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**Role of Stem Cell Factor in the Development of
Alzheimer's Disease**

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To my beloved family

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1 Abbreviations

AChE- acetylcholinesterase

AD- Alzheimer's Disease

ADAS-Cog scores- Alzheimer's disease Assessment Scale, cognitive subsection

A β - β -Amyloid protein

BAD- Bcl-2 related protein

BBB-blood-brain barrier

CSF- cerebrospinal fluid

G-CSF- granulocyte-colony stimulating factor

GDNF- Glial-Derived Nerve Factor

Ig- immunoglobulin

MMSE- Mini-Mental State Examination

MRI- magnetic resonance imaging

NGF- Nerve Growth Factor

PET- positron emission tomography

SCF- stem cell factor

SDF-1- stromal cell-derived factor-1

Src kinase- sarcoma kinase

STAT-Signal Transducers and Activators of Transcription

VEGF- vascular endothelial growth factor

VEGFR2/Flk-1- vascular endothelial growth factor receptor 2/ fetal liver kinase 1

2 Introduction

Alzheimer's disease (AD) is the leading cause of dementia in the elderly, affecting more than 35 million people worldwide (Selkoe 2001). Given the rapidly ageing population, the current search for diagnostic and prognostic biomarkers of AD is of vital importance. Despite all the research efforts in this area, the successful treatment or prevention of this disease has not yet been developed and the costs of caring for such patients are growing every day, making a burden for the health care systems of many countries.

2.1 Diagnosis of AD

The diagnosis of AD is made in accordance to the Diagnostics and Statistical Manual of Mental Disorders (DSM-IV), fourth edition (APA 1994), and to National Institute of Neurological and Communicative Disorders and Stroke and the Alzheimer's Disease and Related Disorders Association (NINCDS-ADRDA) (McKhann et al. 1984). The diagnosis is made in two steps: 1) identification of a dementia symptom, and 2) classifying the stage of AD. According to DSM-VI, the diagnosis of AD is made if there is both memory loss and impairment in at least one additional cognitive domain, both of which interfere with activities of daily living. Meanwhile the NINCDS-ADRDA criteria of probable AD do not take into account the disturbance of activities of everyday life. These criteria specify that the onset of AD is not sudden, developing slowly, and no other brain disease that causes memory loss is diagnosed (McKhann et al. 1984). According to these criteria the diagnosis is classified as definite (after histological examination of the brain tissue), probable AD (typical clinical symptoms without histological verification) or possible (atypical clinical features, but no alternative diagnosis, without histological examination). The clinical severity of cognitive impairment is assessed by the Mini-Mental State Examination (MMSE) (Folstein et al. 1975). The example of the MMSE test is displaced at the appendix. The maximal MMSE score is 30, and cognitive decline is estimated at score lower than 24 points, although the performance in MMSE depends on person's age and education (Crum et al. 1993; Doody et al.

2001a; Folstein et al. 1975). The median scores on Mini-Mental State Examination by age and educational level are presented in the table 1 (Crum et al. 1993). Fast cognitive decline is diagnosed if the MMSE score decreases more than 4 points per year, slow cognitive decline is defined if the decrease of MMSE score is less than 4 points per year (Doody et al. 2001a).

Table 1: Median scores on Mini-Mental State Examination by age and educational level. Reprint from Crum et al., 1993.

Age (years)	Educational level, MMSE median score			
	4th grade	8th grade	High school	College
18 to 24	22	27	29	29
25 to 29	25	27	29	29
30 to 34	25	26	29	29
35 to 39	23	26	28	29
40 to 44	23	27	28	29
45 to 49	23	26	28	29
50 to 54	23	27	28	29
55 to 59	23	26	28	29
60 to 64	23	26	28	29
65 to 69	22	26	28	29
70 to 74	22	25	27	28
75 to 79	21	25	27	28
80 to 84	20	25	25	27
>84	19	23	26	27

When the memory loss is not profound the term mild cognitive impairment is used. Mild cognitive impairment is characterized by memory loss, abnormal memory performance for age, normal general cognition and normal activities of daily living. People with mild cognitive impairment present with typically normal MMSE scores from 24 to 28 (Dubois et al. 2007).

According to the cognitive status of a patient, AD can be classified as mild, moderate or severe. The physicians distinguish AD according to the time point when the disease appeared:

-early onset (familial forms of AD) – disease develops in late middle ages before 65 years of age (McKhann et al. 1984).

-late onset – manifestation of symptoms after 65 years of age (McKhann et al. 1984).

2.1.1 Diagnostic biomarkers in AD

2.1.1.1 Neurochemical biomarkers

Measurement of A β 1-42, A β 1-40, tau and phosphorylated tau in CSF is considered as the gold standard of biochemical AD diagnostics, as these biomarkers directly reflect the levels of corresponding pathological substances in the brain tissue (Hampel et al. 2010). Total tau level is a non-specific marker of axonal damage (Blennow and Hampel 2003). The increased levels of total tau and phosphorylated tau together with the decreased levels of A β 1-42 or decreased A β 1-42/1-40 ratio confirm AD or mild cognitive impairment (Blennow and Hampel 2003). Other potential CSF biomarker candidates include A β oligomers, activity and concentration of β -site amyloid precursor protein-cleaving enzyme 1, secreted isoforms of APP and A β degradation products (Hampel et al. 2010). Blood A β 1-40 and 1-42 are used for the AD diagnosis with limited success as they are unlikely to reflect the A β processing in the brain (Hampel et al. 2010).

2.1.1.2 Neuroimaging biomarkers

Structural and functional magnetic resonance imaging (s-/f-MRI) as well as positron emission tomography (PET) have become an essential part of AD diagnostics (Hampel et al. 2010). It is recommended that a patient with dementia undergoes one of the neuroimaging examinations at least once during the course of the therapy (Cummings 2004). sMRI can reveal ventricular

enlargement and brain atrophy, in specific decreased grey matter in the parahippocampal gyrus, the hippocampus, the amygdala, the posterior association cortex and subcortical nuclei (Hampel et al. 2010). fMRI measures the change of neuronal activity upon rest and stimulation. Neuronal activity is impaired in AD (Hampel et al. 2010). The most common method is blood-oxygen level-dependent fMRI, which measures deoxyhemoglobin concentration, which represents the activity of neurons (Reitz et al. 2011). PET measures metabolism levels of different substances. FDG-PET using ^{18}F -2-fluoro-2-deoxy-D-glucose (FDG) shows the levels of neuronal glucose consumption, the process that represents neuronal activity (Reivich et al. 1979). In AD patients neuronal activity is decreased which is proved by reduced FDG uptake in tempoparietal brain areas. Brain $\text{A}\beta$ accumulation can be visualized by PET with the help of ^{11}C -labelled Pittsburg compound B (Lockhart et al. 2007). Amyloid-PET gives the possibility to see $\text{A}\beta$ plaques distribution *in vivo*, which seems to be independent from the structural changes of the brain in AD (Jack et al. 2008). However, this imaging method can depict only $\text{A}\beta$ in classical and diffuse plaques and in cerebrovascular amyloid angiopathy, but not soluble and oligomeric amyloid peptides (Hampel et al. 2010).

2.2 Epidemiology of AD

Western Europe has the leading position after North America in prevalence of dementia in individuals older than 60 years (5.4% and 6.4%, respectively), followed by Latin America (4.9%), China and its western-Pacific neighbours (4.0%). The annual regional dementia incidence rates (per 1,000 individuals in the population) were estimated to be 10.5 for North America, 8.8 for Western Europe, 9.2 for Latin America, and 8.0 for China and its western-Pacific neighbours (Reitz et al. 2011), although the correct numbers might be higher because not all AD cases are diagnosed by general practitioners and family members in Europe and Asia (Callahan et al. 1995; Ross et al. 1997). As the epidemiological data show, the incidence rate for dementia increases exponentially with age, with the most notable rise occurring through the seventh and eighth decades of life (Reitz et al. 2011).

Moreover, Brookmeyer et al. warn us about a coming global epidemic of AD, predicting that in 2050 1 in 85 persons worldwide will have the disease if no measures of prevention or successful treatment would be developed (Brookmeyer et al. 2007).

2.3 Symptoms of AD

Dementia is a clinical syndrome that can characterize a variety of diseases, such as AD, vascular dementia, dementia with Lewy bodies and frontotemporal dementia (Kawas 2003).

Clinical presentation of AD includes progressive loss of memory and cognitive functions leading to aphasia, apraxia, and agnosia, deficits of visual perception; secondary symptoms such as depression, apathy, hallucinations; increasing incapability of accomplishing routine tasks (Cummings 2004; Kumari and Heese 2010; McKhann et al. 1984). A histological examination of brain tissues reveals extracellular amyloid plaques, composed of A β protein and intracellular neurofibrillary tangles, made up from Tau-protein, loss of neurons and white matter, inflammation and oxidative damage (Kumari and Heese 2010).

2.4 Risk factors of AD

The impact of different risk factors in prevalence and incidence of AD is summarized in Table 2.

Table 2: Risk factors of Alzheimer’s disease

Risk factors of AD	Percentage/Odds Ratio	Reference
Age	2-fold every 5 years	Hirtz et al. 2007
APOE4 genotype	4-fold for heterozygous; 10-fold for homozygous	Carter et al. 2001; Eisenstein 2011
Diabetes mellitus	35% of the Mayo Clinic Alzheimer Disease Patient Registry had diabetes and 46% impaired fasting	Janson et al.2004

	glucose;	
Atrial fibrillation	Significant positive associations of atrial fibrillation with both dementia and impaired cognitive function was observed (age- and sex-adjusted odds ratios, 2.3 [95% confidence interval, 1.4 to 3.7] and 1.7 [95% confidence interval, 1.2 to 2.5]), respectively).	Ott et al. 1997
Arterial hypertension	2-fold increased risk in people with moderate and high systolic blood pressure	Havlik et al. 2002
General anesthesia	In a population of 80 years and older odds ratio is 3.22; 95% Confidence Interval: 1.03–10.09; p < 0.05;	Planel et al. 2009; Bucfill et al. 2009
Down Syndrome	8% (between 35 and 49 years) 55% (between 50 and 59 years) 75% (over 60 years old)	Lai and Williams 1989
Mild cognitive impairment (MCI)	12% of patients with MCI convert to AD every year	Petersen 2004

2.4.1 Age

Age is one of the main risk factor of AD. The risk to develop the disease is doubled every 5 years after 65 years of age (Hirtz et al. 2007).

2.4.2 Apolipoprotein E4 genotype

ApoE protein is the main cholesterol transporting protein into the central nervous system. It has been defined that ApoE plays a role in clearance of A β

from the brain (Eisenstein 2011). In addition, it influences A β generation, formation of neurofibrillary tangles, neuronal survival and lipid homeostasis (Rebeck et al. 2002). There are 3 variants of APOE gene: APOE2, APOE3 and APOE4. ApoE2 and ApoE3 proteins are stable and have protective functions against AD, whereas APOE4 gene encodes the least stable protein that impairs transport of cholesterol and amyloid- β within the brain (Eisenstein 2011; Rebeck et al 2002). People, who are heterozygous for this gene, are in fourfold risk to develop the disease, whereas homozygous people are in tenfold risk (Carter et al. 2001; Eisenstein 2011). APOE4 is found in 14-16% of individuals. APOE4 holders have a higher risk of myocardial infarction (Kato et al. 2011) and stroke (Mielke et al. 2011). ApoE4 genotyping to predict risk of AD development in asymptomatic people, whose relatives suffer from AD, is not recommended in the routine practice (Post et al. 1997), although a prospective randomized controlled study indicates that genotyping does not induce psychological disorders as anxiety and depression in people who were informed about their risks to get the disease (Green et al. 2009).

2.4.3 Other genetic factors

Three genes are associated with familial AD (early-onset AD): amyloid precursor protein (APP), presenilin 1 and 2 (PS1 and PS2) (Suh and Checler 2002). APP is located on 21 chromosome, its mutations lead to accumulation of A β (Selkoe 2008). The PS1 and PS2 genes are located on chromosomes 14 and 1, respectively. 182 AD-related mutations have been found in PS1 and 14 mutations in PS2 gene (Reitz et al. 2011). Mutations of PS1 gene lead to excessive A β production by enhancing proteolytic processing of APP (Schenk 2000). PS2 mutation caused early A β accumulation and learning and memory impairment in APP-transgenic mice (Toda et al. 2011).

2.4.4 Cerebrovascular risk factors

All the risk factors for cardiovascular diseases, such as arterial hypertension, hypercholesterolemia, stroke, homocysteine, smoking, atrial fibrillation are also risk factors for Alzheimer's disease (de Toledo Ferraz Alves et al. 2010).

Homocysteine, a natural product of biosynthesis of cysteine, is an independent risk factor for coronary and cerebral atherosclerosis, stroke (Refsum et al. 1998), cognitive decline (Nurk et al. 2005) and onset of AD (Seshadri et al. 2002).

2.4.4.1 Hypercholesterinemia

As experimental studies show, cholesterol takes part in A β production and depletion of membrane cholesterol inhibits secretase cleavage of APP, thereby lowering A β 1–40 and A β 1–42 accumulation (Reitz et al. 2011). Statins, which reduce levels of cholesterol in blood, were thought to be beneficial in treatment of AD, but the results of the clinical studies were controversial, depending on the initial cholesterol level and stage of the disease (Sparks 2011). Statins were shown to be protective in AD patients with elevated cholesterol levels, meanwhile neither beneficial nor harmful effects of statins on patients with normal levels of cholesterol were observed (Sparks 2011).

2.4.4.2 Arterial hypertension

Hypertension is associated with the increased risk of the onset of AD (Reitz et al. 2007). Framingham Heart Study revealed the positive correlation between the high levels of blood pressure and worse cognitive functions in AD patients (Farmer et al. 1990). Midlife hypertension (till 60 years of age) is associated with cognitive impairment in late years, although the results of the studies relating to the association between late-life hypertension and cognitive decline and dementia remain inconsistent (Reitz et al. 2011). The randomized placebo-controlled studies, showing protective effects of antihypertensive drugs on the incidence of cognitive impairment and AD, are in line with previous observations.

2.4.4.3 Diabetes mellitus

Type 2 diabetes is twice common in people with AD compared to those without AD (Janson et al. 2004). AD was proposed to be named as type 3 diabetes

because of the similarities of glucose utilization pathways in diabetes mellitus and AD (Biessels et al. 2006).

2.4.4.4 Atrial fibrillation

An association between dementia and atrial fibrillation has been observed. Rotterdam study reports that the incidence of atrial fibrillation was significantly higher in people with AD (Ott et al. 1997).

2.4.4.5 Smoking

Influence of smoking on the incidence of AD is debatable (Breteler 2000). Some studies showed no correlation of AD with smoking, although studies presenting negative as well as positive correlation of smoking with AD have been published as well (Anstey et al. 2007). The conflict of the results comes from the different age of study populations' and study bias (Breteler 2000). Nevertheless, the protective role of smoking for the carriers of APOE4 was determined (Prince et al. 1994; Wang et al. 1999).

2.4.4.6 Chronic kidney insufficiency

Recent findings indicate that chronic kidney insufficiency is associated with the rate of cognitive decline. Healthy aged people with lower glomerular filtration rate at baseline had more profound cognitive decline (Buchman et al. 2009).

2.4.5 Platelet activation

Recent findings indicate that there is an association between platelet activation and AD. Platelets contain molecules involved in the amyloid- β cascade such as APP, Amyloid- β , alpha-secretase and beta-secretase (Colciaghi et al. 2004). Alterations of these proteins and enzymes can be detected in platelets from the earliest clinically detectable disease stage of AD (Colciaghi et al. 2004). Noteworthy, platelets are the primary source of Amyloid- β peptides in human blood (Casoli et al. 2007; Chen et al. 1995; Li et al. 1998). Numerous studies have described altered platelet functions in AD (Sevush et al. 1998; Colciaghi

2004). Prodan has demonstrated a positive correlation between the degree of platelet activation and the risk for progression of mild cognitive impairment to AD (Prodan et al. 2011). Several studies showed a positive correlation between the degree of platelet activation and the rate of cognitive decline in AD patients (Prodan et al. 2008; Stellos et al. 2010). Cognitive decline in AD patients has been associated with an AD-specific reduction of the platelet APP isoform ratio (Baskin et al. 2000; Borroni et al. 2004; Liu et al. 2007). The potential role of platelets in development and progression of AD is depicted in Figure 1 (Laske et al. 2012).

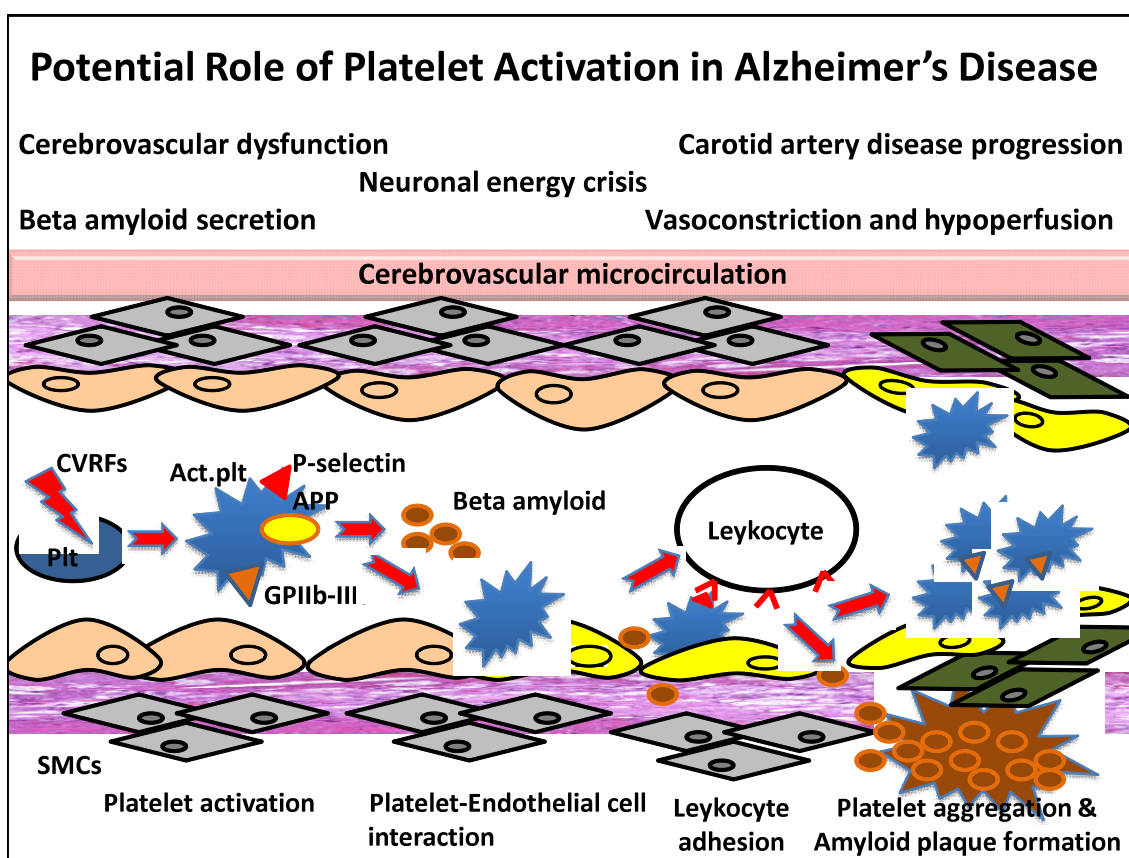


Figure 1: Potential Role of Platelet Activation in Alzheimer's Disease. Reprint from Laske et al., 2012. Cerebrovascular risk factors activate platelets, they in turn secrete Amyloid- β in blood. Activated platelets interact with endothelial cells inducing endothelial dysfunction. These processes induce vasoconstriction, endothelial dysfunction and amyloid plaque formation. SMCs-smooth muscle cells; CVRFs- cerebrovascular risk factors; Act.plt- activated platelet; GPIIb/IIIa - glycoprotein GPIIb/IIIa; APP- amyloid precursor protein.

2.4.6 Down Syndrome

People with Down syndrome who survive to live to their forties develop dementia and in an autopsy of such patients plaques in brain resembling Alzheimer's disease are found (Lai and Williams 1989). In an observational study of the population with Down syndrome the prevalence of dementia was 8% between 35 and 49 years, 55% between 50 and 59 years, and 75% of those over 60 years old (Lai and Williams 1989).

2.4.7 Mild Cognitive Impairment (MCI)

Mild cognitive impairment is a condition when there is either subjective or objective memory loss that does not disturb everyday life, but the memory impairment is more advanced as estimated for normal ageing. MCI is an independent predictor of the development of AD, and 12% of patients with MCI convert to AD every year (Petersen 2004).

2.4.8 Other risk factors

The other risk factors of development of AD include general anesthesia and chronic activation of glucocorticoid axis (Csernansky et al. 2006).

2.5 Pathophysiology of Alzheimer's Disease

A β plaques are a hallmark of AD. They are found in a histological examination of the brain tissue taking the diagnosis from the probable AD to the definite one. A β is a peptide cleaved from amyloid precursor protein by β - and γ -secretases. It exists in two forms: A β 1-40 and 1-42 consisting of 40 and 42 aminoacids correspondingly (Haass and Selkoe 1993). A β 1-42 is believed to be more dangerous, because it is toxic to neurons, prone to aggregate and form the plaques. Mutations in the gene, coding A β precursor protein, cause the early onset of the disease (Chartier-Harlin et al. 1991). The schematic process of A β biosynthesis is presented in Figure 2.

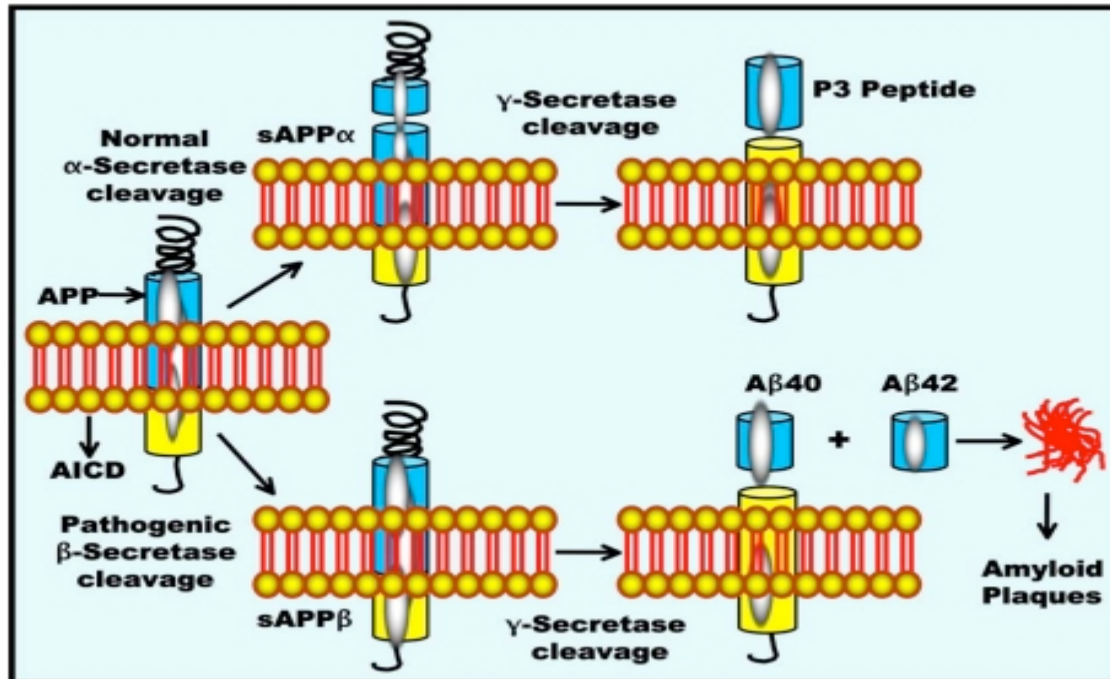


Figure 2: Amyloid plaque development (Reprint from Kumari, 2010). Upon non-amyloidogenic cleavage of APP by α - and β -secretases, soluble APP α (sAPP α), P3 peptide and AICD are released. β -secretase induces amyloidogenic APP metabolism, resulting in sAPP β production, followed by generation of A β 1-40 and 1-42 with the help of γ -secretase. APP-amyloid precursor protein, A β -amyloid- β ; sAPP α - soluble APP α ; sAPP β - soluble APP β ; AICD-Amyloid precursor protein Intracellular Cytoplasmic/C-terminal Domain.

The modern understanding of pathophysiology of AD is depicted in the so called A β cascade hypothesis. This cascade is triggered by the excessive production and accumulation of A β (Cummings 2004). Several factors, such as ApoE4 genotype (Carter, Dunn et al. 2001), mutations in the APP (Selkoe 2008), PS1 and PS2 genes lead to increased A β production (Schenk 2000; Toda et al. 2011). A β can self-aggregate *in vitro*, and these aggregates mediate neurotoxicity and are a compound of A β plaques (Price, et al 1998). Abundant Amyloid- β in turn shows a cytotoxic effect on neurons (Cummings 2004), endothelial cells and smooth muscle cells (Paris et al. 2004; Van Nostrand et al. 1998) and causes secondary changes in the brain. Secondary consequences include formation of neurofibrillary tangles, oxidation and lipid peroxidation, glutamatergic excitotoxicity, inflammation and activation of apoptotic cell death (Cummings 2004). A β cascade is presented in Figure 3.

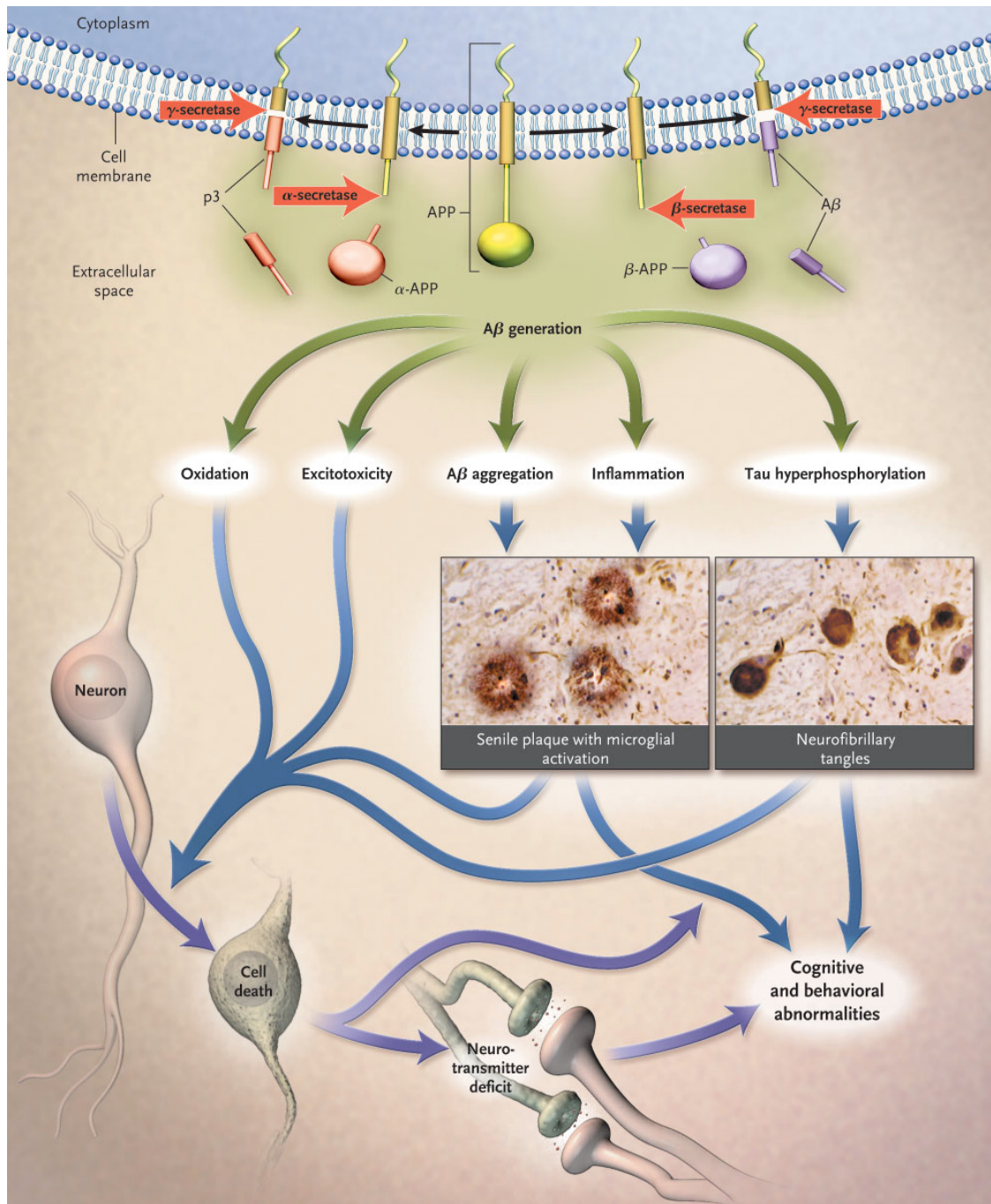


Figure 3: Aβ cascade. Reprint from Cummings, 2004. Explanations of the picture are in the text.

In addition, there are also other hypothesis of AD development such as a cholinergic (Francis et al. 1999), tau- (Kumari and Heese 2010) and an oxidative stress hypothesis (Markesbery 1997).

2.6 Treatment of AD

Till now there has not been discovered a curative therapy of AD. The treatment is inclined to stop the progression of disease and relieve its symptoms. It includes neuroprotection, cholinesterase inhibitors, medications to relieve secondary symptoms (behavioral disturbance) and non-pharmacological strategies (Cummings 2004).

It is intriguing to try to eliminate the accumulation of A β , reducing the multiple damaging actions of this protein in the brain tissue. To date, several anti-amyloid therapies (anti-amyloid vaccinations, passive immunization, inhibitors of β - and γ -secretases) are under development (Cummings 2004).

Neuroprotective strategies against oxidative stress and inflammation, caused by A β , consist of antioxidants vitamin E and C (Engelhart et al. 2002). Memantine, an aspartate antagonist, is thought to prevent glutamatergic excitotoxicity in combination with inhibitors of cholinesterase (Parsons et al. 1999; Tariot et al. 2004). A glutamatergic excitotoxicity is caused by overactivation of glutamine receptors leading to neuronal damage.

Actual gold standard of treatment of mild to moderate AD is acetylcholinesterase (AChE) inhibitors, such as tacrine, donepezil, rivastigmine, galantamine (Small et al. 1997). The mechanism of their action is the blockade of an enzyme that metabolizes acetylcholine, a neurotransmitter deficient in AD patients (Kawas 2003). Donepezil has shown to increase cognitive functions in AD patients assessed by ADAS-Cog Score (Rogers et al. 1998). The initial dose of donepezil is 5 mg per day and after 1 month the dose is usually increased to 10mg per day (Kawas 2003). Behavioural disturbance, which occur in approximately 80% of AD patients, can be managed with non-pharmacological approaches, as listening to music or to the taped voices of the family members, walking and light exercise, sensory stimulation and relaxation (Doody et al. 2001b).

The medications that influence cognition or have side-effects on central nervous system, such as sleeping pills, over-the-counter medications for influenza,

antianxiety medications should be minimized (Kawas 2003). The physician should determine the moment when a patient should stop driving. It is noteworthy to mention that a patient forgets the way home before he or she becomes incapable of the driving process itself (Kawas 2003).

The guidelines developed by the American Academy of Neurology provide the standards of diagnostics and treatment of AD. According to them, an AChE inhibitor should be administered to patients with mild to moderate AD, although the average benefit is limited. Estrogen hormonal therapy should not be administered to treat AD. Antipsychotic medications should be prescribed to treat agitation and psychosis. The guidelines include also the usage of the vitamin E and antidepressants to slow cognitive decline and treat depression respectively. The guidelines give a recommendation of implementation of educational programs for families and health care workers (Doody et al. 2001b; Knopman et al. 2001).

2.7 Stem Cell Factor

Stem cell factor (SCF) also known as mast cell growth factor or kit-ligand, is a hematopoietic cytokine that mediates its effects through its receptor c-kit (Huang et al. 1990). SCF is encoded by the Steel locus on chromosome 12 (Geissler et al. 1991). Mutations in genes, coding SCF and its receptor, show that SCF plays an essential role during development in utero (Broudy 1997). SCF plays role in hematopoiesis, spermatogenesis and melanogenesis (Broudy 1997). SCF intracellular signalling induces activation of phosphatidylinositol (PI) 3-kinase, which leads to Akt-mediated phosphorylation of Bcl-2 related protein (BAD). SCF-induced phosphorylation of BAD suppresses cell apoptosis, promoting cell survival (Blume-Jensen et al. 1998). SCF signals through many other pathways, such as phospholipase C-gamma, Src kinase, Janus kinase, Signal Transducers and Activators of Transcription (STAT), mitogen activated protein (MAP) kinase pathway (Reber et al. 2006). SCF has synergistic activity with other cytokines in megakaryocytopoiesis (Briddell et al. 1991).

2.7.1 Structure

SCF can be present as a soluble and a transmembrane protein (Flanagan et al. 1991). The soluble form has a molecular weight of 18.500 Da (Flanagan, Chan et al. 1991). Both forms are biologically active (Anderson et al. 1990), although the membrane-bound form has a longer life span and can be down-regulated by soluble forms (Miyazawa et al. 1995). The fact that SCF exists in two active forms explains the diversity of the possible co-interactions of its forms with the receptor under different circumstances. The membrane-bound form provides cell-cell interactions. In the immortalized stromal cell line both soluble and transmembrane protein induced cell proliferation, but the effect of transmembrane SCF was significantly longer (Toksoz et al. 1992). The schematic picture of the SCF structure is presented in Figure 4.

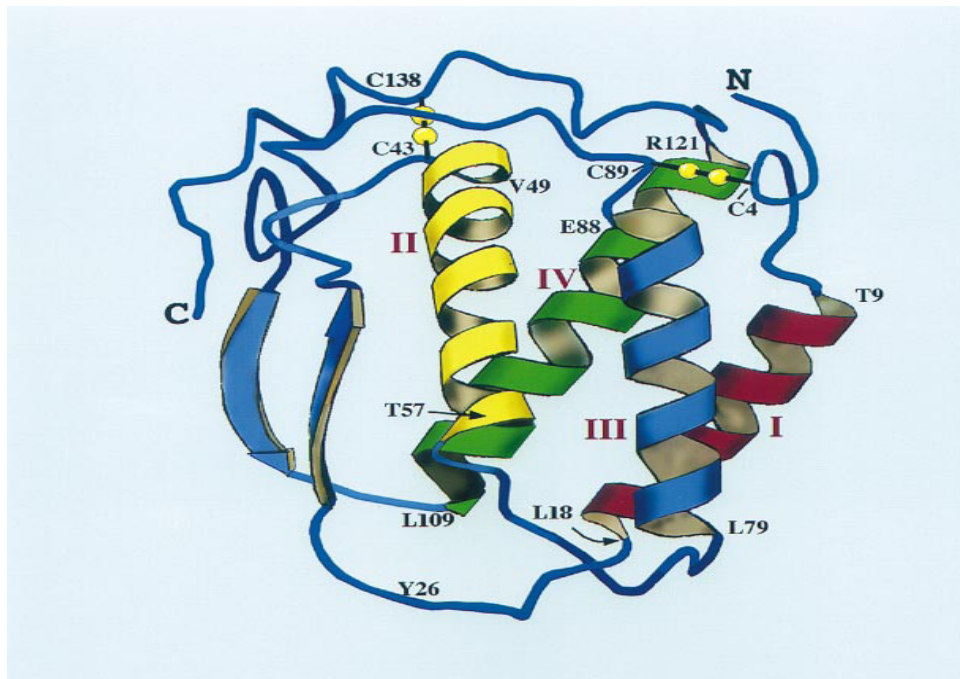


Figure 4: Schematic structure of SCF. Reprint from Broudy et al., 1997. The SCF four helix bundle with two long overhand loops is shown as a ribbon diagram. The location of the two intramolecular disulfide bonds is shown in yellow, and the helix boundaries are indicated in the single amino acid code.

2.7.2 Production

SCF is produced by endothelial cells, fibroblasts, marrow stromal cells (Linenberger et al. 1995), epithelial cells of the gut (Klimpel et al. 1995), neurons, microglia and astroglia (Zhang and Fedoroff 1997).

2.7.3 Role in human development

Absence of SCF protein results in death in utero or in the perinatal period with severe macrocytic anemia (Broudy 1997). Absence of c-kit receptor kinase activity also causes perinatal death with severe macrocytic anemia (Geissler et al 1991).

2.7.4 Role in hematopoiesis

SCF has been shown to induce hematopoiesis by mobilisation, survival, proliferation and differentiation of hematopoietic stem cells (Broudy 1997). SCF acts alone and in synergy with other hematopoietic growth factors (Williams et al. 1992). SCF provides survival for hematopoietic stem cells (Keller et al. 1995). It suppresses apoptosis of mature hematopoietic cells through different mechanisms (Blume-Jensen et al. 1998; Carson et al. 1994; Gommerman and Berger 1998). Decreased levels of SCF induce haematological disorders, such as sickle cell anemia and mastocytosis (Boissan et al. 2000; Croizat and Nagel 1999). SCF receptor C-kit is expressed on many blood cells, like hematopoietic stem cells, myeloid progenitor cells, dendritic cells, mast cells, pro-B and pro-T cells (Qiu et al. 1988).

2.7.5 Role in adult neurogenesis

SCF has neuroprotective effects (Dhandapani et al. 2005). SCF has been reported to promote adult neurogenesis and to modulate microglia (Abrous et al. 2005; Zhang and Fedoroff 1999). SCF has the possibility to get to central nervous system through blood-brain barrier (BBB) (Zhao et al. 2007), where its receptor is expressed in neuroproliferative zones of the brain (Jin et al. 2002a). An extensive thorough insight in the role of SCF in neurogenesis will be presented in Discussion.

3 Objectives and goals

3.1 Objectives

SCF has been proved to stimulate adult neurogenesis, a newly described process of development of new neurons in the adult brain. AD is a neurodegenerative disorder that is characterised by the neuronal cell loss and impaired neurogenesis. The correlation between SCF in plasma and the rate of cognitive decline in AD patients has not been established till now.

3.2 Goals

The goals of the study were to investigate the possible association of cognitive decline and SCF plasma levels in AD patients at baseline and after 1-year follow-up period.

4 Materials and methods

4.1 Subjects and clinical assessment

Forty mild to moderate dementia of AD type outpatients from the Memory clinic at the University Hospital of Psychiatry and Psychotherapy Tübingen were included in the “Pythia” study (Pythia commonly known as the ancient Oracle of Delphi, Greece). The 40 AD patients described in the present study belong to a group of 45 patients which have been consecutively recruited at the Memory clinic. From these originally 45 AD patients, three patients died before the study was completed, one patient denied to participate any longer in the study and one patient did not live any longer in Germany and therefore a follow-up MMSE score could not be obtained.

All AD patients fulfilled the diagnostic criteria of probable AD according to the Diagnostic and Statistical Manual of Mental Disorders, fourth edition (DSM-IV) (APA 1994), the ICD-10 Classification of Mental and Behavioural Disorders (WHO 1992) and the criteria of the National Institute of Neurologic and Communicative Disorders and Stroke and the Alzheimer`s Disease and Related Disorders Association (NINCDS-ADRDA) (McKhann et al. 1984).

The clinical severity of cognitive impairment was assessed by the Mini-Mental State Examination (MMSE) (Folstein et al. 1975). In the present study, patients with mild to moderate dementia (mean MMSE score \pm SD 18.9 \pm 4.1) were examined. A computed tomography or magnetic resonance imaging of the brain were also performed to exclude other causes for dementia such as stroke or bleeding diathesis. Patients with other causes of dementia, with current or a history of depression or psychosis, with major physical illness, alcohol or substance abuse or use of psychoactive medications were not included at the study.

The study was performed according to the ethical principles of the Declaration of Helsinki (sixth revision, 2008) and was approved by the local institutional ethics committee. A written informed consent from AD patients themselves or by legally authorized representatives was obtained.

4.2 Sample Collection

Blood was obtained in the morning (8.00-9.00 A.M.; in the fasting state) after 30 min rest into heparin plasma tubes and then immediately placed in ice for 20 min. The tubes were then centrifuged at 2500 x g for 30 min at 4°C, and the top third of the volumes of the resultant plasma supernatants were collected and frozen at -20°C for evaluation.

4.3 ELISA

Soluble SCF levels in plasma were measured using an ELISA kit (R&D Systems, Wiesbaden, Germany) according to the manufacturer's instructions. Every ELISA Kit contained a 96-microplate pre-coated by the manufacturer with a monoclonal antibody (Ab) specific for the antigen. The ELISA Kits for detection SCF (cat no. DCK00) work with a monoclonal Ab from mouse as capture and with a polyclonal Ab from goat conjugated to horseradish peroxidase (HRP) as detection Ab. All concentrations of reagents in these ready-to-use ELISAs are proprietary information of the manufacturer. Recombinant soluble E. coli expressed SCF (KL-1 variant) was reconstituted in an animal serum with preservatives named Calibrator diluent (5 ml) and from this stock solution a dilution series was produced. SCF stock solution: 2000

pg/ml; dilution series: 2000–31.2 pg/ml. The plasma samples in these tests needed no dilution. The assay procedure was as follows. First, 100 µl of a buffered protein base (assay diluent) was added to each well, followed by pipetting 100 µl of standard or undiluted sample (serum or plasma) in duplicates. Next, the plates were incubated for 2 h at room temperature. After the incubation period, each well was aspirated and washed with 400 µl wash buffer per well, containing buffered surfactant, repeated 3–4 times. Subsequently, 200 µl of conjugate solution were added to each well, containing a recombinant lyophilized human polyclonal antibody conjugated to HRP. Again, the microplate was incubated for 2 h at room temperature. After the incubation period the wash step was repeated as described above. Then 200 µl freshly prepared substrate solution composed of stabilized hydrogen peroxide and tetramethylbenzidine as chromogen was added to each well and incubated for 20–30 min at room temperature protected from light. A blue colour developed in proportion to the amount of antigen in the sample/standard. Finally, the colour development was stopped by addition of 50 µl of 2 N sulphuric acid, which resulted in a change of colour from blue to yellow. The optical density was determined within 30 min using a microplate reader (Tecan Sunrise, Switzerland) at a wavelength of 450 nm (correction at 540 nm). All samples and standards were measured in duplicates, and the means of the duplicates were used for statistical analyses. The intra- and interassay coefficients of variation of SCF in AD patients were <10%.

5 Data analysis

The data are presented as the mean \pm standard deviation (SD). Continuous variables were tested for normal distribution with the Kolmogorov-Smirnov test. The two-tailed t-test was used to assess differences between two groups in case of normal distribution. The Mann-Whitney U-test was used to assess differences between two groups in case of non-normal distribution. The Fisher's exact test was used to compare two groups in case of categorical variables. Correlations between variables were determined using Spearman test. In case of significant correlations between the rate of cognitive decline and examined parameters, we performed a multiple linear regression analysis to identify significant independent predictors for the rate of cognitive decline. In a next step, previously published factors from our cohort that predict cognitive decline were included in the multivariate analysis (Laske et al 2011; Laske et al 2010; Stellos et al 2010).

A binary regression analysis was done to evaluate the performance of SCF plasma levels to differentiate between fast versus slow cognitive decliners.

Significance for the results was a priori set at $p \leq 0.05$. All statistical analyses were carried out using the statistical analysis software package SPSS 17.0 (Munich, Germany).

6 Results

SCF plasma levels are decreased in AD patients with fast cognitive decline compared to AD patients with slow cognitive decline

The baseline demographic and laboratory parameters of AD patients with fast versus slow cognitive decline are displayed on Table 3. AD patients with fast cognitive decline were significantly younger ($p < 0.001$) and showed a trend towards higher baseline MMSE scores (according to a Mann-Whitney U test; $p = 0.05$) than the slow cognitive decline group (Table 3).

All the patients were followed up prospectively and were re-examined clinically 1 year later (range 11-13 months). Among AD patients, 28 patients showed a slow cognitive decline (decrease of MMSE score ≤ 4 /year) and 12 patients displayed a fast cognitive decline (decrease of MMSE score > 4 /year) (Table 3). The threshold was set at 4 points/year according to a previously published paper by Doody (Doody et al. 2001a).

AD patients with fast cognitive decline showed significantly lower baseline SCF plasma levels compared to AD patients with slow cognitive decline (fast vs. slow cognitive decline: mean \pm SD: 1051.1 \pm 178.7 versus 1237.9 \pm 274.2 pg/ml; $p = 0.037$) after 1 year follow-up period (Table 3; Figure 5). SCF plasma levels in all AD patients did not significantly correlate with age ($r = 0.210$; $p = 0.194$) or with MMSE scores at baseline ($r = -0.206$; $p = 0.202$).

The rate of cognitive decline (defined as the change Δ of MMSE scores from baseline to 1 year follow-up) was significantly associated with SCF plasma levels ($r = 0.315$; $p = 0.048$; Figure 5), age ($r = 0.315$; $p = 0.040$) and initial MMSE scores ($r = -0.378$; $p = 0.012$). According to a multiple linear regression analysis, SCF plasma levels ($B = 0.279$; $p = 0.041$), age ($B = 0.393$; $p = 0.006$) and MMSE scores ($B = -0.420$; $p = 0.002$) at baseline were independent predictors for the rate of cognitive decline. In multiple linear regression analysis, these 3 factors together explained 38 % of the corrected variance (Corr. $R^2 = 0.379$) of the rate of cognitive decline (Table 4).

Table 3: Comparisons of clinical and laboratory features between the slow and fast cognitive decliners among Alzheimer's disease (AD) patients.

Characteristics	AD patients with slow cognitive decline (n=28)	AD patients with fast cognitive decline (n=12)	p value
Female/Male, n	21/7	5/7	0.071 ^a
Age (years), mean±SD	74.8±7.7	71.8±9.0	<0.001 ^c
Education (years), mean±SD	11.3±3.1	11.8±2.6	0.711 ^b
MMSE, mean±SD	18.1±3.8	20.8±4.4	0.050 ^c
Change MMSE baseline to 1 year follow-up, mean±SD	0.4±2.7	-11.2±4.4	<0.0001 ^c
BMI, mean±SD	24.4±2.8	24.6±3.7	0.587 ^c
ChEI treatment, n (%)	22 (78.6)	10 (83.3)	1.0 ^a
SCF, mean±SD (pg/ml)	1237.9±274.2	1051.1±178.7	0.037 ^b

^aFisher's exact test; ^btwo-tailed t-test for unpaired samples; ^cMann-Whitney U-test for unpaired samples; MMSE, Mini-Mental State Examination; BMI, body mass index; ChEI, cholinesterase inhibitor.

Table 4: Predictors of change in MMSE from baseline to one year follow-up in multiple linear regression analysis

Model						Coefficients		
Variance			Analysis			Analysis		
DV	R^2	Corr. R^2	F	df	p	B	T	p
SIV								
Change MMSE baseline to 1 year follow-up	0.427	0.379	8.940	3	<0.0001			
SCF						0.279	2.119	0.041
MMSE						-0.420	-3.271	0.002
Age						0.393	2.950	0.006
Change MMSE baseline to 1 year follow-up	0.694	0.635	11.772	5	<0.0001			
SCF						0.412	3.401	0.002
MMSE						-0.304	-2.381	0.025
Age						0.362	3.084	0.005

DV- dependent variables; SIV- Significant independent variables.

Including SCF plasma levels, age and MMSE score at baseline and the previously identified markers: BDNF serum levels, A β 1-42 plasma levels and the degree of platelet activation (activated GP IIb-IIIa and P-selectin) (Laske et al. 2011; Laske et al 2010; Stellos et al 2010) in the multivariate analysis, the only independent predictors of the rate of cognitive decline in our study cohort were age, MMSE scores at baseline, SCF plasma levels, BDNF serum levels and activated GPIIb/IIIa, explaining together 64 % of the corrected variance (Corr. R 2 = 0.635) of the rate of cognitive decline (Table 4).

According to a binary regression analysis, SCF plasma levels allow differentiation between AD patients with fast versus slow cognitive decline with 72.5 % accuracy, 66.7 % positive predictive value and 73.0 % negative predictive value.

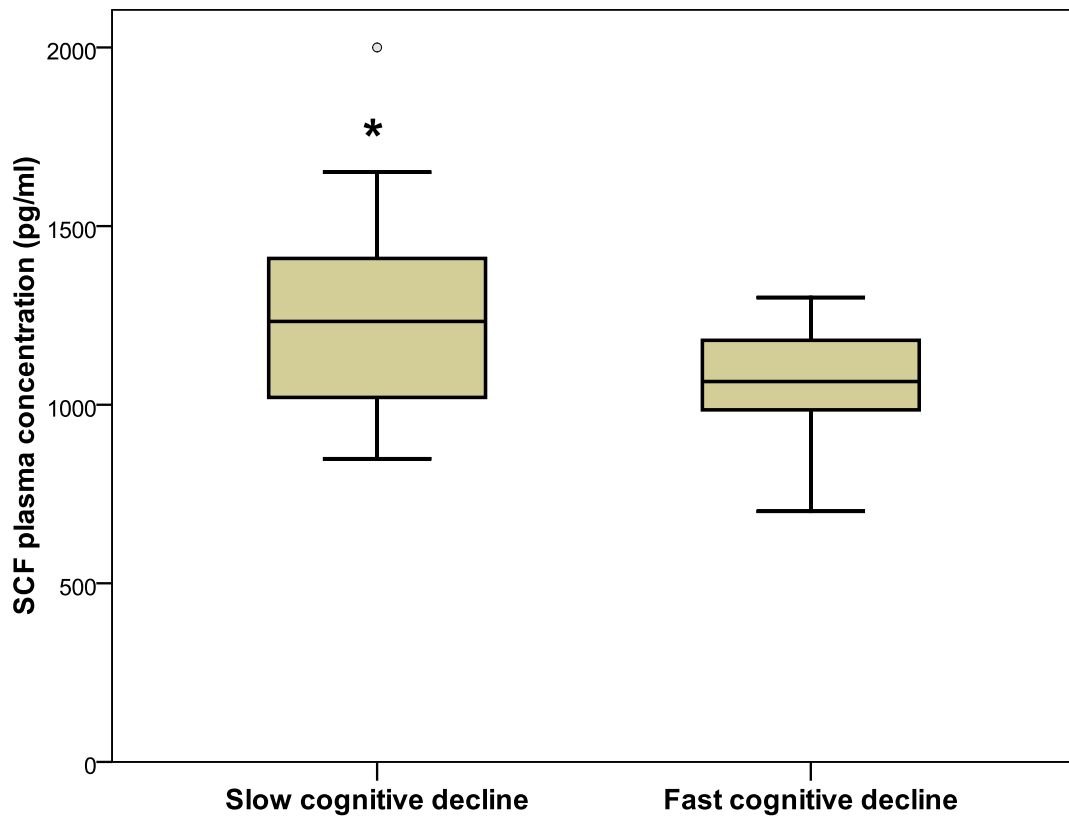


Figure 5: Plasma SCF concentrations are significantly higher in patients with slow cognitive decline: Boxplot showing SCF plasma levels in Alzheimer's disease patients with fast versus slow cognitive decline defined after 1 year follow-up period (*p=0.037)

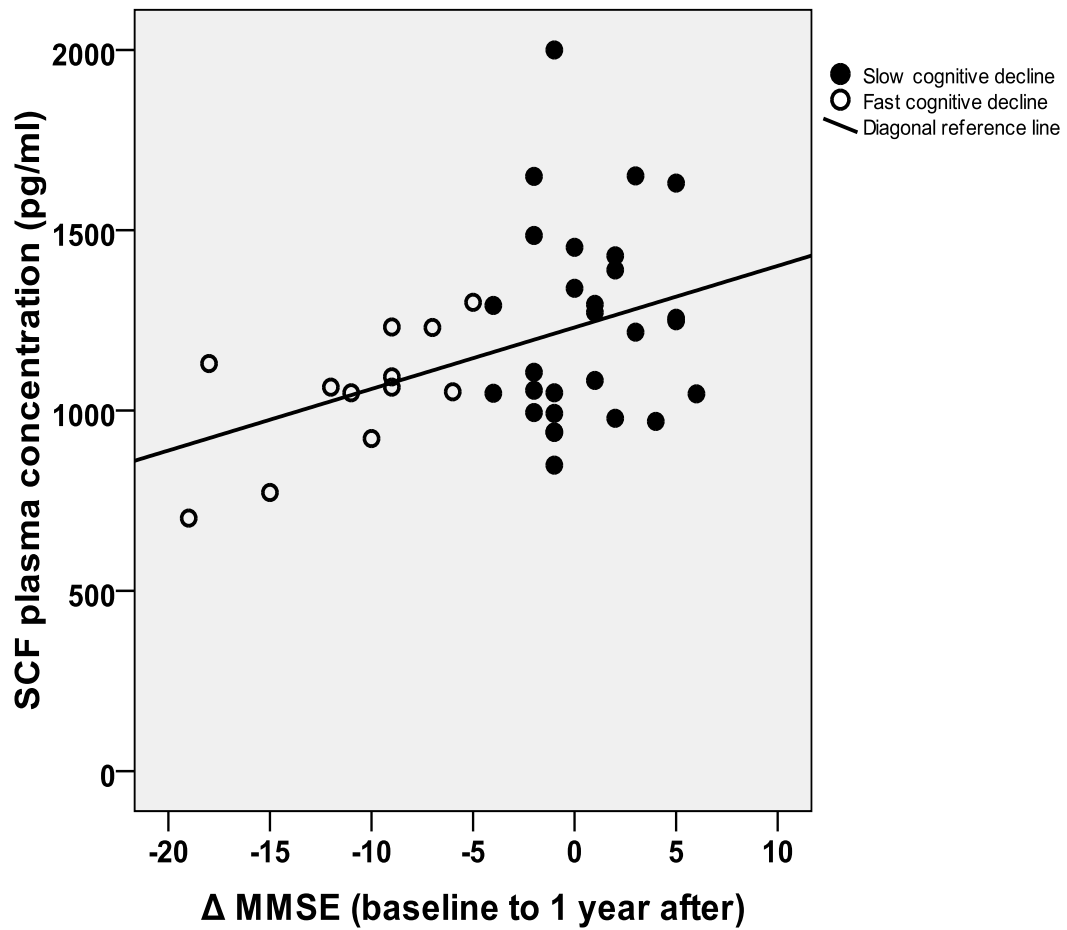


Figure 6: Correlation between change of MMSE Scores (Δ MMSE) and SCF plasma level. Boxplot showing correlation between the change of Mini-Mental State Examination (MMSE) score after 1 year and baseline SCF plasma levels in AD patients ($r=0.315$; $p=0.048$).

7 Discussion

The major findings of the present study are: 1) baseline SCF plasma levels are significantly higher in AD patients with slow cognitive decline compared to AD patients with fast cognitive decline during one year follow-up period; 2) baseline SCF plasma levels are significantly associated with the rate of cognitive decline in AD patients; 3) age, MMSE scores at baseline, SCF plasma levels, BDNF and activated GP IIb/IIIa are independent predictors of the rate of cognitive decline in the study cohort.

SCF- a novel candidate biomarker that predicts cognitive decline in AD patients?

Summarizing the findings of the present study, we identified plasma SCF as a candidate biomarker that predicts the rate of cognitive decline in AD patients. These findings are very important in determining the AD prognosis. The rate of cognitive decline can vary greatly from almost stable MMSE scores to large cognitive decline over one year (Kraemer et al. 1994), and prediction which population of AD patients declines rapidly is of interest of both clinicians and patients together with their relatives. Knowing that a certain person is at higher risk to develop fast cognitive decline will allow the physician to start more aggressive treatment, which may be beneficial to such a patient. At the same time this knowledge will give a patient the possibility to arrange his or her business matters and make decisions about his/her future.

In the “Pythia” cohort the patients with fast cognitive decline are significantly younger than those with slow cognitive decline. This observation stands in line with the work of L. Teri, (Teri et al. 1995), who reported that younger age and lower SCF levels are associated with fast cognitive decline.

Other biomarkers as predictors of AD progression

The previous works of Prof. Laske and co-workers have shown, that BDNF serum levels, A β 1-42, and degree of platelet activation are associated with the

rate of cognitive decline in the cohort “Pythia”, the same cohort used in the present study (Laske et al. 2011; Laske et al. 2010; Stellos et al. 2010).

In order to evaluate the independent predictors of the rate of cognitive decline in our cohort, multiple linear regression analysis was performed including the variables: age, MMSE scores at baseline, SCF plasma levels, BDNF serum levels, activated GPIIb/IIIa (as platelet activation marker). According to this analysis, age, MMSE scores at baseline, SCF plasma levels, BDNF serum levels, activated GPIIb/IIIa turned out to be significant independent predictors of the rate of cognitive decline in AD patients in the study cohort.

The most studied predictors of the rate of cognitive decline are A β 1-42, tau and phosphorylated tau proteins, which are measured in CSF. The measurement of these biomarkers in CSF has many disadvantages, such as need of spinal puncture, high costs of this intervention, frequent complications, making the CSF biomarkers uncomfortable to use in everyday clinical practice. The search for new plasma predictors of cognitive decline in AD patients is an important mission.

Protective role of SCF in AD patients

SCF is broadly expressed in the central nervous system, in specific in neuronal cells, astroglia at an early stage of culture and microglia (Zhang and Fedoroff 1997). The neural progenitor cells express c-kit on their surface, a receptor to SCF (Ashman 1999). SCF can pass through BBB via receptor-mediated transport (Schänzer 2004). SCF has been shown neuroprotective effects against glutamate excitotoxicity in rat cortical neurons (Dhandapani et al. 2005). Its neuroprotective effects are mediated through c-kit receptor inducing the activation of MEK/ERK or PI3K/Akt signaling pathways (Dhandapani et al. 2005). SCF and its receptor are upregulated in the response to brain injury (Zhang and Fedoroff 1999). Erlandsson and colleagues have shown that SCF induced migration of neuronal stem cells (NSC) from the embryonic rat cortex, serving as a survival and migration factor of NSC (Erlandsson et al. 2004). SCF plays a central role in mobilization, survival, proliferation and differentiation of

hematopoietic stem cells (Broudy 1997). An interesting work from Corti et al. revealed that SCF together with G-CSF induced mobilisation of progenitor cells from bone marrow with their subsequent development to neuronal cells and incorporation into the brain of mice (Corti et al. 2002). Jin et al found that SCF stimulates neurogenesis *in vitro* and *in vivo* (Jin et al. 2002a). In addition, SCF is important in spatial learning (Motro et al. 1996).

Increasing evidence suggests that SCF also interacts with microglial cells (Zhang and Fedoroff 1998), which represent a subtype of brain cells that have essential functions of protection and maintenance the balance of neuronal microenvironment (Kreutzberg 1996). Microglial cells are the core players not only in physiological processes but in numerous pathological conditions, such as neurodegeneration and inflammation (Kreutzberg 1996). Zhang and Fedoroff showed that SCF facilitated microglial survival in cultures, but did not induce microglial proliferation and even dose-dependently inhibited the effect of colony-stimulating factor 1, a well-defined proliferation factor for microglia (Zhang and Fedoroff 1998). It is noteworthy to mention that overactivated microglia becomes cytotoxic (Kreutzberg 1996) and SCF inhibits microglial overactivation. Thus, SCF is reducing the neurotoxic innate immune response (Zhang and Fedoroff 1998). In the same work Zhang and Fedoroff observed another neuroprotective mechanism of SCF: Pretreatment of microglia with SCF induced the expression of BDNF and NGF genes (Zhang and Fedoroff 1998), whose coding proteins are known to support the survival of neurons (Mehler and Kessler 1995).

Recent evidence indicates that microglia plays an essential role in the clearance of A β from the brain and thereby restricting A β plaque formation (Simard and Rivest 2006). Simard showed that A β 1-40 and 1-42 triggered chemotaxis of microglia derived from bone marrow. Bone marrow-derived microglia but not the resident microglia phagocytosed amyloid deposits and thereby could be a very efficient therapeutic target (Simard et al. 2006).

A long-lasting protective role of SCF in combination with G-CSF in APP/PS1 transgenic mice was revealed by Li and colleagues (Li et al. 2011). They

showed that SCF together with G-CSF induced mobilisation of bone-marrow cells into circulation with the subsequent differentiation into microglial cells. These results provide the neuroprotective role of SCF by increasing the number of microglial cells and thereby enhanced clearance of A β from the brain.

Another particularly interesting feature of SCF is the ability to induce the release of stromal cell-derived factor-1 (SDF-1) from platelets. SDF-1 is a potent inducer of mobilisation and domiciliation of progenitor cells to sites of injury (Stellos and Gawaz 2007). SDF enhances neovascularization through mobilization of CXCR4+VEGFR1+ hemangiocytes (Jin et. al 2006), supports hematopoiesis (Jin et. al 2006) and possesses neuroprotective properties (Nicolai et al. 2010; Khan et al.2010).

The present study revealed that AD patients with higher SCF plasma levels have significantly less cognitive decline compared to the AD patients with low levels of SCF. Therefore altered SCF levels might play an essential role in pathogenesis of AD and may partly explain the mechanism of impaired neurogenesis and cognitive decline in AD patients. The findings of our study stand in line with a recently published work by Laske, who revealed significantly lower levels of SCF in CSF compared to healthy age-matched controls and a significant inverse correlation with dementia severity as measured by ADAS-Cog score (Laske et al. 2008b).

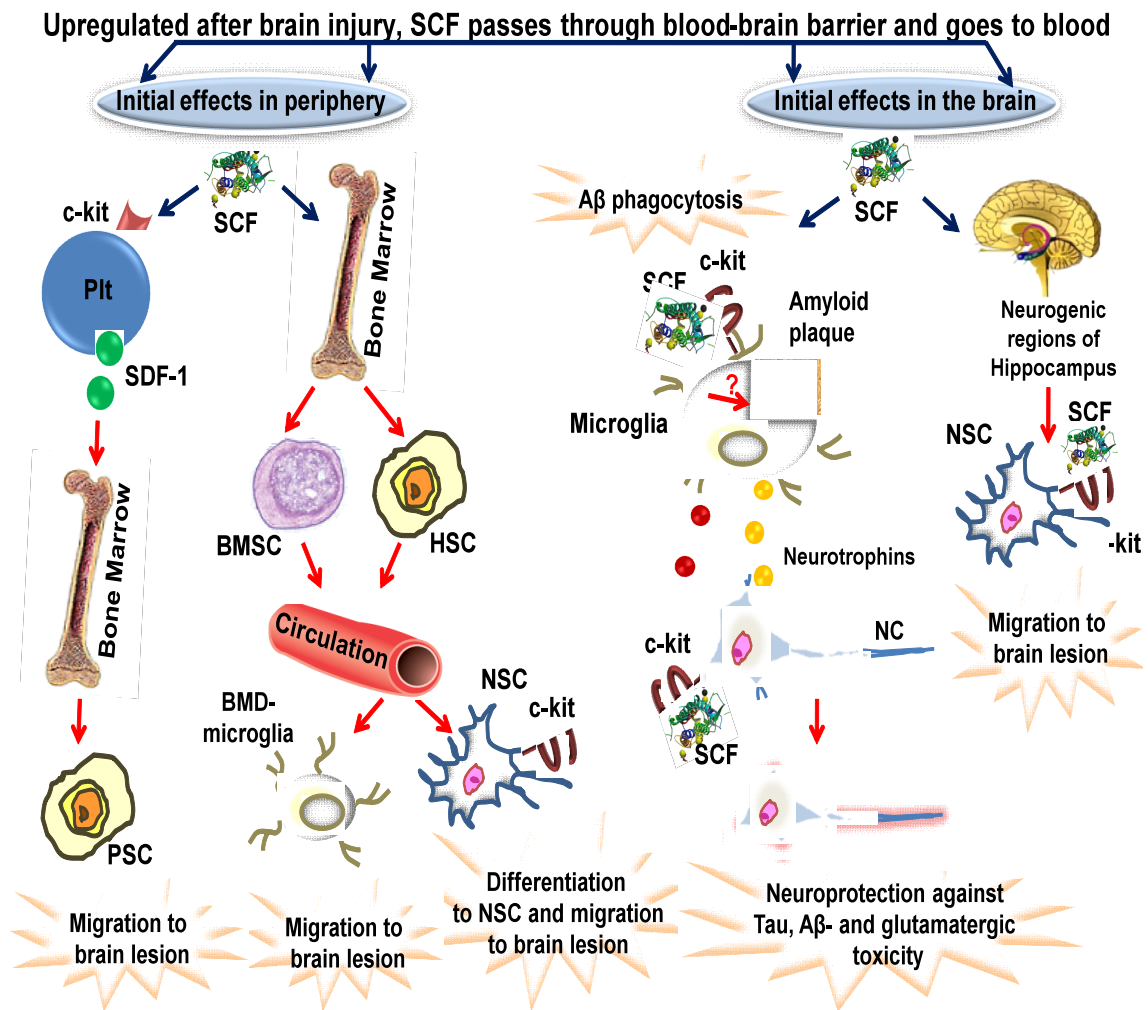


Figure 7: Potential neuroprotective effects of SCF in AD. SCF is expressed in the regions of brain injury. Protective functions of SCF include mobilization of bone-marrow derived stem cells with their subsequent differentiation to neuronal stem cells and microglia, which migrate to the sites of brain lesion. SCF protects neurons against Aβ-, tau- and glutamatergic excitotoxicity. SCF induces microglia to secrete neurotrophins and platelets to release SDF-1, which has neuroprotective functions. Bone marrow-derived microglia phagocytose amyloid plaques, a process in which SCF might also be involved. NSC-neuronal stem cell, NC- neuronal cell, HSC-hematopoietic stem cell, BMSC-bone-marrow stem cell, BMD-microglia- bone marrow-derived microglia; Plt-platelet; SDF-1-stromal cell-derived factor-1; PSC- pluripotent stem cell.

Donepezil treatment of AD increases SCF plasma levels

Donepezil is an approved acetylcholinesterase (AChE) inhibitor for treatment of mild-to-moderate AD (Doody et al. 2001b). As the trials show, Donepezil moderately improves cognition in AD patients (Cummings 2003). Another proof

that SCF levels have a big impact on the cognitive functions is a recently published work from Leyhe et al., showing that treatment with AChE inhibitor donepezil for 15 months increases plasma levels of SCF (Leyhe 2009a). Interestingly, the level of other hematopoietic growth factors (HGFs) (SDF-1, G-CSF, VEGF) remained the same after treatment with donepezil. Such a selective effect of donepezil on induction of SCF production can be clinically important. As it was mentioned above, the higher plasma levels of SCF correlate with slow cognitive decline in AD patients, and donepezil treatment increases SCF production, which may protect against cognitive decline in AD.

Adult brain has the capacity to generate new neurons, but this ability is limited and could depend on the sufficient availability of neurotrophic growth factors

Despite all the efforts, we still do not have methods to inhibit neurodegeneration and progression of cognitive decline in AD patients. It was believed that during adult life no new neurons are born, but recent findings indicate that this is not true. The process of generating new neurons, called neurogenesis, happens in two regions of brain, in olfactory bulb and the dentate gyrus of the hippocampus (Alvarez-Buylla et al. 2001; Eriksson et al. 1998). Hippocampus has been poetically named as 'gateway to memory' because of its crucial role in memory and learning. Understanding the process of adult neurogenesis and revealing the molecular substances involved maybe of invaluable help to fight AD. Although two animal AD models (Jin et al. 2004b; Lopez-Toledano and Shelanski 2007) and an active form of AD (Jin et al. 2004a) have shown increased neurogenesis, no increase was observed in pre-senile AD (Boekhoorn et al. 2006) and a significant reduction of neuronal progenitor cells was detected in AD patients in one of the main regions of neurogenesis (Ziabreva et al. 2006). The controversial work of Jin, regarding neurogenesis in AD (Jin et al. 2004a) has received many doubts. For example, Boekhoorn and colleagues argue that the marker Doublecortin, used as a marker of proliferation and a proof of neurogenesis in the study of Jin, is not a specific neuronal precursor cell marker and it might be increased due to reactive gliosis

(Boekhoorn 2006). Another study revealed that Doublecortin is not a reliable and selective marker of neurogenesis as besides neuroblasts this marker is also expressed in astrocytes (Verwer et al. 2007). These findings show how complicated the processes in a brain of AD patients are besides the fact that neurogenesis in adulthood in non-neurodegenerated brain is incapable to fully restore impaired functions after brain trauma or stroke.

Several growth factors are neuroprotective and can stimulate adult neurogenesis

As experimental data shows, neurotrophic growth factors such as *brain-derived neurotrophic factor* (BDNF) (Schabitz et al. 2007) and *glial-derived nerve factor* (GDNF) (Piltonen et al. 2009) and hematopoietic growth factors such as *vascular endothelial growth factor* (VEGF) (Schanzer et al. 2004) and *granulocyte- colony stimulating factor* (G-CSF) (Kawada et al. 2006) are neuroprotective and play a role in adult neurogenesis, e.g. by domiciliation of neuronal progenitor cells to sites of brain injury.

Vascular endothelial growth factor

VEGF is a growth factor that is induced by hypoxia through the transcription factor HIF-1 (hypoxia-induced factor 1) (Goldberg and Schneider 1994) activates angiogenesis and its neuroprotective effects have recently been revealed. VEGF stimulates axonal outgrowth, enhancing cell survival and Schwann cell proliferation in cultured superior cervical and dorsal root ganglia from adult mice (Sondell et al. 1999). VEGF increased growth and survival of dopaminergic neurons (Silverman et al. 1999). Matsuzaki and colleagues have shown that VEGF protects neurons from glutamate-induced toxicity, a condition when constant activation of a neuron by its transmitter, e.g. glutamate, leads to the death of a neuron (Matsuzaki et al. 2001). Inhibition of VEGF signalling in cortical neuron cultures leads to neuron apoptosis (Ogunshola et al. 2002). VEGF dose-dependently stimulates neurogenesis *in vitro* through interaction with its receptor vascular endothelial growth factor receptor 2/fetal liver kinase 1 (VEGFR2/Flk-1), and *in vivo* inducing the proliferation of neuronal progenitor

cells (Jin et al. 2002b). *In vivo* VEGF administration reduced cognitive impairment in the mice model of Alzheimer's disease (Wang et al. 2011). VEGF levels are significantly decreased in patients with AD compared to healthy age-matched individuals (Mateo et al. 2007). Interestingly, the AChE inhibitor donepezil treatment does not increase the levels of VEGF in AD patients (Leyhe et al. 2009a).

Stromal cell-derived factor-1

Both receptors for SDF-1 are expressed on the surface of neurons (Shimizu et al. 2011). SDF-1 regulates neuronal cell survival through histone deacetylase-dependent pathway (Nicolai et al. 2010) and promotes neuroprotection (Khan et al. 2010). Laske and colleagues have recently discovered significant positive correlation between SCF and SDF-1 in plasma of healthy individuals, and between SCF and SDF-1 in CSF of AD patients (Laske et al. 2008a; Laske et al. 2008b). These findings can be interpreted in the way that SDF-1 and SCF parallel regulate neurogenesis and they are both down-regulated in AD.

Recent evidence indicates that in the central nervous system there are specific growth factors with neuroprotective characteristics, e.g. *Nerve Growth Factor* (NGF), *Glial-Derived Nerve Factor* (GDNF), which are named neurotrophins.

Glial-Derived Nerve Factor

GDNF is a growth factor from the transforming growth factor β (TGF β) superfamily that protects nigral dopaminergic neurons *in vitro* (Piltonen et al. 2009). Straten showed, that GDNF concentrations in CSF were significantly increased in patients with AD compared with healthy subjects, whereas GDNF concentrations of AD patients in serum were significantly decreased compared with the healthy subjects (Straten et al. 2009). The authors explain increased GDNF concentrations in CSF as an adaptive process of the impaired brain to enhance neurotrophic support and decreased serum concentration of GDNF due to altered function of the blood brain barrier thus disturbing clearance of metabolites (Straten et al. 2009). A work of Straten, where he showed that

Lithium treatment significantly increased serum levels of GDNF in early AD patients, could be of a clinical interest (Straten et al. 2011).

Brain-derived neurotrophic factor

BDNF is produced by neurons upon glutamate stimulation, one of the neurotransmitters in central nervous system (Marini et al. 1998). BDNF is known to promote neurogenesis (Lee et al. 2002). Laske and colleagues have observed decreased serum levels of BDNF in AD patients (Laske et al. 2007) and postulated serum BDNF as a predictor of slower cognitive decline in AD patients (Leyhe et al 2009a). This team also observed the increase of serum BDNF concentration after 15 months donepezil treatment in patients with early AD (Leyhe et al. 2008) and after lithium treatment (Leyhe et al. 2009b). These results indicate that a lack of neurotrophic support plays a role in neurodegeneration and increasing levels of BDNF can be an option of treatment of AD.

Granulocyte-colony stimulating factor

G-CSF is a potent growth factor that stimulates myeloid cell line differentiation (Schneider et al. 2005). G-CSF and its receptor are expressed in the central nervous system both of animals and humans (Schneider et al. 2005). VEGF expression is increased upon ischemia (Kleinschnitz et al. 2004; Schneider et al. 2005). Administration of G-CSF to a mouse model of stroke ameliorates motor performance, inducing homing of bone marrow-derived progenitor cells and proliferation of intrinsic neuronal progenitor cells (Kawada et al. 2006). Several studies confirmed a significant neuroprotective effect of G-CSF against cerebral ischemia (Schabitz et al. 2003; Shyu et al. 2006). Treatment of AD with G-CSF has been investigated on Amyloid-beta driven acute and chronic animal models of AD. G-CSF administration induced neurogenesis around Amyloid-beta aggregates and increased level of acetylcholine (Tsai et al. 2007).

According to a recent study, systemic administration of SCF and G-CSF to APP/PS1 transgenic mice increases circulating bone marrow stem cells and augments bone marrow-derived microglial cells in the brains of APP/PS1 mice,

resulting in a long-term reduction of Amyloid-beta deposition in the brain (Li et al. 2011).

Neurotrophin deficiency hypothesis in AD

As recent studies demonstrated, AD patients show decreased BDNF (Laske et al. 2007), GDNF (Straten et al. 2009), SDF-1 (Laske et al 2008a), SCF (Laske et al. 2008b) and G-CSF (Laske et al. 2009) blood levels. As all named factors mediate neuroprotective effects in the brain, these results are supporting the hypothesis of a deficient neurotrophic and hematopoietic brain support in AD. This lack of neurotrophic support may increase neuronal vulnerability in the brain towards neurotoxic agents such as Amyloid-beta and hyperphosphorylated tau and thus could favour neurodegeneration in the brain with consecutive development and accelerated progression of AD. Thus, these growth factors may constitute potential new treatment targets in AD.

Conclusions

Results of the present study give insight into the mechanism of hematopoietic and neurotrophic support in AD. Causes of SCF down-regulation in AD should be further investigated. Treatment of AD with hematopoietic growth factors could be a successful strategy and further investigations are needed.

8 Summary

Alzheimer's disease (AD) is the most common cause of cognitive decline in the elderly and is characterized by massive neuronal cell loss in the brain. Stem cell factor (SCF) is a hematopoietic growth factor (HGF) that promotes neuroprotective effects and supports neurogenesis in the brain. Decreased SCF plasma levels have been described in AD patients. Whether SCF plasma levels are also associated with the rate of cognitive decline in AD patients has not been reported so far.

In the present study significantly lower SCF plasma levels (AD patients versus healthy controls: 908.5 ± 181.7 pg/ml versus 1058.3 ± 221.5 pg/ml) in AD patients were observed.

SCF plasma levels are significantly decreased in AD patients with fast cognitive decline (decrease of Mini-Mental State Examination (Crum et al. 1993) score >4 after one year; $n=12$) compared to AD patients with slow cognitive decline (decrease of MMSE score ≤ 4 after one year; $n=28$) (fast versus slow cognitive decline: mean \pm SD: 1051.1 ± 178.7 vs. 1237.9 ± 274.2 pg/ml; $p=0.037$). Moreover, SCF plasma levels correlated with the rate of cognitive decline after one year follow-up period ($r=0.315$; $p=0.048$). In a multiple linear regression analysis, independent predictors of the rate of cognitive decline in our study cohort were age, MMSE scores at baseline, SCF plasma levels, as well as brain-derived neurotrophic factor (BDNF) and activated glycoprotein (GP) IIb/IIIa.

These results suggest that lower SCF plasma levels are associated with a higher rate of cognitive decline in AD patients. Thus, treatment strategies increasing SCF plasma levels could be useful for delaying the progression of AD and slowing the cognitive decline and in this way increasing the quality of life of AD patients. Further prospective studies are needed to elucidate the value of plasma SCF in a multimarker approach determining AD prognosis.

9 Zusammenfassung

Die Alzheimer-Erkrankung ist die häufigste Ursache von Demenzerkrankungen bei älteren Menschen und ist gekennzeichnet durch einen massiven Verlust von Nerven-Zellen im Gehirn. Der Stammzellsfaktor (SCF) ist ein hämatopoetischer Wachstumsfaktor mit neuroprotektiven Effekten und mit Förderung der Neuroneogenese. Alzheimer-Patienten weisen erniedrigte SCF-Blutspiegel auf. Ob zwischen den SCF-Plasmaspiegeln und der Verschlechterung der kognitiven Leistungsfähigkeit bei Alzheimer-Patienten ein Zusammenhang besteht, ist bisher nicht bekannt.

In der gegenwärtigen Studie fanden sich bei Alzheimer-Patienten signifikant erniedrigte SCF-Plasmaspiegel (Alzheimer-Patienten vs. gesunde Kontrollen: 908.5 ± 181.7 pg/ml vs. 1058.3 ± 221.5 pg/ml).

Die SCF-Plasmaspiegel waren bei Alzheimer-Patienten mit rascher Abnahme der kognitiven Leistungsfähigkeit signifikant erniedrigt (Abnahme von 4 Punkten und mehr der MMSE Score (rasche vs. langsame kognitive Verschlechterung: 1051.1 ± 178.7 vs. 1237.9 ± 274.2 pg/ml; $p=0.037$). Außerdem korrelierten die SCF-Plasmaspiegel signifikant mit der Geschwindigkeit der kognitiven Verschlechterung nach einem Jahr.

Mittels Datenanalyse konnten als unabhängige Prädiktoren der Geschwindigkeit der kognitiven Abnahmen Alter, MMSE-Scores bei Studienbeginn, die Blutspiegel von SCF und des Wachstumsfaktors BDNF, und aktiviertes Glykoprotein (GP) IIb/IIIa identifiziert werden.

Diese Ergebnisse unterstützen die Hypothese, dass erniedrigte SCF-Plasmaspiegel mit einer beschleunigten Rate des kognitiven Abbaues bei Alzheimer-Patienten assoziiert sind. Daher könnten Behandlungsstrategien vielversprechend sein, die eine Erhöhung der SCF-Plasmaspiegel induzieren. Dadurch könnte das Fortschreiten der Alzheimer-Erkrankung verlangsamt und die Lebensqualität von Alzheimer-Patienten verbessert werden.

Weitere prospektive Studien sind notwendig, um den Wert der Plasmaspiegel-Bestimmung von SCF für die Prognose der Alzheimer-Erkrankung aufzuklären.

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11 Appendix

MMSE-Fragebogen

Punkte

- (1-5) (0 /1) 1. Was für ein Datum ist heute?
(0 /1) 2. Welche Jahreszeit?
(0 /1) 3. Welches Jahr haben wir?
(0 /1) 4. Welcher Wochentag ist heute?
(0 /1) 5. Welcher Monat?

Zuerst nach dem Datum fragen, dann gezielt nach den noch fehlenden Punkten (z. B. „können Sie mir auch sagen, welche Jahreszeit jetzt ist?“)

- (6-10) (0 /1) 6. Wo sind wir jetzt? Welches Bundesland?
(0 /1) 7. Welcher Landkreis/welche Stadt?
(0 /1) 8. Welche Stadt/welcher Stadtteil?
(0 /1) 9. Welches Krankenhaus?
(0 /1) 10. Welche Station/welches Stockwerk?

Zuerst nach dem Namen der Klinik, dann nach Station/Stockwerk, Stadt/Stadtteil usw. fragen. In Großstädten sollte nicht nach Stadt und Landkreis, sondern nach Stadt und Stadtteil gefragt werden, in jedem Fall nach dem aktuellen Aufenthaltsort und nicht nach dem Wohnort.

- (11-13) (0/1) 11. Bitte merken Sie sich: Apfel
(0/1) 12. Pfennig
(0/1) 13. Tisch

Anzahl der Versuche: _____

Der Untersucher muss zuerst fragen, ob der Patient mit einem kleinen Gedächtnistest einverstanden ist. Er wird darauf hingewiesen, dass er sich 3 Begriffe merken soll. Die Begriffe langsam und deutlich — im Abstand von jeweils ca. einer Sekunde nennen. Direkt danach die 3 Begriffe wiederholen lassen, der erste Versuch bestimmt die Punktzahl. Ggf. wiederholen, bis der Untersuchte alle 3 Begriffe gelernt hat. Die Anzahl der notwendigen Versuche wird notiert (max. sind sechs Versuche zulässig). Wenn nicht alle 3 Begriffe gelernt wurden, kann der Gedächtnistest nicht durchgeführt werden.

(14-18) Ziehen Sie von 100 jeweils 7 ab oder— falls nicht durchführbar - buchstabieren Sie Stuhl rückwärts:

- (0/ 1) 14. 93 L
(0/1) 15. 86 H
(0/1) 16. 79 U
(0/1) 17. 72 T
(0/1) 18. 65 S

Beginnend bei 100 muss fünfmal jeweils 7 subtrahiert werden. Jeden einzelnen Rechenschritt unabhängig vom vorhergehenden bewerten, damit ein Fehler nicht mehrfach bewertet wird. Alternativ (z. B., wenn der Untersuchte nicht rechnen kann oder will) kann in Ausnahmefällen das Wort STUHL rückwärts buchstabiert werden. Das Wort sollte zunächst vorwärts buchstabiert und — wenn nötig — korrigiert werden. Die Punktzahl ergibt sich aus der Anzahl der Buchstaben, die in der richtigen Reihenfolge genannt werden (z. B. „LHTUS“ = 3 Punkte).

(19-21) Was waren die Dinge, die Sie sich vorher gemerkt haben?

(0/1) 19. Apfel

(0/1) 20. Pfennig

(0/1) 21. Tisch

Der Untersuchte muss die drei Begriffe nennen, die er sich unter 11 bis 13 merken sollte.

(22,23) Was ist das?

(0/1) 22. Uhr

(0 /1) 23. Bleistift/Kugelschreiber

Eine Uhr und ein Stift werden gezeigt, der Untersuchte muss diese richtig benennen.

(24) Sprechen Sie nach:

(0 /1) 24. „Kein wenn und oder aber.“

Der Satz muss unmittelbar nachgesprochen werden, nur ein Versuch ist erlaubt. Es ist nicht zulässig, die Redewendung „Kein wenn und aber“ zu benutzen.

(25-27) Machen Sie bitte folgendes:

(0 /1) 25. Nehmen Sie bitte das Blatt in die Hand,

(0 /1) 26. falten Sie es in der Mitte und

(0/1) 27. lassen Sie es auf den Boden fallen.

Der Untersuchte erhält ein Blatt Papier, der dreistufige Befehl wird nur einmal erteilt. Einen Punkt gibt es für jeden Teil, der korrekt befolgt wird.

(28) Lesen Sie und machen Sie es bitte:

(0 /1) 28. "Augen zu" Die Buchstaben (AUGEN ZU) müssen so groß sein, dass sie auch bei eingeschränktem Visus noch lesbar sind. Ein Punkt wird nur dann gegeben, wenn die Augen wirklich geschlossen werden.

(29) (0/1) 29. Schreiben Sie bitte einen Satz (mind. Subjekt und Prädikat)!

Es darf kein Satz diktiert werden. Die Ausführung muss spontan erfolgen. Der Satz muss Subjekt und Prädikat enthalten und sinnvoll sein. Korrekte Grammatik und Interpunktion ist nicht gefordert. Das Schreiben von Namen und Anschrift ist nicht ausreichend.

(30) (0 /1) 30. Kopieren Sie bitte die Zeichnung (zwei Fünfecke)

Auf einem Blatt sind zwei sich überschneidende Fünfecke dargestellt (siehe Anlage: Erhebungsbögen), der Untersuchte soll diese so exakt wie möglich abzeichnen. Alle 10 Ecken müssen wiedergegeben sein und zwei davon sich überschneiden, nur dann wird ein Punkt gegeben.

12 Publications

The results of this dissertation are subject to publication as follows:

Laske C, Sopova K, Hoffmann N, Stransky E, Hagen K, Fallgatter AJ, Stellos K, Leyhe T (2011) Stem cell factor plasma levels are decreased in Alzheimer's disease patients with fast cognitive decline after one-year follow-up period: the Pythia-study. *J Alzheimers Dis.*;26(1):39-45.

Other publications not being related to the dissertation:

Original articles

Laske C, Sopova K, Gkotsis C, Eschweiler GW, Straten G, Gawaz M, Leyhe T, Stellos K. (2010) Amyloid-beta peptides in plasma and cognitive decline after 1 year follow-up in Alzheimer's disease patients. *J Alzheimers Dis.*;21(4):1263-9.

Bigalke B, Schreitmuller B, Sopova K, Paul A, Stransky E, Gawaz M, Stellos K, Laske C. (2011) Adipocytokines and CD34+ Progenitor Cells in Alzheimer's Disease. *PLoS One.*;6(5):e20286.

Stellos K, Rahmann A, Kiliyas A, Ruf M, Sopova K, Stamatelopoulos K, Jorbenadze R, Weretka S, Geisler T, Gawaz M, Weig HJ, Bigalke B. (2011) Expression of Platelet-Bound Stromal Cell-Derived Factor-1 in Patients with Nonvalvular Atrial Fibrillation and Ischaemic Heart Disease. *J Thromb Haemost.* Nov 1. doi: 10.1111/j.1538-7836.2011.04547.x. [Epub ahead of print]

Stellos K, Ruf M, Sopova K, Kiliyas A, Rahmann A, Stamatelopoulos K, Jorbenadze R, Geisler T, Gawaz M, Bigalke B (2011) Plasma levels of stromal cell-derived factor-1 in patients with coronary artery disease: Effect of clinical presentation and cardiovascular risk factors. *Atherosclerosis*: 219(2):913-6

Nekrasa A.I, Sopova T.O, Odud O.M, Baranov I.G., Sopova K (2010) Efficacy of thrombolytic therapy in patients with myocardial infarction. *Ukrainian Journal of Emergency Medicine*: 11/3

Review articles

Sopova K, Tatsidou R, Stellos K. (2012) Platelets and platelet interaction with progenitor cells in vascular homeostasis and inflammation. *Curr Vasc Pharmacol*. Feb 15. [Epub ahead of print]

Bigalke B, Schuster A, Sopova K, Wurster T, Stellos K. (2012) Platelets in Atherothrombosis - Diagnostic and Prognostic Value of Platelet Activation in Patients with Atherosclerotic Diseases. *Curr Vasc Pharmacol*. Feb 15. [Epub ahead of print]

Stellos K, Sopova K, Gnerlich S, Panagiota V, Paul A, Gawaz M. (2011) Expression of stromal cell-derived factor-1 on the surface of circulating platelets: from molecular interactions to clinical significance. *German Medical Science*. DOI: 10.3205/10dgaf04

Congress abstracts as a first author

Sopova K, Perk A, Panagiota V, Paul A, Gawaz A, Stellos K. (2011) CXCR7 is expressed on the surface of platelets and mediates their adhesion on vascular wall after ischemia/reperfusion injury in vivo. *European Heart Journal* 32 (Abstract Supplement), 28. ESC Congress 2011, Paris.

Sopova K, Sobolewska B, Paul A, Erbel C, Stellos K. (2011) Interleukin-17 receptor-A is expressed on the surface of platelets and induces GPIIb/IIIa activation, but not platelet aggregation, and enhances adhesion over endothelial cells in vitro. Joint Meeting of the European Society for Microcirculation and the German Society of Microcirculation and Vascular Biology, 13-16 October 2011, Munich, Germany. *J Vasc Res*;48(Suppl.1):4-356 (DOI:10.1159/000332604)

Congress abstracts as a co-author

Stellos K, Sopova K, Bigalke B, Gawaz M. (2011) Platelet interaction with circulating progenitor cells is increased in patients with acute coronary syndromes and enhances the adhesive capacity of progenitor cells on vascular wall after ischemia/reperfusion injury in vivo. Joint Meeting of the European

Society for Microcirculation and the German Society of Microcirculation and Vascular Biology, 13-16 October 2011, Munich, Germany. J Vasc Res;48(Suppl.1):4-356 (DOI:10.1159/000332604)

Stellos K, Langer H, Sopova K, Paul A, Borst O, Bigalke B, Geisler T, Gawaz M. (2011) Circulating platelet - CD34+ cell coaggregates are increased in patients with acute coronary syndromes. European Heart Journal 32 (Abstract Supplement), 263. ESC Congress 2011, Paris

Panagiota V, Sopova K, Perk A, Paul A, Gawaz A, Stellos K. (2011) Essential, but not differential role of CXCR7 and CXCR4 on platelet-derived SDF-1-mediated adhesion of human CD34+ cells in vitro and after vascular injury in vivo. European Heart Journal 32 (Abstract Supplement), 820. ESC Congress 2011, Paris

Stellos K, Sopova K, Stakos D, Bigalke B, Laske C, Gawaz M (2011) Plasma levels of amyloid beta peptides 1-40 and 1-42 are novel biomarkers of coronary artery disease Clin Res Cardiol 100, Suppl 1. 77 Annual meeting of the German Cardiac Society, Mannheim, Germany.

Stellos K, Sopova K, Perk A, Gawaz M. (2011) Binding of oxidized LDL on circulating platelets is increased in patients with acute coronary syndromes and augments platelet adhesion on vascular wall. Clin Res Cardiol 100, Suppl 1. 77 Annual meeting of the German Cardiac Society, Mannheim, Germany.

Stellos K, Paul A, Polzelbauer C, Sopova K, Gawaz M. (2011) Human parvovirus B19 non-structural protein NS-1 induces endothelial inflammation and subsequent adhesion of circulating platelets in vitro. Clin Res Cardiol 100, Suppl 1. 77 Annual meeting of the German Cardiac Society, Mannheim, Germany.

Panagiota V, Sopova K, Perk A, Paul A, Gawaz M, Stellos K. (2011) The novel SDF-1 receptor CXCR7 is expressed on platelets and regulates their adhesion after vascular injury in vivo. P644. American Heart Association, Arteriosclerosis, Thrombosis, Vascular Biology 2011, Chicago, USA

Panagiota V, Sopova K, Perk A, Paul A, Gawaz M, Stellos K. (2011) Essential, but not differential, role of CXCR7 and CXCR4 on Platelet-derived Stromal Cell-Derived Factor-1-mediated adhesion of human CD34⁺ cells in vitro and after vascular injury in vivo. P495. American Heart Association, Arteriosclerosis, Thrombosis, Vascular Biology 2011, Chicago, USA

Stellos K, Stakos D, Sopova K, Laske C, Gawaz M. (2010) Plasma levels of amyloid beta peptides 1-40 and 1-42 are novel biomarkers of coronary artery disease. European Heart Journal 31 (Abstract Supplement), 443-444 8. ESC Congress 2010, Stockholm.

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14 Curriculum Vitae

PERSONAL INFORMATION

Name Kateryna Sopova
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EDUCATION AND TRAINING

1994-2000 Primary and Junior High School, Lugansk, Ukraine
2000-2004 Senior High School, Lyceum of Foreign Languages,
Lugansk, Ukraine
09.2004- 06.2010 Medical Faculty of Lugansk State Medical University,
Lugansk, Ukraine. Diploma with honours.
2010-2012 Dissertation "Role of Stem Cell Factor in the
Development of Alzheimer's Disease" under the
supervision of Prof Dr. Christoph Laske, Eberhard-
Karls University of Tübingen, Tübingen, Germany.
1.10.2010- Research assistant at the "Vascular and Regenerative
30.09.2011 Cardiology" Reseach Group (PI: Dr. K.Stellos)
6.03.2012 Baden-Wurtemberg State Medical Licence
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ELECTIVES & RESEARCH WORK

February 2007- Voluntarily clinical research work in Coronary Care
June 2010 Unit, Lugansk State Hospital No.1, Ukraine
August 2008, Research elective at the Department of Experimental
July 2009 Cardiology, Medizinische Klinik III, Kardiologie und
Kreislaufferkrankungen, Eberhard-Karls University of
Tübingen, Germany.

PERSONAL SKILLS & COMPETENCES LANGUAGES

German (TestDaf C1 level)
English (University of Cambridge Certificate in
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**NON FORMAL
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- 09.2005-06.2010 Group leader of the Medical Faculty of Lugansk State Medical University
- 2005-2006 Correspondent of the Magazine "Eskulap" of Lugansk State Medical University

TECHNICAL SKILLS Microsoft Office 2007, Adobe Photoshop, SPSS v. 13 statistical program, Endnote X5

CONFERENCES

- 3-4.07. 2009 SFB -Transregio 19 - Inflammatory Cardiomyopathy Molecular Pathogenesis and Therapy-Summer School Tübingen 2009, Germany
- 28.08.-01.09. 2010 European Society of Cardiology Congress in Stockholm, Sweden
- 08.10.2010 Workshop Hemodynamic Monitoring, Eberhard Karls University of Tübingen
- 17-19.12.2010 Advanced Learning on Platelets International Course in Metsovo, Greece
- 25.-26.03.2011 5th Heart and Circulation Days, University Clinic Tübingen
- 27.-30.04.2011 77th Annual Meeting of German Cardiac Society, Mannheim, Germany
- 28.-31.08.2011 European Society of Cardiology Congress in Paris, France
- 4.-7.09.2011 Cell Volume Regulation Meeting, Tübingen, Germany
- 13.-16.10.2011 Joint Meeting of the European Society for Microcirculation and the Society of Microcirculation and Vascular Biology, Munich, Germany

AWARDS RECEIVED

- April 2008 1st Prize in Student Scientific Conference in Cardiology of Lugansk State Medical University
- April 2008 1st Prize in Student Scientific Conference in Medical Physics and Informatics of Lugansk State Medical University
- May 2008 1st Prize in Student Scientific Conference in General Medicine of Lugansk State Medical University
- June 2008 Letter of Commendation for excellent results in studies
- April 2009 2nd Prize in Ukrainian Contest of Student and Young Scientist Research Works
- April 2010 3rd Prize in Student Scientific Conference in Cardiology of Lugansk State Medical University