors by themselves for dementia at very old ages, whereas possession of both these entities increases the risk for both AD and vascular dementia [118].

AIMS

The general purpose of this thesis was to study different aspects of non specific white matter changes on MRI, and to investigate the clinical significance of such changes in subjects with cognitive impairment or dementia.

In the different papers the specific aims were

I. To evaluate if, and to what degree, MRI either under- or over-estimates the extent of histopathological findings in WMC, and to histologically characterize areas where the methods disagree.

II. To investigate if an increased blood-brain barrier permeability could be shown in WMC areas, in demented patients, using a contrast enhanced MRI technique.

III. To explore the possible relation between WMC and APOE genotype in Alzheimer's disease.

IV. To investigate if WMC in a specific region affect cognitive functions related to that area, and also to test the hypothesis that the early and late phases of the "word fluency test" depend on different mechanisms.

V. To evaluate if the presence of pronounced WMC predicts a more rapid global cognitive decline in patients with memory disturbances or dementia

MATERIAL AND METHODS

SUBJECTS

The MRI-dementia project has been running at Huddinge University Hospital since 1992. In this project, all patients referred for an MRI of the brain from the memory clinic are uniformly examined and all data are stored for research use. Up until the end of 1997, 1264 MRI examinations had been performed. However the project continues to run also after this date. Among the MRI examinations, 476 have been visually rated with regard to the amount and location of white matter changes in the brain, according to a semiquantitative rating scale (see below). All patients have also participated in a comprehensive clinical investigation procedure including physical, neuropsychologic and psychiatric examinations together with blood- serum-, urine- and cerebrospinal fluid analyses as well as tests such as EEG and SPECT. All patients have done the MMSE test, a global cognitive test assessing different aspects of cognition. The sum score ranges from 0-30, where 30 denote a full score on all parts of the test. All dementia diagnoses were made according to the clinical criteria DSM IV / ICD 10 [119], NINCDS-ADRDA [103], NINDS-AIREN [117], or post-mortem according to the CERAD [120] criteria for neuropathology. Subjects with the diagnosis MCI had an objective cognitive decline but did not fulfil dementia criteria. The subjects with "subjective memory disorder" (SMD) had memory complaints but no objective cognitive dysfunction.

In *study I*, the brains from 6 patients who died with a history of memory impairment, and with autopsy diagnoses of possible or probable AD (CERAD criteria), were obtained from the Huddinge brain bank. To increase the possibility of finding WMC the brains were obtained from patients of high age (range 81-101) and / or with a history of cardiovascular risk factors.

Study II included 10 patients from the memory clinic who had previously done an MRI or CT that had shown WMC. The patients were diagnosed as AD (n=3), VaD (n=4), frontal lobe dementia (n=1), MCI (n=1) or SMD (n=1). 5 patients had elevated CSF/serum albumin ratios (3 VaD, 1 AD and 1 frontal lobe dementia) indicative of BBB dysfunction. The remaining 5 had normal CSF albumin levels.

Study III included 60 consecutive patients with a diagnosis of AD. To minimize the influence of cerebrovascular risk factors, patients with a history of hypertension, diabetes or cerebrovascular disease were excluded. The patients were of the APOE genotypes $\varepsilon 4/4$ (n=13, mean age 66.2 y), $\varepsilon 3/4$ (n=19, mean age 63.7 y), $\varepsilon 3/3$ (n=24, mean age 63.6 y), $\varepsilon 2/4$ (n=1, age 56) and $\varepsilon 2/3$ (n=3, mean age 65.3 y).

In *study IV*, 46 patients with different degrees of memory impairment were included, restricted however to patients without pronounced symptomatology. The diagnoses were; mild AD (n=12), MCI (n=24) or only SMD (n=10). The mean age was 61.4 ($\pm$

8.0) y and the educational level was 11.0 (± 3.3) y of formal schooling. The three groups differed in MMSE scores (AD = 22.4 ± 5.3, MCI = 26.5 ± 2.3, SMD = 28.3 ±1.7).

Study V included 48 patients from the memory clinic with different degrees of memory impairment. 24 of the patients were chosen on the basis that their MRI examination had shown extensive WMC. The rest were matched controls i.e. patients with the same degree of memory disturbances but without WMC. The cases and controls were matched pair-wise according to age, education, score on MMSE-test at initial investigation, the initial diagnosis (fulfilling dementia criteria or only MCI or SMD) and the length of follow up. The follow up ranged between 2 and 4 years in the 24 matched pairs (for details see table 1 in study V).

METHODS

Magnetic Resonance Imaging and procedures (studies I-V)

All MRI investigations were performed using the same 1.5 T system (Magnetom SP, Siemens, Erlangen). All axial slices were aligned parallel to the bi-commissural line as defined in a mid-sagittal scout image. The coronal images were perpendicular to the axials.

The MRI sequence used for all white matter ratings in ***studies III, IV and V*** were a PD/T2 weighted double echo sequence, a fast spin-echo (TR 3500, effective TE 19/93) with an echo train length of 2, and with 19 slices in the axial plane (slice thickness 5 mm, interslice gap 1.5 mm). The field of view was 230 mm (rectangular 3/4) with a matrix of 192 x 256, and 1 excitation. Also available on all patients in studies III, IV and V was a T1 weighted 3D gradient echo (GE) sequence (Siemens MPRAGE) (TR/TE: 10/4 ms, FA: 10°), in the coronal plane with 64 partitions, each 2.8 mm thick. Since the MRI system could not perform turbo FLAIR sequences, no such images were included.

In ***study I*** the fixed brain specimens were removed from the formalin solution, put in a plastic box and thereafter positioned in the head coil of the MRI scanner. The same PD/T2 weighted fast spin-echo sequence as for all white matter ratings, was performed, however in the coronal plane, to match the neuropathology gross slices.

In ***study II*** all sequences were acquired in the coronal plane. The same PD/T2 weighted sequence, as described above, was performed to identify the WMC. To assess contrast enhancement a T1 weighted SE sequence (TR/TE: 600/14 ms) with 5 mm slices, with 1.5 mm gap, field of view 230 mm (rectangular 3/4) and a matrix of 192 x 256 was thereafter performed. In addition, we acquired the same T1 weighted 3D GE sequence (Siemens MPRAGE), as described above, also in the coronal plane. All three sequences (PD/T2, T1-SE and T1-GE) were first performed before injection of the contrast agent. The patient then received a double standard dose (0.2 mmol/kg body weight) of GdDTPA-BMA (Omniscan®, Nycomed) a non-ionic, low osmolality gadolinium chelate of low molecular

weight (570 Daltons), which is highly water soluble. GdDTPA-BMA distributes in the extracellular fluid and does not bind to plasma proteins. After injection the patient was imaged repeatedly during 30 minutes with the SE sequence starting at times 5, 15, and 25 minutes post-injection and the GE sequence starting at 10 and 20 minutes post-injection. The WMC were identified as hyperintensities on the PD/T2 weighted images and the same areas were thereafter identified on the corresponding pre-injection T1 weighted images. Between 2 and 6 regions of interest (ROI) were selected in each patient. Corresponding to every chosen ROI another ROI was placed in the same image over an area of normal appearing white matter. The MR-signal was then measured in all ROI´s in all T1 weighted sequences, before and after contrast injection. The signal change over time was analysed separately for the two MR-sequences and ratios between the signal in the lesions and the signal in the corresponding normal white matter areas were calculated.

Rating of WMC (studies III, IV and V)

All WMC ratings were done using the PD and T2 weighted images, by a rater blind to clinical data and diagnosis, and using a scale for visual rating developed by Scheltens et al [121]. We modified the scale slightly in order to rate the right and left hemispheres separately (**Table 1**). The scale is detailed and gives information about both number, size and location of lesions and is referred to as being semiquantitative. According to this scale the lesions were divided into periventricular hyperintensities (PVH), deep white matter hyperintensities (DWMH) and basal ganglia hyperintensities (BGH). Round and well-defined hyperintensities smaller than 2 mm, were regarded as dilated perivascular spaces and were not counted. The Scheltens scale, although more complex than other scales, is shown to have better intra- and interobserver reliability than other commonly used more simple scales [121]. The reliability was given by Scheltens et. al. as measures of "standardised reproducibility" which are the standard deviations of the differences between first and second ratings divided by the range of the scale. The intrarater reliability in our study was calculated in the same way and the DWMH had a standardised reliability of 7.5% (compared to Scheltens study that had 15.3%), the PVH 4.2% (Scheltens 5.6%) and the total sum score had a standardised reliability of 1.8% (not calculated in Scheltens study). Hence, our reproducibility was somewhat greater than in Scheltens study, which probably relates to the fact that after our modifications, the scale had a wider range.

In *study III* scores of PVH, DWMH (except for occipital lobes which were excluded after factor analysis) as well as BGH were used. In *study IV*, the PVH and DWMH scores were used. In *study V* the total sum score was used.

Table 1. *Visual rating scale of WMC*

		Right	Left
Periventricular hyperintensities (PVH)[1]			
Caps	Frontal	0-2	0-2
	Occipital	0-2	0-2
Bands	Lat. Ventricles	0-2	0-2
	PVH sum score	0-12	
Deep white matter hyperintensities (DWMH)[2]			
Frontal		0-6	0-6
Parietal		0-6	0-6
Occipital		0-6	0-6
Temporal		0-6	0-6
	DWMH sum score	0-48	
Basal Ganglia hyperintensities (BGH)[2]			
Caudate Nucleus		0-6	0-6
Putamen		0-6	0-6
Globus pallidus		0-6	0-6
Thalamus		0-6	0-6
	BGH sum score	0-48	

A modified Scheltens scale with separate ratings of the right and left cerebral hemispheres.
[1] 0 = absent; 1 = ≤ 5 mm; 2 = 6-10 mm
[2] 0 = No abnormalities; 1 = ≤ 3 mm, n ≤ 5; 2 = ≤ 3 mm, n > 5; 3 = 4-10 mm, n ≤ 3;
 4 = 4-10 mm, n > 3; 5 = > 10 mm, n ≥ 1; 6 = confluent.

Neuropathology (study I)

The brains were removed at autopsy within 24 h after death and fixed in 4% buffered formaldehyde for at least 4 weeks. Following fixation and subsequent MRI scanning and by using a specially designed brain slicer, each brain could be cut in coronal slices closely corresponding to the MR image planes. In the slicer the brain was put in a plastic chamber, on a movable tray, and angled in accordance to the MRI scout image. The chamber was thereafter filled with 4% agar. After hardening, the agar maintained the correct position of the brain in the slicer. The position of the knife was fixed while the specimen, resting on the sliding tray was moved. The accuracy of the slice thickness was obtained using a ruler, fixed to the side of the instrument. The brains were cut in 6.5 mm slices where the distance between slices corresponded to MRI slice thickness plus the interslice gap. Gross sections of the whole brain slices were embedded in paraffin, and stained by Luxol Fast Blue (LFB) and haematoxylin-eosin.

For each brain, three MRI slices in which white matter hyperintensities could be seen were chosen and the area of WMC as well as of the total white matter were quantified on the PD images, using stereological methodology (see below). The PD and not the T2 images were chosen because they provided a better delineation of the border between

grey and white matter [122] while the hyperintensities were shown equally good. Separate measurements were made for each hemisphere and a total of 33 areas with signal hyperintensities were analysed. On the neuropathological gross sections corresponding to the chosen MR images, the areas of myelin pallor seen on visual gross inspection were quantified using the same method as for the MR images. To overcome the problem of specimen shrinkage during the embedding and staining processes, only relative data (affected area as % of total white matter) was used when comparing the MRI and neuropathological examinations.

For further analysis, the white matter regions were classified into three different category types, according to their appearance on MRI and neuropathological gross sections. The first type (area type 1 = AT1) was that of white matter areas with normal appearance on both MRI and neuropathology. The second area (type 2 = AT2) appeared normal on MRI, but showed white matter pallor on the pathology slices (see fig 2a in study I). The third area (type 3 = AT3) was considered abnormal on both MRI and neuropathology (see fig 2b in study I). In these three different area types, the white matter was microscopically evaluated and the glial density estimated. A total of 8 regions of interest were chosen for this analysis. An unbiased 2D-quantification technique [123] was applied for estimating glial cell density per square millimetre. Since LFB stains the nuclei of all cells, the total number of all glial cell types was calculated, while omitting other easily recognizable cells such as endothelium. The achieved counts were considered to represent cellularity of the white matter.

Stereology

The different white matter areas were quantified using the stereological principles of area counting [123]. A grid-point test system with a set of regularly spaced points was superimposed over the region of interest. The number of grid-points hitting either total white matter or pathological white matter area, respectively, was counted on each particular slice. The areas of white matter changes were then expressed as a percentage of the total white matter area in the same slice. So as to achieve a coefficient of error (CE = standard error of mean/mean) of less than 0.10 in the estimation of areas, the grid size was chosen so that a minimum of 30 observations (point hits) per area of interest was reached. The stereological grid point method is easy to use and more reliable than the delineation of area method [123]. Every counting was repeated three times and the mean values calculated for further analysis.

Assessment of word fluency (study IV)

In the FAS test [124], used to assess letter word fluency, the patient produced as many words as possible, in 60 s, all beginning with the same letter. This was repeated for the three different letters F, A and S. The number of words was recorded for every 10 s period, which resulted in 18 scores.

APOE genotyping (study III)

DNA was extracted from peripheral white blood cells and the APOE genotype was determined using a microsequencing method on microtitre plates (Affi-Gene[®] APOE, Sangtec Medical, Bromma, Sweden).

Statistical analyses

All statistical analyses were performed using the STATISTICA[©] software, versions 4.1, 5.1 and 5.5 (StatSoft, Inc, Tulsa).

The non-parametric Wilcoxon matched pairs test was used for comparing variables (*studies I and V*) consisting of ordinal data such as the MMSE scores and results from stereology. Also the Spearman rank order correlations (*studies III, IV and V*), as well as Kruskal Wallis ANOVA by ranks followed by Mann-Whitney U-test (*study III*) were used for ordinal data.

Analyses of variance (ANOVA) were used for interval data such as cell counts (*study I*) and MR signal ratios (*study II*) as well as for the semiquantitative Scheltens scores if the variances were homogeneous and the distribution of data not highly skewed (*study III*).

For interval data the Pearson product moment correlation was used.

When a principal factor analysis was performed (*studies III and IV*) the standard criteria for inclusion of factors were used, i.e. explained variant > 10 %, more than one factor loading > 50%, and having "eigenvalues" above or at the break-point in the distribution plot.

Multiple linear regression analyses with forward stepwise inclusion of variables as well as simultaneous regression analyses were used (*study IV and V*). The forward inclusion model was preferred if the analysis included more than a few variables.

MAIN RESULTS

The principal findings were:

In *study I* the WMC were more extensive on neuropathology than on a routine MRI sequence in all of the brains, with a mean of > 50 % larger areas. The correlation between measures from MRI and pathology was high. The pathology not depicted by MRI represented however only minor changes with lower intensity of myelin staining and accentuation of the distance between fibres but with preserved axonal network and glial cell density

In *study II* no leakage of the MR contrast agent across the BBB could be measured in the WMC areas.

In *study III* of AD patients we found that individuals with the APOE $\varepsilon 4/4$ genotype had more extensive white matter changes. Moreover, in subjects carrying the $\varepsilon 4$ allele, the deep white matter changes were not age related, as they were in the others.

Study IV showed a relation between WMC in the frontal lobes and poor performance on the initial, but not on the late part of the FAS word fluency test. The correlation between regional white matter scores and poor initial word fluency was most pronounced for periventricular WMC in the left frontal lobe.

Study V showed that extensive WMC did not predict a faster global cognitive decline in a group of memory-disturbed patients.

RESULTS AND METHODOLOGICAL DISCUSSION
STUDY I, POSTMORTEM MRI AND PATHOLOGY

By using the specially designed brain slicer we could get a good anatomic matching between MRI scans and neuropathological sections and were thus able to compare the extent of WMC as seen on MRI, to the areas of WMC seen on neuropathology. The correlation between measures from the two methods was high although neuropathology revealed significantly more extensive WMC in all brains, than did MRI ($p<0.00005$). In the 6 brains neuropathology showed between 16 % and 111 % (mean 54 %) larger areas than did MRI (fig 2). On average 26 % of the total white matter area in the actual neuropathogical slice was affected compared to 18 % on MRI.
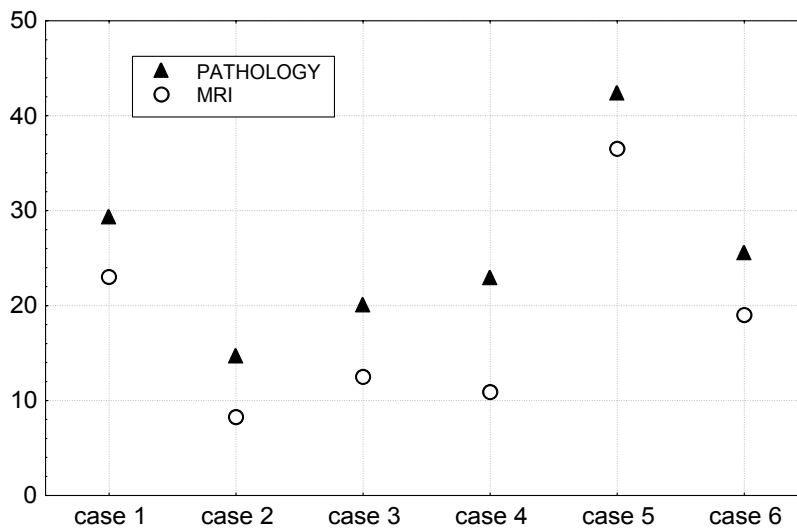
Figure 2.

The mean area of white matter pathology in each of the six brains, as measured on MRI scans and on neuropathologic gross sections and expressed as percentage of the total white matter area. Neuropathological examination showed larger areas of white matter change, in all six cases than did MRI.

There are some fundamental differences between the two methods that might influence the results. The spatial resolution is much higher in the neuropathological sections than on the MRI scan making the delineation of borders between grey and white matter as well as between normal and abnormal white matter easier. Moreover, the partial volume effect, present in all MR images implies that each scan contains information from a thick slice,

making all borders that are not perpendicular to the slice appear blurred. The MRI sequence chosen for the study is widely used in the clinical routine but not specifically optimised for the detection of white matter changes. FLAIR sequences were not available on our equipment at the time. These mainly have the advantage of giving superior contrast between lesions and the surrounding white matter. A PD sequence without "turbo" effect would also have yielded a slightly better contrast and spatial resolution. An improved contrast would have made the quantification easier and at the same time would have made the effects of partial volume work more in the favour of MRI. However, it is unlikely that lesion areas would be adjusted more than marginally, and not approaching the 50 % difference we observed.

Post mortem MRI may not have the same sensitivity to WMC as in vivo MRI studies. Although many authors have claimed it to be a reliable method [12, 125-128] it is also reported to have a somewhat lower sensitivity to WMC as compared to in vivo studies [15, 21]. Moreover, the fixation process reduces both T1 and T2 relaxation times [129] and the rate appears to be different in different kinds of tissue [130] explaining the reversed contrast seen between grey and white matter, in T1 images. Studies comparing in vivo to in vitro MRI are desirable but will always meet obstacles because of the time delay between the two examinations as well as unknown perimortal events.

In this study microscopic analyses were made of different types of areas, as categorized by their appearance on MRI and LFB slices (figs 2 a and b in study I). AT1 (fig 3a, in study I), that appeared normal with both techniques, showed white matter, heavily stained by LFB, with dense packed cells, corresponding to normal tissue. AT2 (fig 3b, in study I), with normal appearance on MRI but abnormal on neuropathology, showed a lower intensity of myelin staining with an accentuation of the wide spaces between fibres in a fairly intact axonal network and with well-preserved nuclear shapes and glial cell density. AT3 (fig 3c, in study I) that was abnormal with both techniques correlated to areas of variable atrophic appearance with severe myelin pallor, intense vacuolation and decreased cell density. Different degrees of reduction of the axonal network were seen together with some irregularly shaped and fragmented axons. Dilated perivascular spaces were common findings, but no areas of gliosis or infarction. Only one of the brains displayed fibrohyaline wall thickening of small arterioli in the white matter, while in the others only smooth muscle degeneration without any sign of extravasated bleeding or phagocytosis could be seen. In two brains, minor cortical and leptomeningeal amyloid angiopathy changes were seen. The glial cell numbers per mm^2 were compared and were lower in AT3 as compared to both AT1 and AT2 while there was no significant difference between AT1 and AT2.

STUDY II, BBB EVALUATION

There was no signal change in the WMC areas in any patient after contrast agent injection, in spite of a prolonged scanning time, a doubled contrast dose and measures of MR signal change over time in lesions as compared to normal areas. Even in the group of

patients with elevated CSF/serum albumin ratios, indicative of increased BBB permeability, no contrast enhancement in the WMC could be found, and there were no differences compared to the group of patients without signs of BBB dysfunction.

The lack of evidence for contrast leakage in the WMC might be explained by an intact BBB. On the other hand, there might also be explanations of methodological and physiological nature. The BBB dysfunction caused by microvascular ischemic injury is probably caused by defective tight junctions and basal membranes [3] giving a general increase in permeability. Other cerebrovascular conditions such as chronic hypertension and impaired autoregulation of cerebral perfusion can also open up the tight junctions [3]. In the case of a general permeability increase, the small Gd-DTPA-BMA molecule would be expected to pass the defective barrier and the MRI technique used in this study is sensitive to a diffuse signal increase throughout the lesion areas. However, the dysfunction caused by microvascular ischemic injury has also been described as variable and focal and it might under some circumstances, be reversible [3, 131]. The leakage may thus appear in the form of "microleaks" [131], i.e. only in small areas or transiently over short time periods, and when measuring signal changes in regions including large areas of WMC, there is a high possibility to miss a contrast enhancement occurring only in small parts of a lesion. Furthermore, when comparing different patient groups we used mean signal values from all measures in each patient. This also limits the possibility to discover local changes that do not include the WMC as a whole. Moreover, although the MRI procedure was designed to detect small degrees of enhancement, a diffuse but very low-grade leakage could still be missed. Such BBB leakage might be detected with a more delayed imaging (up to 2 h after injection) and by using a higher dose of the contrast agent [132]. The MR sequences might also be further optimised with an improved SNR or a slightly reduced TR.

STUDY III, APOE INFLUENCE

After controlling for age and degree of dementia (estimated by MMSE score) a significant effect of APOE genotype was found on the MRI white matter scores, both for the DWMH and PVH. The subjects with the homozygous $\varepsilon4/4$ genotype had more extensive DWMH, PVH and BGH than those with other genotypes. The differences were significant for DWMH scores when $\varepsilon4/4$ subjects were compared to those with one or no $\varepsilon4$ allele, and for PVH and BGH only when compared to those with one $\varepsilon4$ allele (Figs 3 a and b). The effect of the APOE $\varepsilon4$ allele was thus found only in homozygous individuals. This might be explained by a threshold effect or hypothetically that one $\varepsilon4$ allele is sufficient to prevent the process leading to WMC.
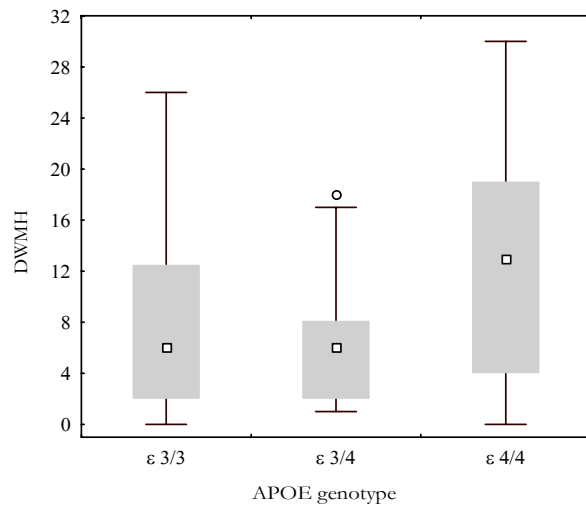
Figure 3a. *Box and Whisker plot showing scores for DWMH in AD patients with different genotypes. The differences between ε4/4 and the other groups are significant*
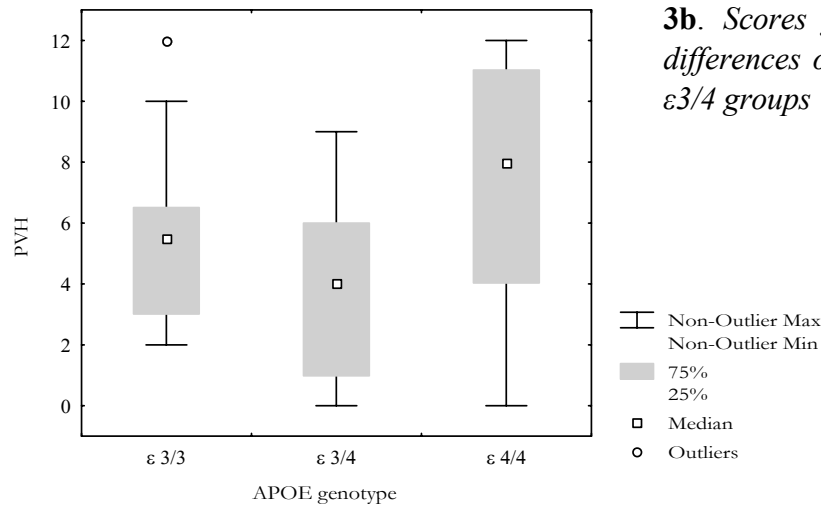
3b. *Scores for PVH. Significant differences only between ε4/4 and ε3/4 groups*

In most studies of healthy individuals the WMC correlate strongly to advancing age, while in AD this correlation is not clear with disparate results being reported in previous studies. In our study of AD patients the correlations between age and white matter ratings were different in the different genotype groups. Only subjects without the $\varepsilon4$ allele showed a correlation between advancing age and scores for DWMH and BGH, while for the PVH there was no age correlation in any patient group. The finding that age correlation in AD is dependent on the APOE genotype supports the hypothesis that in AD subjects carrying the $\varepsilon4$ allele the white matter changes have a different origin, one that might be related to the aetiology of the disease.

STUDY IV, WORD FLUENCY AND WMC

The scores on the word fluency FAS test were factor analysed which resulted in two factors, one relating to the *initial* and one to the *late* test performance. The WMC scores were also factor analysed, which yielded one *anterior*, and one *posterior* factor.

According to a regression analysis (R=0.60, p<0.001) the anterior WMC factor and the level of education predicted the initial, but not the late performance on the FAS test. In further analyses of the individual WMC scores from the Scheltens scale, mainly PVH in the left frontal lobe, i.e. left frontal cap and left lateral band, predicted performance on the initial part of the FAS test (p<0.01). According to Spearman rank correlation PVH in the left frontal lobe had the highest correlation to initial FAS factor, however, also PVH in the right frontal lobe was significantly, but less correlated. The late FAS factor did not significantly relate to any of the variables, neither WMC factors, WMC scores, or education (although a tendency was seen). However, since there was less variation between the patients in performance on the late part of the test, the possibility to detect correlations with other variables is reduced. The relation between left frontal PVH and initial FAS test was on the other hand highly significant, which indicates that WMC in this region affects initial word fluency.

STUDY V, PROGNOSIS

When comparing two groups of memory-impaired patients with and without extensive WMC, no difference in outcome, as measured by decrease in MMSE score, during the observation period could be found. The mean decrease was 3.9 points (median 1) among cases, i.e. patients with WMC, and 4.0 points (median 3) among the controls (fig 4). The only factor that was correlated to the outcome measure was the initial MMSE score (R= -0.41, p<0.01) where a high initial score correlated to a lesser decrease. This relates to the fact that among those with the highest scores were subjects who did not develop any further symptoms. Although the number of patients was limited, the extent of WMC among the cases was pronounced which should increase the chances to detect possible effects. No tendency for the subjects with WMC to decline faster could however be detected. In contrast, the outcome was similar for both groups. The MMSE does not specifically reflect the subcortical dysfunctions typical of WMC but indicates the global cognitive level. On the other hand, some previous studies have shown WMC to also affect MMSE performance [30, 68, 70, 74]. The purpose of this study was to evaluate the prognostic significance, in the clinical situation, of the finding of extensive WMC during the dementia investigation. The issue being to evaluate if such changes predict a poorer clinical outcome in this patient group.
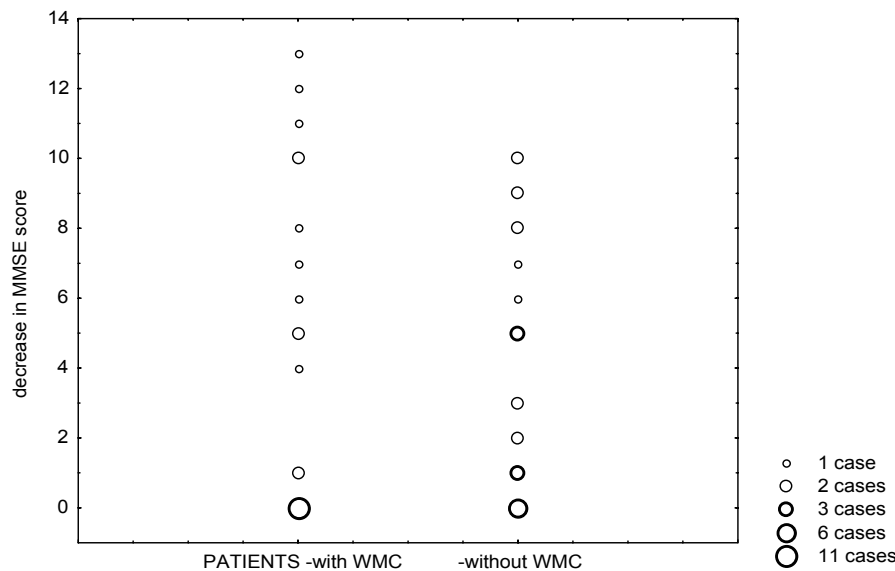
Figure 4

Frequency scatter plot of the outcome measure, decrease in MMSE score at follow up, in the two patient groups; patients with extensive white matter changes in the brain and patients with minor WMC. No significant difference was found between the groups.

The patient group was heterogeneous and because of this, the cases and controls were pair wise closely matched according to age, educational level, initial performance on MMSE and initial diagnosis. The MMSE is a scale of ordered categorical data, which is not linear. According to Mendiondo et al [133] the rate of decline differs between various ranges of the scale. In addition, younger and more educated patients progress more rapidly, while gender has little impact on disease progression as measured with MMSE. Thus a matching between cases and controls is important regarding age, education and MMSE level but not gender. Regarding the patients initial diagnose only the fulfilment of dementia criteria or not was noted, as well as evidence or not of MCI. Since the presence of severe WMC influences the specific dementia diagnoses (e.g. AD, VaD or mixed) these were not considered. Length of follow up, ranging from 2 to 4 years, differed between pairs but was similar in cases and controls in every matched pair.

GENERAL DISCUSSION AND CONCLUSIONS

The results of *study I* showed a systematic difference between MRI and neuropathology where the less pronounced pathology, interpreted as early changes, was not seen on MRI. These changes consisted mainly of areas with reduced myelin density and a concomittant

increase in tissue water content. More severe changes that included cell loss were clearly visible on MRI. Although the MRI technique is sensitive to an increase in water content these early changes were not depicted by a conventional fast-spin echo MRI sequence. Diffusion weighted MRI is a new technique that has a high sensitivity for microscopic structural changes in the white matter related to volume of the extracellular space and to integrity of cell membranes. This technique has depicted changes in the otherwise normal appearing white matter in AD patients, indicating a pathology in these areas not visible on routine MR imaging [134-136]. The changes in diffusion indicated a decreased fibre density with higher extracellular water content, well corresponding to the findings in our study. The MR diffusion technique is not applicable in the post-mortem situation and cannot be used in comparison with neuropathology.

MR spectroscopy (MRS) is another promising method, which reveals metabolic compounds in the tissue and this technique is possible to use both in vivo and in vitro. In vivo studies have shown AD patients to have abnormal spectra not only in grey but also in white matter [137], indicating diffuse axonal injury and membrane alterations. An MRS study of WMC in non-demented elderly also showed a neuronal/axonal loss in such areas [138]. None of these studies included neuropathologic correlation.

Another new MR imaging method that has been applied for brain white matter studies is magnetisation transfer imaging (MTI). MTI is related to relaxation properties associated with the presence of macromolecules in tissue membranes. In brain tissue, myelin is regarded as the major macromolecule responsible for the MT phenomenon and reduced MT ratios are thought to reflect changes in the amount and constitution of myelin present in white matter [139]. Abnormal MT ratios have been found in the normal appearing white matter in MS patients [140]. Moreover, differences in MT ratios have been shown when comparing WMC in demented patients to those in non symptomatic individuals [141, 142] supporting the hypothesis of different types or grades of damage in these two groups.

Previous studies comparing WMC as seen on post-mortem MRI and histopathology dealt with the qualitative analysis of the pathological correlates to the MRI lesions. One study also compared the extent of pathology depicted with the respective method [11] and claimed that MRI might be more sensitive than microscopic evaluation of WMC. Others [12] found a poor correlation between findings on MRI and pathology in the deep white matter, while again others reported a high correspondence between the methods [13]. In most studies the lesions observed were reported to be more extensive on pathology than on MRI [12, 14-16] although none of these studies made a quantitative comparison. In our study all patients had a diagnosis of probable AD, but some of them also had cerebrovascular risk factors. The white matter lesions could thus have varying causes and the interpretation of our results may be difficult to generalise. The neuropathological findings are however considered to mainly be of the same nature although different pathophysiological mechanisms may underlie the changes seen in AD and cerebrovascular disease [7, 11]. A limitation of our study is the small number of brains included. However, the pattern was similar in all of the cases, regardless of concomitant

cerebrovascular disease. We therefore conclude that WMC are more extensive on neuropathologic examination than on post-mortem MR images but that the lesions not identified on conventional MRI only represent minor changes with slight myelin pallor but with preserved axonal network and glial cell density.

The BBB dysfunction occurring in demented patients seems related to vascular factors [143] and is suggested to be a consequence of small vessel disease [144]. In line with this, a number of authors have reported that WMC are related to elevated CSF albumin levels, taken as an indication of disturbed BBB function [57, 59]. In addition, pathology studies using immunohistochemistry have shown an increased amount of extravasated serum proteins in WMC areas indicative of BBB dysfunction [54, 60, 145]. In *Study II,* using contrast enhanced MRI; no general BBB leakage was found in the WMC areas, not even among the patients with elevated CSF albumin. There might however still be a dysfunction in the form of microleaks, limited in space and/or time and as such difficult to demonstrate with this kind of method. On the other hand the results might reflect a true integrity of the BBB in the WMC and the elevated CSF albumin might have another origin. Only some patients with WMC have elevated CSF albumin and the albumin source might be located to other areas in the CNS and thus not caused by the small vessel disease of the white matter. Since the primary source of protein in the CSF is the choroid plexus [146], then it is possible that elevated CSF albumin levels could be due to a dysfunction in these areas with an increased leakage or decreased reabsorption of proteins. Another explanation for high CSF albumin levels in the lumbar sac, from where the CSF samples are taken, is that of a slowed CSF-circulation. Normal pressure hydrocephalus (NPH) is a condition that is characterised by disturbances in the CSF-circulation and which can give cognitive decline. NPH can also cause extensive WMC probably due to disturbed CSF dynamics. As pointed out by Pantoni et al [54] CSF-circulation disturbances might explain the link between WMC and high CSF/Serum albumin ratios. Our results are consistent with such a hypothesis. Finally, in contrast to a general increase in BBB permeability, the impairment might appear as a more selective dysfunction of the barrier with an increased pinocytic protein transport across the barrier not affecting the Gd-DTPA-BMA molecule. This type of mechanism has been described in chronic hypertension in animal models [147].

In conclusion, this study provided no evidence for a major increase in BBB permeability in the WMC areas. The protein leakage into the CSF in patients with WMC either occurs by some other mechanism, like pinocytosis, or in some location other than the white matter lesions, or it is of such low grade, or limited to such small areas that it is not detectable with the method used. Another possibility is that disturbed CSF circulation causes a higher concentration of proteins in the lumbar sac from where the samples usually are taken.

No connection between WMC and the APOE ε4 allele has been shown in studies of healthy elderly [77, 148]. In ***study III*** we concentrated on WMC in AD patients, under the hypothesis that WMC in these patients might be of a different nature. To minimise the atherosclerotic influence we excluded all patients with a cerebrovascular history as well as those with hypertension and diabetes. We found that AD patients homozygous for the APOE allele ε4 had more WMC than patients with other genotype groups, the results being most significant regarding changes in the deep white matter. Recently a number of other papers addressing the same issue have been published [37, 149-151] without reproducing our results. However, in two of these studies WMC were only reported as present or absent [37, 150] an approach that reduces the sensitivity. In the other two studies [149, 151] the extent of WMC were, as in our study, recorded using the Scheltens scale. In both of these studies AD patients carrying the APOE ε4 allele had higher white matter scores than other groups, although the differences did not reach significance. Consequently, there seems to be a relation between APOE genotype and WMC in AD although this relation is not prominent. A difference between our study and the others is that we excluded patients with the common risk factors for WMC. Together with the young mean age in our study, this gave us a different AD population where the chances of having WMC connected to arteriolosclerosis, was reduced. Our subjects with ε4/4 not only showed more extensive changes, in patients carrying the ε4 allele the changes were unrelated to advancing age in contrast to the findings in patients without ε4, as well as in most populations previously studied. However, previous findings in AD populations have been inconclusive. Together our results suggest that in AD patients of the ε4/4 genotype some of the WMC might be related to the aetiology of the disease. The same patient group is previously shown to have more severe cerebral amyloid angiopathy than others [115, 116, 152]. CAA is a condition proposed by some to cause white matter changes [39, 40] a theory however disputed by others [11, 26]. At present, no connection between CAA and WMC has been established. Our results could be interpreted as supporting the hypothesis that CAA causes white matter changes in Alzheimer patients.

Study IV: In demented patients it has been difficult to demonstrate the neuropsychological syndrome that in healthy subjects has been associated with WMC [88-90]. This has been explained by overlapping of more severe symptoms caused by dementia. There are however a few studies that have reported cognitive disturbances related to the presence of WMC similar to those described in non-demented individuals [65, 91, 92].

Most studies exploring the cognitive correlates of WMC, either in healthy subjects or in patients with dementia, have not separately evaluated lesions in different brain regions but only looked for general effects [65, 92]. These effects have mainly been found to be reduced mental speed and attention and the typical findings are suggestive of fronto-subcortical brain dysfunction [96]. Only few have reported that WMC in specific regions seem to influence performance in specific neuropsychological tests [86, 153, 154]. In such cases the tests were mainly time dependent or reliant on executive functions. Some authors have separated periventricular from deep white matter changes and claimed that mainly the periventricular changes are associated with cognitive impairment [38] and

motor changes [73]. In contrast, it has also been claimed that only subcortical and not periventricular location gives cognitive deficits [155].

Letter based word fluency tests have been regarded as indices of left frontal lobe functioning [156]. This anatomic relation has been shown in studies of patients with brain infarcts in this region, as well as in fMRI and PET studies. However, all reports have not been in full agreement and other parts of the brain appear to also be involved. The presence of WMC in general has been shown to affect verbal fluency [66, 86, 157] but it is not known whether this effect is a general effect on attention and speed of mental processing, or a specific one, due to impaired function of the actual subcortical pathways that mediate the verbal fluency tasks.

In the present study, only periventricular WMC, and most apparent those in the left frontal lobe, predicted a poor performance on the initial part of the FAS test. Since the initial part of the test is more dependent on attention and speed of mental processing than the late phase our results could be explained by a general effect of WMC. On the other hand, since periventricular lesions in the left frontal lobe were most significantly related to the test performance, this might indicate a more specific effect of WMC, since word fluency performance is considered to mainly rely on the left frontal lobe. Previous observations [38, 73] showing that mainly periventricular changes are related to cognitive deficits are supported by our results. This might hypothetically be explained by the pattern of the subcortical neural pathways.

The significant correlation found in this study between a test considered to reflect left frontal lobe functioning and the presence of WMC in the same area is an indication that WMC might have specific effects in different brain regions and that they do not just exert a generalised effect by reducing attention and speed of mental processing. In addition, the fact that the late FAS factor did not significantly relate to any of the WMC variables can be taken as support for the hypothesis that the FAS test should be divided into an initial and a late phase. These two phases might to some extent rely on different brain regions, and maybe represent different modes of retrieval.

In the heterogeneous group of memory-disturbed patients used in the retrospective **study V** the presence of extensive WMC did not predict a poorer outcome, with respect to further decline in global cognitive performance. Only a few papers have previously been published on the prognostic significance of WMC in memory-impaired patients. Most of them included only a very limited number of patients, most of whom were without pronounced white matter pathology [93-95], or they had a very short follow up time (i.e. 1y) [90]. Among these studies, only one [93] indicated that WMC might accelerate the cognitive decline, the main finding being that MCI patients who declined into dementia had more frequent and extensive WMC. Also in our study the number of patients was somewhat limited and another drawback is the heterogeneity of the patient group. A

carefully designed, prospective study might give additional information. However, to enhance the chances of detecting a possible impact of WMC in our study we used cases with extensive white matter pathology and used a follow up ranging from 2-4 y. Despite this, the two matched groups were very similar in outcome, suggesting that the pathological process causing WMC does not affect the rate of cognitive decline in patients with dementia or mild memory disturbances.

FINAL REMARKS AND CONCLUSIONS

There seems to be a great complexity regarding both causes and effects of WMC. This might be explained by the inability of the neuroimaging techniques to separate changes of different severity as well as changes of different origin and potentially also of different significance. There might be threshold effects that confound the picture if lesions of different severity have the same appearance in the images. With MRS, Brooks et al [158] have shown that the MR proton spectra of WMC differ between symptomatic and asymptomatic patients, mainly by exhibiting lower N-acetylaspartate (NAA) peaks (a neuronal marker) in the symptomatic group. Similarly by using MTI, differences in MT ratios between WMC in demented and in non-symptomatic individuals have been shown [141, 142]. This suggests either different types or grades of damage in these two groups corresponding to the different clinical expression. There are also indications that WMC in AD might be of a different nature than changes of similar appearance in patients with cerebrovascular disease. Only the latter group seems to exhibit BBB impairment [159]. Also our observation that the extent of WMC in AD patients are influenced by APOE genotype, something that has not been found in population based studies, indicates partly different mechanisms. In the future, the new MRI applications including MRS, MTI and diffusion weighted MRI might bring new insights into the pathology of brain white matter. These techniques have the potential of revealing very subtle pathology. In addition they give quantitative measures which might allow us to separate different degrees of pathology and maybe also to depict changes with different underlying pathology as well as with different patophysiological background.

In conclusion; the present study has shown minor pathological changes in the white matter, not visible on conventional MRI. These changes represent mainly a slight myelin degradation with concomittant increased tissue water content but no reduction of the glial cell counts or the axonal network. We have also shown that there is no major general increase in BBB permeability in areas of WMC. In addition, homozygosity with regard to the APOE ε4 allele implies an increased extent of WMC in patients with Alzheimer's disease, and in AD patients carrying the ε4 allele WMC seem to be not only age related phenomena, but might be related to the aetiology of the disease. We also claim that WMC in a specific location might impair cognitive functions that rely on those particular pathways. In contrast, WMC do not seem to have any prognostic value in the dementia investigation, in predicting the rate of global cognitive decline in patients at a memory clinic.

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