Vascular Mechanisms in Dementia
with Special Reference to Folate and Fibrinolysis
Thee whom stops getting better, stops being good.

*Oliver Cromwell, 1599–1658*
Örebro Studies in Medicine 33

Nils-Olof Hagnelius

Vascular Mechanisms in Dementia
with Special Reference to Folate and Fibrinolysis
**ABSTRACT**


Örebro Studies in Medicine 33, 65 pages.

The aim of this thesis was to study the biomarker homocysteine and other novel potential vascular risk factors for dementia.

In an out-patient based study of a cohort of 926 consecutive subjects referred to our Memory Unit during 1996—2000, serum-folate was lower and total plasma homocysteine (tHcy) and serum methyl malonate were higher in subjects being prescribed with B12. In the subgroup diagnosed with dementia and with a positive family history of dementia, tHcy was higher than in the subgroup diagnosed as non-demented. It is necessary to supplement subjects with vitamin B12 deficiency with B12, but our results indicate that it is not sufficient with B12 alone because this gives rise to intracellular folate deficiency. We also found indications of a genetic component in dementia because tHcy was higher in the group with a positive family history of dementia. These findings prompted further studies of homocysteine metabolism.

The frequency of mutations in the gene for folate receptor-α (FOLR-1), and the fibrinolytic pattern in dementia and non-dementia were studied in the two cohorts DGM (n=300) and AS (n=389). The DGM cohort is a consecutive series of subjects attending our Memory Care Unit for investigation of suspected cognitive problems or dementia between 2003 - 2007. The AS (= active seniors) cohort comprises retired, apparently healthy subjects from central Sweden, actively participating in study circles. A rare haplotype in the FOLR-1, with mutations in two nearby loci, was discovered, possibly associated with lower serum-folate and higher tHcy concentrations and was more frequent in the DGM group.

The transport of folate to the CSF was studied in the DGM-cohort. Dementia with a vascular component was associated with a lower CSF to serum folate ratio indicative of reduced transport of folate to the CSF and further to the brain. The vascular endothelial derived fibrinolytic markers tPA, tPA/PAI-1-complex, and vWF were not only higher in vascular dementia (VaD) but also in Alzheimer’s Disease (AD) when compared to the AS group. The impaired fibrinolytic activity in both vascular dementia and in AD is a novel finding, signifying a vascular component in the development of dementia.

In conclusion we found that both hereditary and nutritional background factors were linked to dementia and furthermore that a dysregulated fibrinolysis was linked to both VaD and AD.

**Keywords:** dementia, Alzheimer’s disease, vascular dementia, folate, homocysteine, vitamin B12, CSF/Serum folate ratio, fibrinolysis, tPA, PAI-1.
LIST OF PUBLICATIONS

I  Hagnelius N-O, Nilsson T K, Wahlund L-O. High homocysteine and methylmalonate among demented and non-demented elderly receiving vitamin-B12 prescription and home help service
   Submitted for publication

II Böttiger A K, Hagnelius N-O, Nilsson T K. Mutations in exons 2 and 3 of the FOLR1 gene in demented and non-demented elderly subjects.

   Dement Geriatr Cogn Disord 2008;25:516-23

   Submitted for publication
### Abbreviations

<table>
<thead>
<tr>
<th>Abbreviation</th>
<th>Description</th>
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<tbody>
<tr>
<td>AchEI</td>
<td>Acetylcholine-esterase inhibitors</td>
</tr>
<tr>
<td>AD</td>
<td>Alzheimer’s disease</td>
</tr>
<tr>
<td>AS</td>
<td>Active seniors</td>
</tr>
<tr>
<td>B-CSF-B</td>
<td>Blood-Cerebrospinal fluid-Barrier</td>
</tr>
<tr>
<td>CBS</td>
<td>Cystathionine beta synthas</td>
</tr>
<tr>
<td>CNS</td>
<td>Central nervous system</td>
</tr>
<tr>
<td>CP</td>
<td>Choroid plexus</td>
</tr>
<tr>
<td>CSF</td>
<td>Cerebrospinal fluid</td>
</tr>
<tr>
<td>FR-α</td>
<td>Folate receptor alfa</td>
</tr>
<tr>
<td>Hcy</td>
<td>Homocysteine</td>
</tr>
<tr>
<td>MCI</td>
<td>Mild cognitive impairment</td>
</tr>
<tr>
<td>Mixed dementia</td>
<td>Mixed Alzheimer’s and vascular dementia</td>
</tr>
<tr>
<td>MMA</td>
<td>Methylmalonic acid</td>
</tr>
<tr>
<td>MMSE</td>
<td>Mini mental state examination</td>
</tr>
<tr>
<td>MS</td>
<td>Methionine synthas</td>
</tr>
<tr>
<td>MTHFR</td>
<td>Methylenetetrahydrofolate reductase</td>
</tr>
<tr>
<td>NMDA</td>
<td>N-methyl-D-aspartate</td>
</tr>
<tr>
<td>PAI-1</td>
<td>Plasminogen activator inhibitor</td>
</tr>
<tr>
<td>PCFT</td>
<td>Proton-coupled folate transporter</td>
</tr>
<tr>
<td>RCF</td>
<td>Folate CSF/Serum gradient</td>
</tr>
<tr>
<td>RFC</td>
<td>Reduced folate carrier</td>
</tr>
<tr>
<td>SAM</td>
<td>S-adenosyl methionine</td>
</tr>
<tr>
<td>tHcy</td>
<td>Total plasma homocysteine</td>
</tr>
<tr>
<td>tPA</td>
<td>Tissue plasminogen activator</td>
</tr>
<tr>
<td>VaD</td>
<td>Vascular dementia</td>
</tr>
<tr>
<td>vWF</td>
<td>Von Willebrand factor</td>
</tr>
</tbody>
</table>
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ORIGINAL PAPERS
Preamble

When I was first introduced to the world of cognitive and dementia problems in the late 1980’s, there were no specific therapeutic agents available. But at that time there was a growing interest in deficiency of first and foremost vitamin B12 in the nervous system. Addison [1] already in the mid 19th century had described that the mind occasionally wandered in patients with pernicious anemia. In the mid 1990’s, I decided to establish a database to monitor the quality of our medical service in the Memory Unit of the Department of Geriatrics.

When analyzing that database my interest in B-vitamins and the new marker, homocysteine, really increased. This also meant that folate, the “poor relative”, gradually became the vitamin in focus, of course together with vitamin B12.

This thesis, and the work behind it, has its origin in this context.
INTRODUCTION

In the first century A.D. the Roman encyclopedist and physician Celsus made one of the first descriptions of dementia. Dementia is derived from the Latin de- for “apart, away” and mens for “mind” (genitive mentis). Dementia is thus an old condition, but it has come more in focus the last decades. There are, however, no data indicating that the incidence rate per age-group has risen [2]. Since the population gets older the dementia diseases today affects growing numbers of the elderly. Dementia is one of the most common diseases in aged people and the prevalence of dementia doubles almost every 5-year cohort [3]. If it was possible to delay the onset of AD with 5 years, then the prevalence of AD would be reduced with 50% [4].

Dementia Background

Epidemiology

During the last 5 decades the average life expectancy has grown dramatically [5]. Prognosis from United Nations predict a continued substantial rise until at least 2050. The population pyramid is no longer broadest at the base but at the top.


The main dementia risk factor is old age [6]. Therefore, dementia diseases have become a curse to both individuals and to the society because of their enormous
expenses. The total cost-of-illness for the dementia diseases to society is substantially greater than the collective cost for all cancer disease and all cardiovascular disease, including stroke, together, see Figure 2 [7]. Interestingly a Spanish paper found that VaD causes an even greater indirect costs than AD [8]. Dementia diseases have also become one of the great causes of death in the US [9]. One man in 6, and almost 1 woman in 3, will suffer from dementia during their lifetime [10].

**Figure 2.** Data from a paper concerning the cost of AD in the UK [7].

**Etiology**

The etiological factors of AD are not clearly understood, although hypotheses have included genetics and environmental factors. ApoE ε4 is considered a major susceptibility marker for AD. It is associated with an earlier age of AD onset and increased amyloid plaque load [11]. AD is in the broad majority of cases spontaneous, but mutations in presenilins and in amyloid precursor protein may together explain approximately up to 5% of all cases. In spite their rarity, these conditions are of utmost importance since they can serve as model to pathophysiologic understanding of the disease.

Environmental factors that might play a role include: toxins like aluminum, infectious agents like syphilis or Creutzfeld Jacobs disease, head trauma,
malnutrition, B-vitamin deficiency, a stressful life and oxidative stress.

Table 1 lists a wide range of risk factors with literature references. Definite risk factors (scientifically proven to be causal) are: age, ApoE ε4 allele and family history of dementia.

### Table 1. Risk factors for dementia.

<table>
<thead>
<tr>
<th>Risk Factor</th>
<th>Results from the literature</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age</td>
<td>Old age is the most important dementia risk factor</td>
<td>[6, 12]</td>
</tr>
<tr>
<td>Alcohol</td>
<td>J-shaped relationships are reported, and wine may be better than spirits</td>
<td>[13-15]</td>
</tr>
<tr>
<td>Aluminum</td>
<td>Al may be associated with development of AD</td>
<td>[16, 17]</td>
</tr>
<tr>
<td>ApoE ε4 allele</td>
<td>ApoE ε4 increases risk of AD, after age the most important risk factor for AD</td>
<td>[12, 18]</td>
</tr>
<tr>
<td>Atrial fibrillation</td>
<td>Clear association, slightly more pronounced for AD than VaD</td>
<td>[19]</td>
</tr>
<tr>
<td>BMI</td>
<td>BMI over 25 may protect against developing dementia</td>
<td>[20]</td>
</tr>
<tr>
<td>Cholesterol</td>
<td>High serum cholesterol in midlife increases risk of AD in later life</td>
<td>[21, 22]</td>
</tr>
<tr>
<td>Diabetes mellitus</td>
<td>DM is an independent risk factor for post stroke dementia</td>
<td>[23]</td>
</tr>
<tr>
<td>Family history of dementia</td>
<td>History of AD in the family is linked to higher risk</td>
<td>[12]</td>
</tr>
<tr>
<td>Homocysteine</td>
<td>Homocysteine is an independent risk factor for dementia and Alzheimer’s disease</td>
<td>[24-27]</td>
</tr>
<tr>
<td>Sex</td>
<td>Female sex implicates higher risk</td>
<td>[28]</td>
</tr>
<tr>
<td>Smoking</td>
<td>Smoking is associated with increased dementia risk especially in ApoE4 non-carriers</td>
<td>[14, 29]</td>
</tr>
<tr>
<td>Systolic BP</td>
<td>High blood pressure in middle aged is associated with increased risk of dementia in old age</td>
<td>[21, 30]</td>
</tr>
<tr>
<td>Social network</td>
<td>Frequent social network contact seems to be a dementia protective factor</td>
<td>[31]</td>
</tr>
</tbody>
</table>

Furthermore, there are also some factors suggested as protective against dementia. To live an active life, long education, daily exercise, *a glass of red wine/day* [14] and a good social network with many contacts helps to keep your mind better preserved.
**Diagnostic Procedures**

The diagnosis of dementia is based on the overall appraisal of medical history, clinical examination, including neuropsychological assessment, and the result from biochemical assays and neuroimaging. Unfortunately, there are no specific biological markers available that could exclude the element of clinical judgment.

Dementia is a term for a non-specific disease syndrome (set of symptoms) which could be caused by many different disease processes or trauma. Dementia is an acquired protracted (> 6 months according to ICD-10) significant deterioration in cognitive capacities. Memory disturbance is obligate, deterioration in language, attention, thinking and problem solving, and executive problems are the most prominent areas of symptoms. The degree of difficulties should interfere with daily social activities and represent a significant deterioration compared to premorbid level.

The dementias are usually slowly progressive disorders. They are caused by serious acquired lesions to the brain. Above all, dementia is a geriatric condition, but people at any age could suffer from it.

**Primary Degenerative Dementias**

Dementia that does not result from other disease is classified as primary degenerative dementia. Examples of these conditions include AD, frontotemporal dementia and Lewy body dementia.

AD is named after the German neuropathologist and psychiatrist, Alois Alzheimer, who in November 4, 1906, at a psychiatric meeting in Tübingen in Germany, for the first time presented a case, Auguste Deter [32-34]. She was around 50 years old when she first began to suffer from progressive cognitive impairment, hallucinations, delusions, and psychosocial incompetence. She died in Frankfurt on the 8th of April 1906, 56 years old. In 1910, Professor Emil Kraepelin named the condition Alzheimer’s disease.

Kraepelin denoted the disease as “presenile” dementia since Auguste Deter was well below 65 years of age, and although Oscar Fisher already in 1907 had reported 12 cases with “senile” dementia, it was not unusual to recognize also elderly people with dementia as AD [35]. Since the late 1970’s the Alzheimer type of dementia is called AD, also in people older than 65 years [36].

In 1912 a colleague of Alois Alzheimer, Frederick Lewy, described a neuropathological inclusion body in neurons in the substantia nigra, the Lewy body. Dementia with Lewy bodies is considered one of the more frequent primary degenerative dementias after AD and frontotemporal dementia.

AD is usually considered to be the most common of all dementia disorders.
Approximately 60% of all dementia disease can be assigned as AD. There are no exact figures, but in Sweden around 100,000 people suffer from AD, based on epidemiological data. On average, patients with AD live for about 10 years from diagnosis. However, some people live as long as 20 years or more after diagnosis, and others suffer from a more aggressive form, living less than 5 years from diagnosis.

**Secondary Dementias**

Dementia that occurs as a result of another physical disease or injury is classified as secondary dementia. The most common of these disorders is vascular dementia (VaD). It accounts for approximately 20% of all dementias.

VaD usually has its origin in atherosclerotic changes of the vessels inside or outside the brain but it can also be caused by severe vascular inflammation or result from very low blood pressure. Alois Alzheimer in his first case report described that Auguste Deter had suffered from atherosclerotic changes in the larger vascular tissues [32].

Among the secondary dementias we can find diseases that are potentially curable, eg some intracranial tumors or normal pressure hydrocephalus.

Secondary dementias could lead to development of dementia through different mechanisms: space occupation (eg tumors); indirect influence on the brain (eg toxic or metabolic), and direct influence on the brain (eg vascular or infectious).

In recent years it has been argued that AD is perhaps not the most common dementia disease, but rather a combination of both AD and VaD has come to take the role of the most frequent dementia disease [37]. Figure 3 gives a clue about the association between AD and VaD. Several papers have argued that traditional vascular risk factors also are risk factors for AD [38-40]. However, a recent review does not support the influence of traditional vascular risk factors on AD with the exception of exercise and physical function, APOE e4, diabetes, and cholesterol [41].
Figure 3. Balance between Alzheimer disease (AD) and cerebrovascular disease and the mapping of clinical diagnoses on the pathologic findings. Diagnostic labels at the bottom of the graph depict current common use. Diagnostic areas are not drawn to the scale of the relative prevalence of the disorders. From Knopman DS. Dementia and cerebrovascular disease. Mayo Clin Proc. 2006;81(2):223-230. Used with permission.

Comorbidity

Since dementia is primarily a condition of old age it is of course accompanied by other usual geriatric conditions eg ischemic heart disease, renal failure, cancer, stroke and osteoporosis. Depression is also common in older people. Especially the early stages of AD may be accompanied by depression, but it could be present in any stage and any kind of dementia disease.

Treatment

Today, there are two different types of pharmacological symptomatic treatments of AD. The first one, AchEI, was registered in Swedish market 1995. AchEI’s are indicated when the disease is mild to moderate and give a small relief through supporting the cholinergic transmitter system. The other kind of symptomatic drug is the N-methyl-D-aspartate (NMDA) receptor antagonist, aimed to be used in the moderate to severe phase of AD.

There are several ongoing clinical trials were new therapeutical strategies are
tested. One of the most interesting points of action is active or passive immunization against amyloid plaques. This concept is attractive and new variants of immunogenic active parts of the \(\beta\)-amyloid molecule have been developed, besides antibodies for passive immunization. The first trial with vaccination against AD was interrupted due to severe adverse events, in particular encephalitis [42]. However, up to April 11 2009 on PubMed, not any truly positive results were reported from this kind of trials.

Non-pharmacological treatment is also reported to give some positive results, eg cognitive stimulation made the intervention group significantly better, the effect was comparable to the effect of AchEI’s and NMDA-receptor antagonists [43].

To be able to discover more and better treatments, we need more knowledge about the mechanisms that initiate and propel the various forms of dementia.

**Mechanisms**

The basic mechanisms behind AD and other primary degenerative dementias are not known.

Malnutrition leading to deficiency of macro- and micronutrients has been recognized as the cause of dementia in some cases [44], eg B-vitamin deficiency. Atrophic gastritis, a usual condition in the elderly, may cause vitamin B12 deficiency due to malabsorption. Deficiency of B-vitamins could also be due to side effects of pharmacotherapy, eg methotrexate treatment of rheumatoid arthritis or psoriasis.

Inflammation in the CNS is a possible pathway for dementia to develop. In brain specimens from newly diseased AD patients there are high concentration of interleukin IL-6, but also of acute phase reactants like CRP and \(\alpha\)-1-antichymotrypsin [45]. There has been a dispute between scientists whether the inflammatory processes is present in AD brain to remove detritus of already existing damage or whether it is a primary processes. As in many common peripheral inflammatory disorders (eg, asthma, arthritis), the AD brain provides numerous opportunities for chronic inflammation to do more damage than the primary pathologic events that induced it.

Vascular mechanisms are by some scientists regarded as risk factors not only for VaD but also for AD [37-39, 46]. Risk factors that VaD and AD share include age, atherosclerosis, stroke and TIA, diabetes mellitus, smoking, ApoE \(\varepsilon\)4 and raised homocysteine [47]. A recent paper found an association between atrial fibrillation, hypertension, and angina with greater decline in AD, while the use of antihypertensive medication, CABG and diabetes mellitus were associated with
less decline [48]. Small vessel dementia is probably caused by changes in the small penetrating end arteries in the depth of the brain, these changes probably relate to eg hypertension [49]. Large vessel dementia is caused by infraction (or bleeding) in the large cortical vessels.

In recent years, total plasma homocysteine (tHcy) has been proposed as an independent risk factor for dementia and AD [27, 50, 51]. Hemostasis has been proposed as a possible pathogenetic factor in dementia, especially vascular dementia [52-54].

The Blood Brain Barriers
The CNS is protected from harmful influence in several ways. A part of this protection is constituted from special kinds of blood vessels with a high density of tight junctions. Generally we refer to these special vessels as the blood brain barrier (BBB). The transport of nutrients to the neurons and other cells in the brain has to manage the BBB. Some micronutrient utilizes active transportation eg folate and vitamin C. Folate transport occurs in the choroid plexus, a small tissue in the brain ventricles that also produces the vast majority (~ 90%) of cerebrospinal fluid (CSF). This way is the only route to support the brain with folate and vitamin C. The function of CSF is also to reduce the brain weight, protect from impinge, to supply the brain with nutrients, and to remove products from brain metabolism.

There are three main compartments in the central nervous system, blood, CSF, and brain tissue. These compartments are separated by four barriers, see Figure 4. Failure to the integrity of BBB occurs in cerebral vascular disease such as stroke and might be of importance in the development of dementia disease, especially VaD.
Figure 4. The brain barriers can be divided into four principal interfaces: 1) the endothelium of the cerebral blood vessels (blood-brain barrier), 2) the epithelium of choroid plexus tissue in the brain ventricles (blood-CSF barrier), 3) the meningeal barrier (a second blood-CSF barrier), and 4) the ependymal lining of the ventricles (CSF-brain barrier). Picture with kind permission from C.J Ek.
Homocysteine is a non-proteic sulphydryl amino acid derived from the metabolic conversion of methionine. It was first described in 1932 by Butz and du Vigneaud [55] as an intermediary sulfur metabolite in the methionine cycle.

Homocysteine is metabolized through two pathways: remethylation to the essential amino acid methionine or transsulfuration to the sulfur amino acid cysteine (Figure 6). In 1962 two research groups independently of each other, discovered an inborn error of homocysteine metabolism in mentally retarded children, homocystinuria [56, 57]. The clinical characteristics of these children included thrombembolic disease, osteoporosis, mental retardation, and ocular lens dislocation. These children had very high plasma homocysteine levels. In 1964 S Harvey Mudd described the most common cause of homocystinuria, mutation in cystathionine β synthase (CBS), i.e. in the transsulfuration pathway [58]. The birth prevalence of this mutation is approximately 1:10000.

Extremely high tHcy levels (>150 μmol/L) has also be seen in children with the missense mutation 1129C>T in MTHFR gene, representing the other part of the homocysteine metabolism, the remethylation pathway. A case report has showed that supplementation with betaine has clinically significant effect [59]. A common polymorphism in the gene for MTHFR, 677C>T, causing a thermolabile form of the enzyme with a slightly reduce enzyme activity. In case of good folate intake, the 677C>T variant will not give higher homocysteine concentrations, while a sparse intake results in a moderate rise in the homocysteine concentration. The prevalence of MTHFR 677C>T polymorphism varies widely between countries and regions; it is about 8% in Sweden while it is around 20% in Italy and up to 38% in French Canadians [60].

In 1969 the American pathologist Kilmer McCully developed a theory that homocysteine is deleterious to the vascular system and possibly a marker of atherosclerosis [61]. The theory was further developed by McCully in a paper 1975 [62]. In 1976, Wilcken [63] observed elevated homocysteine levels after methionine loading in 7 out of 25 patients with coronary artery disease (CAD) and concluded that persons with premature onset CAD have reduced ability to metabolize homocysteine. The association between renal failure and homocysteine was first described by Wilcken in 1979 [64].

Most of homocysteine in plasma is bound to albumin or covalently bound to the amino acid cysteine (Figure 5). Only a small fraction exists that is free and unbound. It is easy to be confused by the names homocysteine and homocystin. Homocystin is constituted of two covalently coupled homocysteine molecules.
Homocystin is the molecule excreted in the urine of subjects with homocystinuria.

Methylmalonic acid (MMA) is widely regarded as a sensitive and specific biomarker of intracellular vitamin B12 deficiency. MMA serum concentration rises in vitamin B12 deficiency, in renal impairment and in rare inborn errors affecting methylmalonate-CoA mutase activity. However, it has not received the same attention as homocysteine, probably owing to the fact that it has not been as strongly associated with atherosclerosis or cognitive decline as homocysteine has been. Hvas et al [65] in a Danish study found that P-MMA does not predict clinical manifestations related to vitamin B12 deficiency. They concluded that MMA could be challenged as a clinical biomarker of B12 deficiency.

Lars Brattström et al for the first time described an association between moderate hyperhomocysteinemia and ischemic stroke in 1984 [66]. There is unequivocal epidemiologic evidence that elevated tHcy both predicts and precedes cardiovascular disease.

In the extensive Norwegian homocysteine study, the Hordaland homocysteine study, homocysteine was found to be a strong predictor of both cardiovascular and non-cardiovascular mortality in a general elderly population [67].
One-Carbon Metabolism

The one carbon cycle is an important metabolic cycle. Methyl groups (\(-\text{CH}_3\)), one carbon groups, are essential in the synthesis of many substances in the body. In the brain, S-adenosylmethionine (SAM), is the sole methyl-donor for many important reactions involving neurotransmitters, phospholipids for cellular membranes, myelin assembly, nucleoproteins and proteins.

Methyl groups are also of utmost importance in methylation of DNA in order to regulate gene expression. A simple example to illustrate this is the acute phase protein C-reactive protein (CRP), which rises rapidly and dramatically as a response to inflammation. However, the gene for CRP is always present, but the expression is regulated by the inflammatory state in the body and gene methylation.

![Diagram of One-Carbon Metabolism](image)

Figure 6. *The one carbon metabolism.* The remethylation pathway is represented by MS = methionine synthase and the transsulfuration pathway is indicated by CBS= cystathionine \(\beta\) synthase. 2= methyltransferases, 3= Methylene-THF-reductase (MTHFR)

MS is blocked by the anesthetic gas nitrous oxide. Thus extensive abuse of nitrous oxide can cause neurological lesions similar to that seen in vitamin B12 deficiency [68].

Betaine Pathway

Substitution with betaine has a potential to reduce tHcy, even extremely high tHcy concentrations, for example in inborn errors of metabolism. It is not known whether it can reduce the risk of cardiovascular disease [69]

However, the enzyme *betaine homocysteine methyl transferase* (BHMT) is not expressed in the CNS, so this metabolic pathway is not available in the CNS. Furthermore, the other metabolic pathway able to clear homocysteine from the tissues, the transsulfuration pathway, is very strongly reduced in the brain [70]. It appears that only *Cystathionine beta synthas* (CBS) is expressed in the human...
brain. The brain is thus completely dependent on the *methionine synthase* remethylation, a vitamin B12 (and folate) dependent enzyme.

**Causes of Hyperhomocysteinemia**

Elevation in S-creatinine concentration is, apart from vitamin and/or enzyme deficiency, the most common cause of an increased homocysteine concentration. Hypothyreoidism is also linked to hyperhomocysteinemia [71].

There are several other determinants of the plasma tHcy concentration (Table 2a).

**Table 2a.** Homocysteine determinants.

<table>
<thead>
<tr>
<th>Determinants</th>
<th>Effect on Hcy concentration</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Genetic factors</strong></td>
<td></td>
</tr>
<tr>
<td>CBS homzygoity</td>
<td>†††</td>
</tr>
<tr>
<td>Thermolabile MTHFR</td>
<td>†</td>
</tr>
<tr>
<td><strong>Physiologic factors</strong></td>
<td></td>
</tr>
<tr>
<td>Increasing age</td>
<td>(†)</td>
</tr>
<tr>
<td>Male sex</td>
<td>(†)</td>
</tr>
<tr>
<td>Increased muscle mass</td>
<td>(†)</td>
</tr>
<tr>
<td><strong>Lifestyle factors</strong></td>
<td></td>
</tr>
<tr>
<td>Smoking</td>
<td>(†)</td>
</tr>
<tr>
<td>Coffee consumption</td>
<td>(†)</td>
</tr>
<tr>
<td>Ethanol consumption</td>
<td>††</td>
</tr>
<tr>
<td>Physical activity</td>
<td>†</td>
</tr>
<tr>
<td><strong>Clinical conditions</strong></td>
<td></td>
</tr>
<tr>
<td>Folate deficiency</td>
<td>††</td>
</tr>
<tr>
<td>Vitamin B12 deficiency</td>
<td>†††</td>
</tr>
<tr>
<td>Vitamin B6 deficiency</td>
<td>†</td>
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<tr>
<td>Renal failure</td>
<td>††</td>
</tr>
<tr>
<td>Hyperproliferative disorders</td>
<td>†</td>
</tr>
<tr>
<td>Hypothyreoidism</td>
<td>†</td>
</tr>
</tbody>
</table>


† = Reduction of tHcy concentration, (†) = increase within normal reference range, ††, †††, †††† = moderate hyperhomocysteinemia (15-30μM), intermediate hyperhomocysteinemia (30-100μM), and severe hyperhomocysteinemia (>100μM), respectively.
Several drugs can also influence the tHcy (Table 2b).

**Table 2b. Drug influence on tHcy.**

<table>
<thead>
<tr>
<th>Drug class/Drug</th>
<th>Effect on Hcy concentration</th>
<th>Mechanism of effect</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Lipid lowering drugs</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Fibrates</td>
<td>Increases</td>
<td>Renal impairment, altered creatinine metabolism</td>
</tr>
<tr>
<td>Nicotinic acid</td>
<td>Increases</td>
<td>Inhibition of pyridoxal kinase, decreased vitamin B6 levels, decreased activity of CBS</td>
</tr>
<tr>
<td>Cholestyramin</td>
<td>Increases</td>
<td>Interference folate absorption</td>
</tr>
<tr>
<td>Simvastatin</td>
<td>Decrease</td>
<td>Not known</td>
</tr>
<tr>
<td><strong>Diabetes drugs</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Metformin</td>
<td>Increases</td>
<td>Decreased intrinsic factor secretion, binding of free calcium in the gut, possible folate lowering effect</td>
</tr>
<tr>
<td>Insulin</td>
<td>Decreases</td>
<td>Increased activity of MTHFR, decreased activity of CBS</td>
</tr>
<tr>
<td><strong>Sex hormones</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Estrogens</td>
<td>Decreases</td>
<td>Mechanism not clear</td>
</tr>
<tr>
<td>Androgens/testosterone</td>
<td>Increases</td>
<td>Increased creatinine synthesis, differences in steroid balance</td>
</tr>
<tr>
<td><strong>Anti-estrogens</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Tamoxifen/raloxifene</td>
<td>Decreases</td>
<td>Modest elevation in folate levels, estrogen receptor mediated?</td>
</tr>
<tr>
<td><strong>Anti-rheumatic drugs</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Methotrexate</td>
<td>Increases</td>
<td>Inhibition of dihydrofolate reductase</td>
</tr>
<tr>
<td>Sulfasalazine</td>
<td>Small non-sustained acute rise</td>
<td>Mechanism not clear</td>
</tr>
<tr>
<td><strong>Anti-epileptic drugs</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Phenytoin</td>
<td>Increases</td>
<td>Folate depletion, possible decreased activity of 5-MTHFR, decreased activity of methionine synthase, hepatic enzyme induction</td>
</tr>
<tr>
<td>Carbamazepine</td>
<td>Increases</td>
<td>Folate depletion, possible hepatic enzyme induction</td>
</tr>
<tr>
<td>Valproic acid</td>
<td>Non-significant effect</td>
<td></td>
</tr>
</tbody>
</table>
### Other drugs

<table>
<thead>
<tr>
<th>Drug</th>
<th>Effect</th>
<th>Impact</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cyclosporin</td>
<td>Increases</td>
<td>Interference with renal function, possible interference in folate dependent remethylation</td>
</tr>
<tr>
<td>Theophylline</td>
<td>Increases</td>
<td>Pyridoxal kinase inhibition</td>
</tr>
<tr>
<td>Levodopa</td>
<td>Increases</td>
<td>Acts as a substrate for S-adenosylmethionine (SAM) dependent transmethylation</td>
</tr>
<tr>
<td>Acetylcysteine</td>
<td>Decreases</td>
<td>Thiol-disulfide exchange, lower plasma protein binding</td>
</tr>
<tr>
<td>Alcohol</td>
<td>Increases</td>
<td>Possible depletion of folate</td>
</tr>
<tr>
<td>Trimetoprim</td>
<td>Increases</td>
<td>Inhibition of dihydrofolate reductase</td>
</tr>
<tr>
<td>Nitrous oxide</td>
<td>Increases</td>
<td>Inactivation of methionine synthase</td>
</tr>
<tr>
<td>Betaine</td>
<td>Decreases</td>
<td>Enhanced remethylation</td>
</tr>
</tbody>
</table>

In diabetes mellitus there is an association with hyperhomocysteinemia, most likely due to impaired renal function since eg insulin if anything decreases homocysteine [72].

Cigarette smoking interfere with pyridoxal phosphate giving the smokers significantly lower vitamin B6 concentrations than non-smokers [73].

**Homocysteine: Therapeutical Trials and Vascular Disease**

In the US and Canada folate fortification of grain products is mandatory since 1998 and has coincided with a lower mortality of cerebrovascular lesions when comparing 1990 with 2002. In the UK, were folate fortification of grain products not was carried out, no reduction in mortality has been seen [74].

The VISP study [75] included 3680 stroke survivors with elevated tHcy. No significant effect was found on recurrent stroke, on ischemic heart disease, and there were no reduction in mortality rate in the two year long study. Supplementation with vitamin B12, folate, and vitamin B6 did however reduce homocysteine levels with approximately 25%. There was, however, a tendency towards lower mortality in the treatment group. If the study duration had been longer it probably would have shown significant difference between the two study groups, most likely favoring the high dose arm.

In the NORVIT study [76] 3749 patients, who survived myocardial infarction and who had hyperhomocysteinemia, were treated in four arms with different combinations of folate, vitamin B12, and vitamin B6 for three years. Homocysteine were effectively lowered (approximately 25%, depending on the supplement combination) but there were no significant effect on mortality or on
re-infarction compared with the control group. The arm with high doses of folate, vitamin B12 and B6 instead had a trend towards higher mortality, however not significant.

The HOPE-2 study [77] comprised 5522 patients with vascular disease/diabetes mellitus who were treated with folate (2.5 mg), vitamin B12 (1 mg) and B6 (50 mg) and were followed up for five years. Homocysteine levels were reduced approximately 27%. The odds ratio (OR) for cardiovascular event was 0.94 (95% CI: 0.82–1.07) but it appeared to reduce stroke event: OR 0.75 (95% CI: 0.58–0.96).

**Homocysteine and Dementia**

In 1983 Goodwin et al [78] for the first time described epidemiological evidence linking low vitamin B12 and folate status in blood with decline in neurocognitive function in the elderly. Homocysteine was, however, not analyzed and discussed in their paper. Lindenbaum et al linked hyperhomocysteinemia, vitamin B12 deficiency and neuropsychiatric symptoms in 1988 [79]. Karin Nilsson et al [80] and Andrew McCaddon et al [50] were also early in linking hyperhomocysteinemia and dementia in their case-control studies. In 1998 Clarke et al [81] found that PAD confirmed AD cases, after adjustment for age, sex, social class, smoking and Apo E ε4 allele, were associated with low levels of the vitamins folate and B12 and elevated serum homocysteine concentrations. They concluded that the homocysteine level was stable over time and it had a lack of relationship with disease duration, suggesting that the findings of higher serum homocysteine concentration hardly could be a consequence of the disease. Thus, 1998 is a memorable year in the dementia – homocysteine relationship context.

In a prospective study based on 1092 non-demented subjects from the Framingham Cohort, Seshadri et al found that cognitively intact persons with the highest baseline quartile of homocysteine (>14 μmol/L), 8 years later almost doubled their risk of developing dementia or AD [27]. Adjustments were made for age, sex, ApoE genotype, vascular risk factors other than homocysteine (smoking, alcohol, diabetes mellitus, systolic blood pressure and BMI), and plasma levels of the B-vitamins folate, B12, and B6. They concluded that hyperhomocysteinemia is an independent risk factor for the development of dementia and AD. Ravaglia et al [82] in 2003 reported similar findings as Seshadri.

In a newly published prospective study in the US, Haan et al [24], found significant association between elevated plasma homocysteine and cognitive impairment without dementia (CIND) or dementia with a Hazard Ratio 2.39
The literature contain different opinions in that homocysteine itself can be neurotoxic and that the neurotoxicity can also be related to reactive oxygen species, eg homocysteic acid which is well known to induce neuronal death when interacting with the NMDA-receptors [83].

**Homocysteine: Therapeutical Trials and Dementia**

Today there are, to my knowledge, only two prospective randomized controlled studies that have investigated the effect of B-vitamin supplementation in AD, both of them rather short in the context of dementia.

The first one is the Taiwanese study by Sun et al from 2007, a 26 week double-blind prospective trial of 89 mild to moderate AD patients (45 men, 44 women) with normal vitamin B12, folate and tHcy levels. All patients were receiving AchEI treatment. The subjects were randomized to either supplementation with vitamin B12 (500 μg), vitamin B6 (5 mg), folic acid (1 mg), other vitamins, and iron or to placebo. No statistically significant nor any clinically significant beneficial treatment effects on cognition (measured with ADAS-Cog/11) or ADL function were found between the groups after 26 weeks, but tHcy was significantly reduced by approximately 25% [84].

The second study was presented in late 2008 [85]. This randomized double-blind, placebo controlled multicenter study enrolled 409 participants with mild to moderate AD with normal folic acid, vitamin B12 and tHcy levels; 340 completed the study (202 in active treatment group and 138 in placebo group). During the 18-month long trial period the tHcy level was significantly reduced in the active treatment group (26%) but there were no significant reduction on cognitional decline, as measured with ADAS-Cog. In the placebo group, tHcy was reduced with 9%. Furthermore, the active treatment group had a higher quantity of adverse events, including depression and blurred vision.

Thus, both these studies were performed on patients with no signs of B-vitamin deficiency, they had all normal tHcy, folate and B12 levels at the start of respective study. Therefore, it is not surprising that cognition did not improve from vitamin supplementation.
**Folate**

Folate plays a crucial role in purine synthesis, in monoamine synthesis as well as methyl group donor in methylation of eg DNA in gene regulating reactions and is intimately related with vitamin B12.

Folate was first discovered by the British pathologist Lucy Wills in 1931 [86]. Folic acid was isolated from spinach in 1941 and then received its name (folium is the Latin name for leaf). Folic acid was from 1946 available in its crystalline form.

Around 1950 it was found that folate supplementation made prognosis worse for children with acute lymphatic leukemia. This observation initiated the development of anti-folates. Anti-folates like methotrexate and trimethoprim could cause a rise in plasma homocysteine. Methotrexate is a potent cytostatic agent and it also possesses anti-inflammatory and immunosuppressive properties.

Folate deficiency occurs at all ages and is usually a result of poor diet, alcoholism, malabsorption or the use of certain drugs eg methotrexate, anti-epileptics or trimethoprim.

The methods for folate assay have changed over the last decades. The first method was a microbiological assay using Lactobacillus casei, measuring all possible forms of folate. In a comparison this method showed to give higher values of folate concentration than the newer radio assay method [87]. Assaying Erc-folate is not considered to be the golden standard today since serum folate reflects the intra cellular folate in a better way. In this thesis the folate assays have been performed with the radio assay method.

**CSF-Folate**

The concentration of folate in the cerebrospinal fluid is approximately 2-4 times higher than in serum [88-90]. This relationship remains constant in the presence of serum folate deficiency [91]. To achieve this ratio, folate is actively transported from blood to CSF via folate receptors in the choroid plexus. Folate Receptor-α (FR-α) and the recently described Proton-Coupled Folate Transporter (PCFT) seems to work in tandem and thus both are required to maintain the CSF/Serum folate gradient [92].

**Methyl-Folate Trap**

The methyl folate trap theory, originally formulated by Herbert in 1962 [93], explained the biological protection to methyl group deficiency in kwashiorkor (eg methionine deficiency). A more common problem in the western world is vitamin
B12 deficiency. The folate trap theory explains why vitamin B12 deficiency often results in a functional folate deficiency status, through trapping of 5-THF and thus not letting it be further metabolized in eg the purin synthesis. In a situation with vitamin B12 deficiency, administration of folic acid, which induces cell division and the use of methionine in protein synthesis, impairs methylation of myelin and precipitates or exacerbates subacute combined degeneration of the spinal chord (SCD)

**Folate Receptors**

Folates enter the cells through three carrier mediated transport systems. *Reduced Folate Carrier (RFC)* is a classic facilitated transport protein with micromolar (μM) affinity that transports reduced folates across the cell membrane. The RFC is found in virtually all cells and exists in both soluble and membrane bound forms. Some cells, especially choroid plexus epithelial cells, also express the FR-α which exhibits pM affinity for folic acid. The glycosylphosphatidylinositol (GPI) anchored FR-α actively transports folates from blood to the CSF in a process called endocytosis [94]. The third kind of folate transporter is the recently discovered proton-coupled folate transporter (PCFT) [95]. One function of PCFT is to maintain the CSF folate gradient in a tandem like reaction where FR-α is the other part [92].

**Vitamin B12**

In the mid 19th century the British physician Thomas Addison for the first time described an anemia associated with neurological symptoms – later this disease was named ‘pernicious anemia’. The disease remained incurable until 1926 when Minot and Murphy described that a special diet of liver, meat, and vegetables cured 45 patients with pernicious anemia [96]. Rickes et al [97] in 1948 isolated vitamin B12 from liver extract. In the early 1950's, vitamin B12 was produced industrially and it became possible to treat vitamin B12 deficiency in the general public.

Vitamin B12 is essential for the one-carbon metabolism and works tightly together with folate to accomplish remethylation of homocysteine to methionine via methionine synthase, were B12 acts as coenzyme.

Symptoms of vitamin B12 deficiency are typically revealed by tissues with great cell turnover, like bone marrow, and megaloblastic anemia is one of the key signs. This is attributable to diminished DNA biosynthesis. The folate trap provides a solution for this course of events [98]. There is overwhelming documentation that neurological signs could present well before the hematological signs [99]. Vitamin
B12 is also necessary for the formation of eg myelin and neurotransmitters [100].

Mammalian cells contain two vitamin B12 dependent enzymes, L-methylmalonyl-CoA mutase and methionine synthase (MS). Although the consequence of methylmalonic acid accumulation itself in vitamin B12 deficiency is not exactly known, most of the sequele of vitamin B12 deficiency are attributable to inhibition of MS. However, the accumulation of MMA is specific marker of vitamin B12 deficiency.

Vitamin B12 deficiency is most often observed in the elderly (prevalence ~10-15%). It is most often attributable to malabsorption caused by lack of intrinsic factor, gastric atrophy or disease in terminal ileum. The use of proton pump inhibitors have been described as a potential problem to absorb vitamin B12.

**CSF-B12**

Vitamin B12 is transported to the brain, at least partly, by transcobalamin II. The concentration of vitamin B12 in serum is about 10 — 30 times higher than that in CSF. In 1992 Björn Regland et al [101] published a paper with an interesting concept of comparing CSF/serum ratio of vitamin B12 as a marker for the transport of vitamin B12 from serum to CSF. A group of demented men with subcortical symptoms had lower ratio than AD or the control group. The lower ratio was interpreted as an indication of an impaired transport function for vitamin B12.

**Vitamin B6**

Vitamin B6 was discovered by the Hungarian physician Paul György [102]. The enzyme Cystathionine β synthase (CBS) is the doorkeeper in the crossroads of the one-carbon metabolism between the remethylation pathway and the transsulfuration pathway. CBS has pyridoxal-5’-phosphate (derived from vitamin B6) as coenzyme, as it also is in all transamination reactions.

Sulfur is an essential element in the chemistry of life. It is necessary for the synthesis of the amino acids methionine and cysteine. In the inborn error of metabolism called homocystinuria, there is an impaired function of CBS. Some of the cases with homocystinuria can be cured from their hyper-homocysteinemia with supplementation of vitamin B6, but the main symptoms still remain. In persons with normal CBS activity it does not seem to be necessary to give supplement with vitamin B6 — plasma homocysteine is not affected at all.
Fibrinolysis in Health and Disease

It is necessary for the preservation of life that the blood can coagulate when there is an injury to the blood vessel tree. Furthermore, a badly regulated coagulating system could be dangerous as it can result in thrombosis. The fibrinolytic system is responsible for the degradation and removal of fibrin clots, thereby assuring a satisfying circulation in vessels potentially obstructed. The fibrinolytic system counteracts the coagulation system in a fine-tuned balance act. Fibrinolysis is the process where the fibrous blood clotting protein fibrin, also called Factor Ia, is degraded to soluble products via the activated form of plasmin. The fibrinolytic system is of crucial importance to manage the balance between coagulation and bleeding of the blood. An activation of the fibrinolytic system is seen in atherosclerotic diseases like stroke and myocardial infarction. Impaired fibrinolysis in atherosclerosis is mainly due to an increased release of Plasminogen Activator Inhibitors (PAI) from activated platelets. It could also be due to an insufficient activity of tissue Plasminogen Activator (tPA), or a change of the structure of fibrin which makes atherosclerotic clots less susceptible to fibrinolysis [103]. In a Swedish population sample from Västerbotten county, aged 30, 40, 50 or 60 years, it was found that tPA increased with age in both sexes whereas PAI only increased with age in women [104].

The literature in the dementia/fibrinolysis field, though sparse, indicates that disturbances in the fibrinolytic system leading to hypercoagulability may play a pathogenetic role in stroke and in VaD [54]. There is also increasing epidemiological evidence that cerebrovascular disease and AD are associated. However, there is a scarcity of publications, probably because the clinical criteria for AD exclude overt vascular disease [19]. The Dutch study, Vascular Factors in Dementia, found an association between increased levels of D-dimer and dementia [53]. Endothelial derived fibrinolytic markers in AD vs VaD has to my knowledge so far only been studied by Mari et al [52], who found that von Willebrand factor (vWF) was elevated in both AD and VaD and that PAI-1 was significantly higher in the VaD group compared to both the AD and the control groups.

McCully was the first to suggest a relationship between hyperhomocysteinemia and arterial disease [61, 62]. In the study by Fermo et al, hyperhomocysteinemia was found to be an independent risk factor for both venous and arterial occlusive thrombotic disease, OR: 1.9 (95% CI: 0.5—6.6) [105]. The pathophysiologic mechanism by which hyperhomocysteinemia exerts an adverse effect on blood vessels is unknown, but there may be an interaction with endothelial-derived
relaxing factor [106].

There are partly different mechanisms for the development of venous and arterial thromboembolic disease. In the case of venous thromboembolism there are abnormalities of coagulation and/or fibrinolysis. Arterial thromboembolic disease are usually dependent on abnormalities in the platelet function [107] or in the atheromatous vessel wall.
AIMS OF THE STUDY

- To examine if routine clinical data obtained during medical history taking at a Memory Care Unit predict the diagnostic outcome.
- To study homocysteine and related biomarkers and other novel potential vascular risk markers of dementia, in blood and also in CSF.
- To investigate mutations in FOLR-1 and its/their eventual influence on serum folate and plasma homocysteine.
- To investigate differences in endothelial derived fibrinolytic markers and vWF between dementia and non-dementia and also between dementia groups.


**Subjects and Methods**

The subjects in the present thesis and the papers comprised three different groups of subjects.

**The Case Book Study**

Late 1995 a database, for monitoring of the quality of the medical service in the Memory Unit of Department of Geriatrics, was set up. The material comprises 926 consecutive subjects (560 women; 366 men) collected between 1996 and 2000. Both inpatients and outpatients were included and they were all referred for diagnostic evaluation of suspected dementia. Three consultant geriatricians with special interest in dementia examined the subjects. They had worked together for a long time and the diagnoses set by the three consultants were quite similar. Diagnoses were made in accordance with DSM-III-R [108], and for the AD patients also in accordance with NINCDS-ADRDA [109]. Medical history, age, diagnoses, pharmaceutical use, and analyses of blood chemistry were amongst the registered variables, as well as result of EEG and CT scan of the brain. There are no autopsy data, so the diagnoses were strictly clinical. There are no follow up data. The use of over the counter multivitamins was not widespread at that time, and prescription of B vitamins by doctors was mainly restricted to vitamin B12. The main route of administration route of vitamin B12 was oral [110].

B-vitamin combinations like ‘B-Kombin Forte N’ were launched 2002 and ‘TrioBe’, a drug sold on prescription, was launched July 1999. As a reflection of how I viewed B vitamins at that time, prescription of vitamin B12 was recorded but unfortunately not folate prescription. Since the study was based on the clinical reality back then and primarily was not set up for research purposes, the data completeness is suboptimal from today’s perspective.

**Dementia, Genetics, Milieu (DGM) Study**

The subjects were seen at the Memory Care Unit at the Örebro University Hospital. The material comprises 300 consecutive patients (157 women; 143 men). They were all referred for diagnostic assessment and treatment of suspected cognitive problems. At this time, when the acetylcholine-esterase inhibitors had been on the market for at least 7 years, the subjects referred to the specialist department had considerably milder cognitive symptoms than in the case book study. The inclusion period extended from May 2003 to August 2007. To be
included in the study, the cognitive problems had to be mild or at worst moderate, defined as MMSE [111] score ≥10. At the time of inclusion no one of the subjects were living in sheltered housing. Because subjects not fulfilling the diagnostic criteria for dementia also were enrolled, the upper limit of the MMSE was 30. Everyone in the study group underwent a structured and thorough clinical investigation, including medical history, socio-economic data and family history, physical, neurological, neuropsychological, and psychiatric examination. Ongoing pharmacological use was registered according to the Anatomical Therapeutic Chemical (ATC) Classification System. Blood chemistry tests were done on all. For differential diagnostic purposes lumbar puncture were accomplished in 243 subjects at L3/L4 or L4/L5 level. Analyses of the Csf biomarkers for AD: total tau protein, phosphorylated tau protein and \( \text{A}_\beta_{1-42} \) were performed according to our clinical routine at the Department of Psychiatry and Neurochemistry, Institute of Clinical Neuroscience, Sahlgrenska University Hospital, Mölndal, Sweden. Fasting blood and Csf samples were taken on subjects in sitting position after a minimum of 15 minutes rest in order to diminish effects of acute physical activity on the biomarkers, and always between 08 and 09 AM in order to reduce the effect of circadian rhythm variation (see below). Two psychologists made the neuropsychological examinations and one consultant geriatrician made the other clinical examinations and also made all diagnoses.

There are no follow up data at present.

The grouping of patients was done in the following way:

Subjects were considered non-demented (ND) when they not fulfilled the definition for dementia according to DSM-IV [112]. In the ND group this means that some of the subjects belong to the category mild cognitive impairment (MCI) and do not quite yet fulfill the dementia definition. However, at least some of them, will fulfill the definition for dementia in the future. There was also a considerable part that was not MCI and probably was not in a pre-dementia state at all.

The AD group was categorized according to ICD-10 [113] and ADRDA-NINCDS [109] criteria as probable AD. CSF biomarkers for AD (tau, p-tau and \( \text{A}_\beta_{1-42} \)) were used to sharpen the differential diagnostics along with the CSF biomarkers for the integrity of blood-brain-barrier.

Subjects in the VaD-Mixed group were compiled due to their mutual vascular component. This means that patients with a diagnosis of possible AD (insidious onset and medical history of TIA/stroke without time relation to the start of dementia symptoms, and/or vascular signs on brain CT scan) and CSF
biomarkers consistent with AD, but also a raised albumin-index as sign of an impaired BBB, are listed in the Mixed-VaD group. Our hypothesis is that some kind of vascular components are central in the pathophysiology of dementia development, both of course in vascular dementia, but perhaps also AD.

The VaD group was amassed because they were on clinical grounds considered as “pure” VaD. They fulfilled the VaD criteria according to NINDS-AIREN [114]. The AD CSF biomarkers were all negative.

The manuscript to Paper II was completed during 2006, about a year before the DGM study data collection was finished. So, the DGM group in Paper II consists of a subgroup analyzed at that time, 202 subjects.

Active Seniors Study

Active Seniors study (AS) was recruited by a multi-phase sampling procedure between 2003 and 2004, aimed at an elderly retired population, living in various communities in Central Sweden. The locations for the recruitment were selected to represent a broad range of socioeconomic levels and included rural as well as urban and suburban areas. The sample comprised 389 senior citizens and was recruited from several retired persons organizations, which implied that they were independent and socially active. Being retired, living independently in their own homes in addition to active participation in such organizations meetings, were the sole inclusion criteria, not preset health criteria. The subjects were designated as ‘Active Seniors’ in contrast to elderly persons that do not engage themselves in the mentioned social activity.

All were Caucasians, most of them born in the 1920’s and 1930’s, mean age at sampling was 74 ± 5 years for both sexes, and the sex ratio F/M was 262/127. A subset of the cohort was assessed by the MMSE (n=197) and the clock drawing test (CDT) (n=333) [115]. CDT was scored according to Schulman [115]. Those subjects who were scored according to both MMSE and CDT (n=197) and had MMSE ≥28 and CDT ≥4 (n=107) were designated cognitively intact (n=154), this was the ‘referent’ group in paper IV. Combining MMSE and CDT results improves the positive predictive score according to Thalmann [116].

The blood samples from both the DGM and AS groups are kept in our biobank together with CSF samples from the DGM study.

Statistics

All statistical analyses were done with the SPPS package for personal computers version 11.5 (paper II) and 15 (paper I, III, and IV) except for the power calculation on page 50, which was done in Minitab 14. Statistical significance
was considered with a probability value ≤0.05. Variables that did not follow the normal distribution according to Kolmogorov-Smirnov's test were logarithmically transformed (Lg10). Descriptive figures are presented as mean ±SD and in paper IV as mean ±SEM.

Univariate analyses were done with 'Independent Sample t-Test' procedure in SPSS in two group comparison with continuous variables, and ANOVA with post hoc analyses (Tukey or Bonferroni as stated in tables legend), according to the ‘Compare Means — One-Way ANOVA’ procedure in SPSS in three group comparisons with continuous variables. In group comparisons with dichotomous variables, \( \chi^2 \) tests were used. Correlation coefficients were analyzed with the Bivariate Correlations procedure in SPSS (Pearson’s).

In paper I the odds ratio was calculated in a multivariate model with the Binary Logistic Regression procedure, and dementia/non-dementia as the dependent variable, the Method as Forward:LR.

In paper II, a Chi-squared test was used for test of Hardy-Weinberg equilibrium.

In paper III, the Mann-Whitney U-test was used in comparing differences in the ND group through the non-parametric ‘2 Independent Samples’ procedure.

In paper IV, a multivariate model calculated with ANCOVA analyses were used (General Linear Model, Univariate procedure in SPSS), with each of the four hemostatic variables as dependent variable. The diagnostic group variable was used as a fixed factor in these models. Post hoc analyses according to Bonferroni as stated in Table IV:1.

**Ethics**

All studies in this thesis were performed in accordance with the Helsinki declaration. In the case book study (paper I), ethical approval to use the database and case records for a retrospective patient file analysis was obtained from the Regional Ethical Review Board, Uppsala, Sweden in 2007. The Board also stated that individual informed consent was not applicable to this study.

The Ethical Committee of the Örebro County Council approved the studies II—IV. Permission to store the subjects’ data on files and in digital form was obtained from the Swedish Data Inspection Board. All participants were informed verbally and in writing and all gave informed consent to participate in the study and personally signed an informed consent form. Because of the biobanking legislation in Sweden, only the subject concerned can sign the informed consent form.
Results

Paper I

This clinical material was the starting point of my interest in B vitamins and dementia. In fact, the interest began a few years earlier in the first years of the 1990's. This was the pre acetylcholinesterase inhibitors era. Among the homocysteine-related B-vitamins, B12 was regarded as the most important one. This point of view was founded already in Medical School. The overemphasis on vitamin B12 led to a general relative undervaluation of folate, which is the reason why information about folate prescriptions is missing from the database, while information on B12 prescriptions were kept in the database. Baseline characteristics not presented in paper I are shown in Table I:1.

Table I:1. Baseline characteristics and data completeness of the studied 926 subjects

<table>
<thead>
<tr>
<th></th>
<th>ND</th>
<th>Demented</th>
<th>P*</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>n=240</td>
<td>n=686</td>
<td></td>
</tr>
<tr>
<td>Living alone — no./total no. (%)</td>
<td>123/229</td>
<td>368/664</td>
<td>0.654</td>
</tr>
<tr>
<td>Brain CT done — no./total no. (%)</td>
<td>154/240</td>
<td>406/686</td>
<td>0.174</td>
</tr>
<tr>
<td>EEG done — no./total no. (%)</td>
<td>72/240</td>
<td>175/686</td>
<td>0.176</td>
</tr>
<tr>
<td>EEG back ground activity, Hz</td>
<td>8.5 ±1.0</td>
<td>8.2 ±1.0</td>
<td>0.013</td>
</tr>
<tr>
<td>Education ≤7 years — no./total no. (%)</td>
<td>185/229</td>
<td>566/651</td>
<td>0.023</td>
</tr>
<tr>
<td>Home-help service — no./total no. (%)</td>
<td>86/237</td>
<td>324/669</td>
<td>0.001</td>
</tr>
<tr>
<td>Living in sheltered housing — no./total no. (%)</td>
<td>22/232</td>
<td>132/677</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Smoking — no./total no. (%)</td>
<td>63/217</td>
<td>177/609</td>
<td>0.993</td>
</tr>
</tbody>
</table>

*Pearson Chi-square or t-test as appropriate (calculated on logarithmically transformed variables)
Logistic regression analysis showed that both a prescription, a positive family history of dementia and the presence of home help service significantly increased the risk of having a dementia diagnosis as shown in Table I:2.

Table I:2 Odds ratio (OR) of having dementia vs non-dementia in relation to vitamin B12 prescription, family history of dementia, and home-help service

<table>
<thead>
<tr>
<th>Variable</th>
<th>OR</th>
<th>95% CI</th>
<th>p</th>
</tr>
</thead>
<tbody>
<tr>
<td>Vitamin B12 prescription</td>
<td>2.01</td>
<td>1.29 – 3.13</td>
<td>0.002</td>
</tr>
<tr>
<td>Family history of dementia</td>
<td>1.50</td>
<td>1.02 – 2.19</td>
<td>0.037</td>
</tr>
<tr>
<td>Home-help service</td>
<td>1.47</td>
<td>1.01 – 2.13</td>
<td>0.044</td>
</tr>
</tbody>
</table>

Logistic Regression analysis by the forward stepwise procedure, $R^2$: 0.126

In this paper we wanted to examine whether people attending our Memory Care Unit were fully supplemented with tHcy related B-vitamins. Clinicians had recognized the need for vitamin B12 supplementation in 260 of the subjects. We found that these 260 subjects, with a vitamin B12 prescription, had significantly lower S-folate, significantly higher P-homocysteine and P-MMA, both in subjects with dementia and in those without dementia diagnosis.

When interpreting these results we found it most likely that supplementation with vitamin B12 alone caused a secondary intracellular folate deficiency due to increased demands on folate when vitamin B12 was enhanced.

Significantly higher S-MMA was found in both the D and the ND groups of subjects who were prescribed vitamin B12. This could be due to a couple of different reasons. One possibility is that the substitution with vitamin B12, which in Sweden was mainly orally prescribed in the 1990’s [110], was prescribed in too low dose to fully achieve the required intracellular metabolic effect. Another explanation could be that the compliance, also for vitamins, was as low as it was with other prescriptions [117]. From this point of view the compliance was better when vitamin B12 was administrated as an injection.

Another conceivable explanation is malfunction in the transport of vitamin B12 from serum to the intracellular compartment. This transport is carried out by the carrier proteins haptocorrin and transcobalmin (TC). The latter has also an important role in delivering vitamin B12 over the blood-brain-barrier. The frequent polymorphism in the gene for TC, 776C>G, is associated with a slight elevation in tHcy.

In conclusion, paper I showed that subjects being prescribed vitamin B12 constitute a group which appeared to have unmet needs of nutritional support, paradoxically even including vitamin B12 itself. Home help service did not appear to meet these needs fully.
Paper II

The aim in paper II was to further elucidate FOLR1, exons 2 and 3 for mutations, and if found answer the question whether the mutation/s influence S-folate and or P-tHcy in dementia and/or non-dementia subjects.

We found 4 sequence variations in the 5' part of the FOLR1 gene, g.1314G>A, g.1816delC, g.1841G>A, and g.1928C>T. Pyrosequencing™ genotyping assays were developed for all of them, and 389 active seniors (AS subgroup) and the 202 DGM patients were genotyped for these mutations. The frequency of the mutated allele was, among the AS subjects, 0.068, 0.0026, 0.0026, and 0.024 respectively, and among the DGM subjects, 0.067, 0.0076, 0.0078, and 0.023. The g.1816delC and g.1841G>A mutations thus were approximately three times more frequent in the DGM than in the AS subgroup, but the difference did not reach statistical significance with this sample size. The mutated alleles, FOLR1 1816(-) and 1841A, always occurred together in the same subjects, suggestive of a rare double-mutant haplotype, an interesting finding that will be further explored.

The two common polymorphisms, FOLR1 g. 1314G>A and g.1928C>T did not raise plasma tHcy levels or reduce S-folate concentrations, whereas the double mutated g.1816delC—g.1841A haplotype may possibly have a slight tHcy-raising effect. Further data from additional populations will be studied to confirm this possible effect.

In conclusion, paper II showed that the double-mutated FOLR1 g.1816delC—g.1841A haplotype may possibly have a tHcy-raising effect. This should be further studied in larger cohorts.

Figure II:1. SSCP gel, the arrows point out the frequent g.1314G>A polymorphism.
**Paper III**

The aim in paper III was to study the transport mechanism of folate from serum to CSF in accordance with the concept that Björn Regland introduced for vitamin B12 in a paper 1992 [101]. We studied three subgroups from the DGM study: non-dementia (ND), Alzheimer’s disease (AD) and a group with mixed Alzheimer and vascular dementia (Mixed+VaD).

From Figure III:1 it is clear that the concentration of CSF-folate levels out at serum-folate concentrations around 25 nmol/L, indicating that the process is saturable.

**Figure III:1.** Shows the saturable transport mechanism.

The grouping of the subjects is more extensively described under the heading “Subjects and Methods” on page 35; here I want to give some simple additional details about the ND group.

The ND group consisted of 73 subjects that had not yet deteriorated to the dementia level; this group is sometimes denoted as mild cognitive impairment (MCI). As can be seen in Table III:1, the ND group consisted of 39 subjects with MCI, and 34 subjects that probably were not in a possible pre-dementia state at all. There was a significant difference between both non-dementia groups in clinical dementia rating scale (CDR) [118]. Also the MCI group had mean value below 0.5, considered the limit for dementia.
Table III:1

<table>
<thead>
<tr>
<th>±SD</th>
<th>ND n=34</th>
<th>MCI n=39</th>
<th>p*</th>
</tr>
</thead>
<tbody>
<tr>
<td>$R_{CSF/S}$</td>
<td>2.60±0.67</td>
<td>2.33±0.55</td>
<td>0.203</td>
</tr>
<tr>
<td>CSF-folate</td>
<td>30.52±6.61</td>
<td>30.24±8.1</td>
<td>0.736</td>
</tr>
<tr>
<td>S-Folate</td>
<td>12.73±5.07</td>
<td>14.03±6.4</td>
<td>0.576</td>
</tr>
<tr>
<td>BMI</td>
<td>27.4±4.0</td>
<td>26.3±3.6</td>
<td>0.356</td>
</tr>
<tr>
<td>MMSE</td>
<td>28.3±2.5</td>
<td>26.5±3.0</td>
<td>0.001</td>
</tr>
<tr>
<td>Clock Drawing</td>
<td>4.6±0.7</td>
<td>4.1±1.1</td>
<td>0.103</td>
</tr>
<tr>
<td>CDR</td>
<td>0.28±0.28</td>
<td>0.47±0.20</td>
<td>0.001</td>
</tr>
</tbody>
</table>

* p-values calculated according to Mann-Whitney U-test

In subjects who were considered as non-demented (ND) the mean CSF to serum folate ratio ($R_{CSF/S}$) for folate was 2.46 ± 0.62 (±SD) vs 2.09 ± 0.67 (p=0.008) in the dementia subgroup with a vascular component (VaD+Mixed). The ND subgroup had higher CSF-folate (p=0.001), and lower S-homocystein values (p=0.001) than the VaD+Mixed subgroup. The folate gradient $R_{CSF/S}$ was negatively correlated to S-folate ($p<0.001$, $R^2=0.518$) and to albumin ratio, a B-CSF-B biomarker ($\beta = -0.235$). Alzheimer patients (AD) had $R_{CSF/S}$ and albumin ratios similar to ND subjects.

The folate concentration gradient is achieved by an active transport over the choroid plexus into the CSF, both FR-α and RFC are involved. CSF-folate then passively diffuses through the ventricles ependymal layer to the lower concentration in the brain extra cellular fluid and into the neurons [119]. The recently described proton-coupled folate transporter (PCFT) has been reported to work in tandem with FR-α to maintain the CSF to serum folate gradient [92].

In conclusion, paper III showed that the folate $R_{CSF/S}$ was significantly lower in the VaD+Mixed dementia subgroup than in the ND and AD subgroups, suggestive of a defect in the transport of folate over the choroid plexus that may be characteristic of, and limited to, the VaD+Mixed dementia subgroup.
Paper IV

Vascular risk factors like hypertension and atrial fibrillation may be associated also with AD, in addition to VaD. Only a few studies have dealt with fibrinolytic biomarkers in cross sectional studies. We wanted to look more carefully into the challenging problem – is AD a vascular disease? In paper IV we analyzed the endothelial derived fibrinolytic markers tPA, its inhibitor PAI-1, tPA/PAI-1 complex and von Willebrand factor (vWF). We studied 304 subjects of whom 95 were diagnosed as AD and 55 as VaD, recruited from the DGM study, and 154 subjects from the AS-study comprised the referent group.

The blood samples from the DGM group were taken under standardized conditions after over nights fasting between 08 — 09 am, while the AS samples were taken under less standardized conditions, not fasting between 1 and 4 pm.

We found a significantly higher tPA concentration in both the dementia groups compared to the reference group. Both tPA/PAI-1 complex and vWF were higher in both the dementia groups than in the reference group, but there was no significant difference between the both dementia groups. There were no significant changes in the result when adjusting also for statins, angiotensin converting enzyme (ACE)-inhibitors and angiotensin II receptor blockers (ARB) with the exception that tPA/PAI-1 complex now only differed between AS and VaD with border line significance, as can be seen in Table IV:1.

When evaluating unadjusted values of the endothelial derived fibrinolytic markers and vWF, Table IV:2, we found that tPA mass concentration differed significantly when comparing AD vs VaD. PAI-1 was significantly lower in AD than in AS. Unadjusted tPA/PAI-1 complex differed significantly from the adjusted values when comparing AS vs AD and AS vs VaD where lower tPA/PAI-1 concentration was found in the AS subgroup.

In conclusion, paper IV showed that endothelial derived fibrinolytic factors, tPA/PAI-1 complex and vWF, discriminated between the AD and VaD groups and the reference group of non-demented elderly, but not between AD and VaD. This suggests that there are similar disturbances for endothelial derived fibrinolytic and hemostatic factors among AD and VaD patients, which may reflect common pathophysiological mechanisms.
<table>
<thead>
<tr>
<th>Variable</th>
<th>Group 1 (Mean ± SEM)</th>
<th>Group 2 (Mean ± SEM)</th>
<th>Group 3 (Mean ± SEM)</th>
<th>p*</th>
<th>p**</th>
</tr>
</thead>
<tbody>
<tr>
<td>tPA μg/L</td>
<td>11.6 (±0.4)</td>
<td>11.6 (±0.5)</td>
<td>11.4 (±0.8)</td>
<td>&lt;0.001</td>
<td>0.001</td>
</tr>
<tr>
<td></td>
<td>11.6 (±0.5)</td>
<td>13.9 (±0.5)</td>
<td>14.5 (±0.9)</td>
<td>AD vs VaD: p=1.000</td>
<td>1.000</td>
</tr>
<tr>
<td></td>
<td></td>
<td>AS vs AD: p=0.001</td>
<td>AS vs VaD: p=0.017</td>
<td>0.001</td>
<td>0.010</td>
</tr>
<tr>
<td>PAI-1 μg/L</td>
<td>26.4 (±1.7)</td>
<td>23.6 (±2.1)</td>
<td>21.6 (±3.1)</td>
<td>0.243</td>
<td>0.141</td>
</tr>
<tr>
<td></td>
<td>25.3 (±1.5)</td>
<td>22.2 (±2.0)</td>
<td>21.7 (±3.0)</td>
<td>AD vs VaD: p=1.000</td>
<td>1.000</td>
</tr>
<tr>
<td></td>
<td></td>
<td>AS vs AD: p=0.370</td>
<td>AS vs VaD: p=0.760</td>
<td>0.146</td>
<td>1.000</td>
</tr>
<tr>
<td>tPA/PAI-1 complex μg/L</td>
<td>9.1 (±1.4)</td>
<td>13.2 (±1.7)</td>
<td>10.9 (±2.5)</td>
<td>0.001</td>
<td>0.003</td>
</tr>
<tr>
<td></td>
<td>9.2 (±1.5)</td>
<td>13.3 (±1.7)</td>
<td>11.1 (±2.8)</td>
<td>AD vs VaD: p=1.000</td>
<td>1.000</td>
</tr>
<tr>
<td></td>
<td></td>
<td>AS vs AD: p=0.002</td>
<td>AS vs VaD: p=0.029</td>
<td>0.005</td>
<td>0.054</td>
</tr>
<tr>
<td>vWF %</td>
<td>195.5 (±10.1)</td>
<td>290.8 (±12.0)</td>
<td>308.7 (±18.0)</td>
<td>&lt;0.001</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td></td>
<td>192.6 (±10.5)</td>
<td>289.7 (±12.2)</td>
<td>320.0 (±19.7)</td>
<td>AD vs VaD: p=1.000</td>
<td>1.000</td>
</tr>
<tr>
<td></td>
<td></td>
<td>AS vs AD: p&lt;0.001</td>
<td>AS vs VaD: p&lt;0.001</td>
<td>&lt;0.001</td>
<td>&lt;0.001</td>
</tr>
</tbody>
</table>

ANCOVA statistics. Adjusted for age, sex, BMI, systolic blood pressure, cystatin C, hs-CRP, HDL-cholesterol and HbA1c. p* was calculated with logarithmically transformed variables. Figures in bold italics shows the results when prescription of statins (Y/N), ACE-inhibitors (Y/N), and ARB-blockers (Y/N) was added to the model, corresponding p-values was calculated with logarithmically transformed variables and are show in the column marked p**. Post hoc analyses according to Bonferroni.
ANOVA statistics. The significance data was changed compared to the adjusted data in Table IV:1, see the bold figures. P-values calculated using logarithmically transformed variables. Post hoc analyses according to Tukey.

**Table IV:2. Unadjusted endothelial derived fibrinolytic markers.**

<table>
<thead>
<tr>
<th></th>
<th>Mean (±SEM)</th>
<th>AS with MMSE n=152</th>
<th>AD n=83</th>
<th>VaD N=40</th>
<th>p</th>
</tr>
</thead>
<tbody>
<tr>
<td>tPA μg/L</td>
<td>12.1 (±0.4)</td>
<td>13.0 (±0.6)</td>
<td>15.4 (±0.9)</td>
<td></td>
<td>0.002</td>
</tr>
<tr>
<td></td>
<td></td>
<td>AD vs VaD: 0.040</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>AS vs AD: 0.457</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>AS vs VaD: 0.001</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>PAI-1 μg/L</td>
<td>25.4 (±1.7)</td>
<td>20.9 (±1.5)</td>
<td>23.9 (±1.8)</td>
<td></td>
<td>0.022</td>
</tr>
<tr>
<td></td>
<td></td>
<td>AD vs VaD: 0.173</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>AS vs AD: 0.019</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>AS vs VaD: 0.992</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>tPA/PAI-1 complex μg/L</td>
<td>8.6 (±0.5)</td>
<td>12.6 (±2.4)</td>
<td>13.0 (±1.0)</td>
<td>&lt;0.001</td>
<td>0.057</td>
</tr>
<tr>
<td></td>
<td></td>
<td>AD vs VaD: 0.066</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>AS vs VaD: &lt;0.001</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>vWF %</td>
<td>197.6 (±6.6)</td>
<td>283.4 (±13.5)</td>
<td>327.7 (±19.4)</td>
<td>&lt;0.001</td>
<td>0.083</td>
</tr>
<tr>
<td></td>
<td></td>
<td>AD vs VaD: 0.083</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>AS vs AD: &lt;0.001</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>AS vs VaD: &lt;0.001</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
**General Discussion and Implications**

*Methodological considerations*

To be able to generalize the results of the study it is important to be aware of the possible bias problems [120]. Selection bias is not a major issue in the DGM-study or in the case book study. These persons were referred from doctors outside the Geriatric Department and consecutively enrolled to the study. In the DGM study there is however a divergent sex distribution with an unusual high proportion of men (47.7%) which suggests a possible sex bias.

The Active Seniors study could be biased since the subjects probably represent a part of the retired population that is more active and healthier than the general retired population.

In the Case Book Study the subjects were examined by three different consultants who independently made their own diagnoses, thus reducing the risk of systematic misdiagnosing. On the other hand, in the DGM study all diagnoses were made by one of the above mentioned consultants. This could imply a positive uniformity in the diagnostic process, but there is also a raised risk of systematic misdiagnosing. Taken together the latter procedure has a slight advantage compared to the procedure where multiple physicians made their own diagnoses. The preferred diagnostic procedure would have been a procedure in which a multi professional team set the diagnoses in consensus.

**Paper I**

The main findings in this study were that subjects with a prescription of vitamin B12, both demented and non demented, had significantly lower S-folate levels, and significantly higher tHcy and S-MMA levels, compared to subjects without vitamin B12 prescription. Further studies are needed to confirm this observation. If it holds true, then the design of supplementation needs to be changed in two directions. First, the problem of compliance and doses of vitamin B12, can probably be solved by administering B12 via injections. Secondly, and probably the most important, would be to expand the supplementation to include folate besides vitamin B12.

The time point of blood sampling in relation to start of supplementation and last dose might have played a role. If B12 supplementation was started very recently, laboratory parameters may not have normalized, or if subjects had been treated for a very long time, compliance and follow-up routines might have become insufficient and these subjects might have experienced a relapse.
Another result that ought to be further elucidated was that the nutritional support given by the home help service did not seem to be fully satisfactory with regard to the nutritional status of the receivers. In Sweden the home help service is most often provided by the community and the main part is compensated by taxes. There are some fundamental demands that ought to be met by home help service, one of these is adequate nutrition. Despite the strained economic situation in Sweden (and in the world) and the growing numbers of elderly, the home help service facility has to provide a support that actually prevents the recipient from getting worse, eg due to malnutrition.

A deficiency of intracellular folic acid, as measured by tHcy and S-MMA, may arise through a number of mechanisms which includes inadequate dietary intake, defective absorption due to a generalized malabsorption syndrome, increased requirements, presence of folic acid antagonists, mutations in genes essential to folate and homocysteine metabolism [121] or through autoantibodies directed against the folate receptor [122, 123]. The need to more closely monitor vitamin status in the elderly has been recognized for some time, but the analytical tool to detect deficiencies continue to evolve so that different sets of tests have been proposed [124, 125]. To better survey the B-vitamin situation in this kind of patients it would in the future be preferable to add holo-transcobalamin-II to the arsenal of biomarkers.

Genetic causes of folate deficiency might include mutations in the genes for Reduced Folate Carrier (RFC), Folate Receptor-α (FR-α) or Proton Coupled Folate Transporter (PCFT). FR-α in the choroid plexus is apparently necessary for the active transport of folate from blood to CSF. Folate deficiency has been linked to several conditions in the CNS, neural tube defects, dementia and depression [126].

Due to the central role for FR-α in the folate uptake and transport, it is reasonable to assume that mutations with profound impact on S-folate or CSF-folate would be rare. We wanted to explore the incidence of mutations in FOLR-1, the gene for FR-α. In a previous paper from our group, the 5’-upstream part of the FOLR-1 was screened for mutations and six novel mutations were found [127]. In paper II we examined exons 2 and 3 in FOLR-1 for mutations.

**Paper II**

In paper II we for the first time described a rare double mutant haplotype in FOLR-1, FOLR1 g.1816delC. The FOLR1 g.1816delC mutation has \( q = 0.0026 \) in the AS group and \( q = 0.0078 \) in the DGM group, that is three times as usual in the DGM cohort. However, it was not a statistically significant difference.
Since we had no pre-study opinion about the frequency of the double mutant haplotype or other FOLR1 mutations it was impossible to accomplish a reasonable power calculation. After the study we know the approximate frequency in our cohorts, and based on these new facts we made a power calculation. A frequency of 1.5% in the DGM cohort and 0.005% in the AS cohort implies a sample size of 1553 with 80% power to detect the difference at the 5% significance level.

Based on the literature, it seems not unreasonable to assume that the folate metabolism is impaired to a greater extent in subjects with dementia than in non-demented subjects[128]. Further inquires are needed to confirm or reject this hypothesis. The same kind of studies should also be performed with the RFC1 and PCFT folate receptors. In paper III we examined the CSF/serum folate gradient as a marker of the transport of folate to the CNS.

**Paper III**

We used the concept of a folate gradient CSF/Serum as a benchmark for the folate transport over the blood-CSF-blood barrier. FR-α in choroid plexus has a central role in CNS folate maintenance. To my knowledge there is no other pathway of supplying the brain with folate.

The main finding in *paper III* is that people with a vascular component as cause of their dementia (VaD+Mixed) have significantly lower $R_{CSF/S}$ for folate compared to ND and AD subjects. We interpret the lower ratio as an impaired transport of folate from blood to CSF and thus to the brain, although the complete transport mechanism is not known in detail. The choroid plexus is mainly a vascular tissue, a part of the explanation why dementia subjects with a vascular component appear to have a more affected transport of folate from blood to CSF, might be lesions of the blood vessels also in choroid plexus.

The folate transport mechanism showed to be saturable, implying that there is no need for oversupplementation with folate.

The grouping strategy of the diagnoses used in this paper has its origin in the hypothesis that dementia develops in part by vascular mechanisms. We therefore made the choice to compare three groups of consecutive subjects attending our Memory Care Unit.

The ND subgroup consists of both real non-demented subjects, but also of a group of MCI subjects, of which probably a considerable fraction, will develop dementia in the future, they were just not there when the diagnosis was set. Further explanation of the ND group is given in Table III:1.

The AD group was categorized according to ICD-10 [113] and ADRDA-
NINCDS [109] criteria as probable AD. CSF biomarkers for AD (tau, p-tau and Aβ1-42) were used to sharpen the differential diagnostics along with the CSF biomarkers for the integrity of blood-brain-barrier. Here the CSF biomarkers of AD comprised an elevation of total tau protein and phospho tau along with a decrease in β-amyloid and a normal CSF/serum albumin ratio.

The VaD+Mixed subgroup was joined together by an identified vascular component. That is infarct on CT brain scan, a medical history of stroke or TIA, white matter disease on CT brain scan and elevated CSF/serum albumin ratio.

**Paper IV**

In accordance with epidemiological papers concerning vascular risk factors and diseases associated with dementia we also found similarities between VaD and AD. The review by Skoog et al 1999 [129] documented several vascular risk factors and diseases for late-onset AD, a picture shared by several recent authors [40, 130, 131]. In this paper we bring new data as we also compared the fibrinolytic biomarkers in the two dementia giant diagnose groups, AD and VaD, and also compared with a cognitively intact and healthy reference group in the same age range.

The main result in paper IV is that VaD and AD both have a pathologically raised concentration of endothelial derived hemostatic and fibrinolytic markers as opposed to elderly non-dementia subjects. Our interpretation of this finding is that AD shares a vascular mechanism with VaD. This is in line with several epidemiological studies where other vascular risk markers such as hypertension or atrial fibrillation have been associated with AD. If other studies confirm our finding, then it might be proposed that therapeutic strategies to reduce common vascular risk also would reduce the risk of AD.

There are no neuropathologically confirmed diagnoses in our studies, so all diagnoses are based solely on the clinical findings in accordance with ICD-10 and for AD also NINCDS-ADRDA. Additionally, CSF biomarkers for AD and the albumin index were used to sharpen the differential diagnostic procedure so the AD and VaD diagnoses were reasonably correct. The fasting blood samples from the DGM group were taken under standardized conditions between 8 and 9 am, while the AS samples were taken under less standardized circumstances, not fasting between 1 and 4 pm. Since the circadian rhythm of PAI-1 implicates lower concentration in the afternoon while tPA has higher concentration in the afternoon, the results in our study could be expected to have been even more pronounced if also the AS blood samples had been taken in the morning.
CONCLUSIONS

- In dementia cases with a family history of dementia, there was an association with significantly raised homocysteine.
- Subjects with prescription of vitamin B12 had higher homocysteine and lower S-folate as sign of intracellular folate deficiency. Methylmalonate levels were also higher. It is thus necessary to recognize vitamin B12 deficiency, but it is not enough. Folate and vitamin B12 work so close together that it is unwise to supplement just one of them; they are needed together.
- Two unusual mutations in FOLR-1 were detected in our group of Swedish subjects and were found to always occur together (FOLR1 g.1816delC—g.1841A). The rare double mutation may be more common in the DGM subjects. Low S-folate and raised homocysteine were also associated with the rare double mutation, however not significant. Several other FOLR1 mutations were found, that appeared not to affect tHcy or S-folate levels.
- Patients with vascular component as part of their dementia diagnose (VaD+Mixed) had significantly lower CSF/serum folate ratio ($R_{CSF/S}$) indicating an impairment in folate transport from blood to CSF in these forms of dementia.
- Alzheimer patients had the same impairment in hemostatic and fibrinolytic endothelial derived biomarkers as patients with vascular dementia, indicating an impaired shared common pathway for these diseases, and significantly differentiating both of them from a reference group of healthy active seniors.
**Future perspectives**

The work of this thesis has lead me to want to go on with further scientific work trying to acquire new knowledge first and foremost in the field of dementia, folate and fibrinolysis.

An important finding in this thesis was that single supplementation with vitamin B12 was found to be insufficient. However, this finding has to be confirmed in future studies.

The Case Book Study should be continued by investigating the subjects causes of death diagnoses and dates of death from central registers available in Sweden, and to correlate this data with already known data such as vitamin and homocystein concentration. The same kind of follow up would be reasonable also for de DGM and maybe also for the AS material. Interesting topics to address are, for instance: Is there an association between the fibrinolytic markers and death? and is there an association with CSF biomarkers?

We discovered a rare double mutant haplotype in \( FOLR1 \) that may a) be more common in the DGM material than in AS, and b) may cause elevated plasma total homocysteine concentrations. Our findings did not reach statistical significance, probably due to insufficient power. We plan to extend the studies on this haplotype.

Folate transport across the choroid plexus to the CSF compartment appears to be dysregulated in subjects with VaD+Mixed dementia. Our result that low \( R_{\text{CSF/S}} \) seems to be a biomarker of vascular types of dementia needs further confirmation.

There is evidence that elevated plasma homocysteine is associated with thrombosis. There is also growing evidence that an activated fibrinolytic system is a fundamental factor in the pathogenesis of vascular disease. Fibrinolysis and inflammation are tightly bound in these processes. In this thesis we have studied hs-CRP, haptoglobin, and fibrinogen as markers of inflammation. In the future I would like to study additional markers of inflammation, especially cytokines eg IL-6 and IL 10, in the biobank material from both DGM and AS with regard to vascular mechanisms like activated fibrinolysis and elevated homocysteine in elderly with and without dementia.

A new and exciting perspective that seems to link homocysteine metabolism with the CSF hallmark biomarkers of AD, tau and \( \beta \)-amyloid [132], also indicates a way for future research. Integrative studies are in general still lacking and should be high on our agenda.
I Sverige nyinsjuknar ca 25.000 personer i demenssjukdom årligen. Förutom det stora lidande sjukdomen för med sig för den drabbade och dennes familj så drabbas också samhällsekonomin hårt. Den samlade samhällskostnaden för demenssjukdomarna är större än för all cancersjukvård, all hjärt- och kärlsjukvård inklusive all strokesjukvård tillsammans.


Denna avhandlings syfte har varit att undersöka ärfliga och förvärvade riskfaktorer för demens, speciellt med avseende på vitaminet folsyra. Utgångspunkten har varit uppfattningen att brist på vitaminer kan bero på dåligt näringsintag, på ärfliga problem i vitaminernas transportproteiner eller på en kombination av dessa faktorer. Vår hypotes har varit att intracellulär brist på B-vitaminerna folsyra eller B12, mätt som ett förhöjt homocystein, kan medföra en ogynnsam effekt på hjärnan via mekanismer i blodkärlen.


1 DGM= demens, genetik, miljö
2 AS= aktiva seniorer

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hemtjänst är troligen kroppslikt sjukare än de som inte har hemtjänst vilket i sig kan tänkas ge högre homocystein. Det kan dock inte uteslutas att hemtjänsten på 1990-talet inte riktigt levde upp till målsättningen att ge en god och ändamålsenlig nutrition till sina brukare.

I delarbete II bekräftar vi förekomsten av några vanliga mutationer i FOLR-1\(^1\) också i den mellansvenska befolkningen. Vi fann också en i Sverige relativt ovanlig, tidigare ej beskriven, dubbelmuation i FOLR-1. Denna verkade vara vanligare i DGM- än i AS-materialet. Dubbelmutationen föreföll också att ge lägre folsyravärden samt högre homocysteinvärden i blod. Dubbelmutationen var ovanlig och vårt material räckte ej till i antal forskningspersoner för att statistiskt säkerställa skillnad mellan AS och DGM grupperna.

I delarbete III undersöker vi transportfunktionen av folsyra från blod till cerebrospinalvätska hos personer med ND\(^2\), AD\(^3\) och VaD+Mixed\(^4\) i DGM-gruppen. Vi fann att personer som hade en problematik i blodkärlen som orsak eller delorsak till sin demenssjukdom (vaskulär- respektive mixed demens) hade en nedsatt transportfunktion av B-vitaminet folsyra till CNS\(^5\). Den förändrade transportfunktionen såg vi bara hos VaD+Mixed och ej hos AD och ND.

Vaskulära mekanismer undersöktes också i delarbete IV genom att kartlägga nivåerna av fibrinolytiska makörer\(^6\) i blodet på personer ur AS-gruppen samt på personer med AD respektive VaD\(^7\) ur DGM-gruppen. Vi fann att demenssjukdomarna, AD och VaD, gemensamt skiljer sig från de icke demente äldre personerna i AS-gruppen genom att ha ett påslaget och mer aktiverat fibrinolytiskt system. Således har både VaD och AD en vaskulär problematik mätt som aktiverat fibrinolytiskt system.

Slutsatser: Vi fann att både ärfliga och nutritionsfaktorer var kopplade till demens. Vidare fann vi att blodets fibrinolytiska system var aktiverat och ur balans vid både VaD och AD.

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\(^1\) FOLR-1= genen för folatreceptor-\(\alpha\)

\(^2\) ND= icke demens

\(^3\) AD= Alzheimers sjukdom

\(^4\) VaD+Mixed= vaskulär och mixed demens

\(^5\) CNS = Centrala nervsystemet

\(^6\) Äggvitämnen i blodet som håller blodets levningsförmåga i balans

\(^7\) VaD= vaskulär demens
En historisk blick på hur neuronet blodförsörjs (uppfattning från 1920-talet)

**Blodomloppet i en nervcell.** Enligt undersökningar av den polske forskaren Adamkiewicz försörjes varje nervcell av en artär (röd), som träder till cellen vinkelrätt mot densamma (till vänster på bilden). Denna vidgar sig sedan så att cellen på alla sidor ligger kringfluten av blodströmmen, som en ö i en flod. Mot artärens inträdesställe flyter blodet från cellen in i en ven, som blir allt tunnare (till höger på bilden). Från det omgivande blodet tränga syre- och näringsmolekylerna (röda punkter) in i cellens plasma. Kolsyra och avfallsprodukterna (blå punkter) samlas omkring kärnan i cellens mitt och flyta därifrån i en ven (blå) genom plasman och cellväggen utåt.

*Från Fritz Kahn: Människan del IV, Albert Bonniers Förlag, Stockholm 1930
Översatt till svenska av Professor Ivar Broman och Docent Sture A. Siwe*

(Ivar Broman var professor i anatomi i Lund, far till den kände kompositören och musikkritikern Sten Broman.)
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