



HAL
open science

Growth factors and cytokines/chemokines as surrogate biomarkers in cerebrospinal fluid and blood for diagnosing Alzheimer´s disease and mild cognitive impairment

Lars Olson, Christian Humpel

► To cite this version:

Lars Olson, Christian Humpel. Growth factors and cytokines/chemokines as surrogate biomarkers in cerebrospinal fluid and blood for diagnosing Alzheimer´s disease and mild cognitive impairment. *Experimental Gerontology*, 2009, 45 (1), pp.41. 10.1016/j.exger.2009.10.011 . hal-00547646

HAL Id: hal-00547646

<https://hal.science/hal-00547646>

Submitted on 17 Dec 2010

HAL is a multi-disciplinary open access archive for the deposit and dissemination of scientific research documents, whether they are published or not. The documents may come from teaching and research institutions in France or abroad, or from public or private research centers.

L'archive ouverte pluridisciplinaire **HAL**, est destinée au dépôt et à la diffusion de documents scientifiques de niveau recherche, publiés ou non, émanant des établissements d'enseignement et de recherche français ou étrangers, des laboratoires publics ou privés.

Accepted Manuscript

Growth factors and cytokines/chemokines as surrogate biomarkers in cerebrospinal fluid and blood for diagnosing Alzheimer's disease and mild cognitive impairment

Lars Olson, Christian Humpel

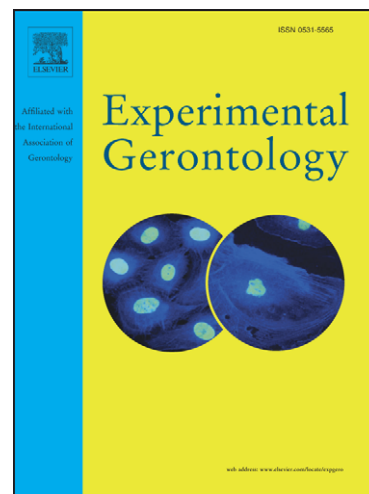
PII: S0531-5565(09)00208-3
DOI: [10.1016/j.exger.2009.10.011](https://doi.org/10.1016/j.exger.2009.10.011)
Reference: EXG 8663

To appear in: *Experimental Gerontology*

Received Date: 2 June 2009
Revised Date: 13 October 2009
Accepted Date: 15 October 2009

Please cite this article as: Olson, L., Humpel, C., Growth factors and cytokines/chemokines as surrogate biomarkers in cerebrospinal fluid and blood for diagnosing Alzheimer's disease and mild cognitive impairment, *Experimental Gerontology* (2009), doi: [10.1016/j.exger.2009.10.011](https://doi.org/10.1016/j.exger.2009.10.011)

This is a PDF file of an unedited manuscript that has been accepted for publication. As a service to our customers we are providing this early version of the manuscript. The manuscript will undergo copyediting, typesetting, and review of the resulting proof before it is published in its final form. Please note that during the production process errors may be discovered which could affect the content, and all legal disclaimers that apply to the journal pertain.



MINIREVIEW, Special Issue Experimental Gerontology, re-revised

131009

**Growth factors and cytokines/chemokines
as surrogate biomarkers in cerebrospinal fluid and blood
for diagnosing Alzheimer's disease and mild cognitive impairment**

Lars Olson¹ and Christian Humpel²

¹ Department of Neuroscience, Karolinska Institutet, Sweden

² Department of General and Social Psychiatry, Innsbruck Medical University, Austria

To whom correspondence should be addressed:

Dr. Christian Humpel, Department of Psychiatry and Psychotherapy,
Anichstr. 35, A-6020 Innsbruck, Austria.

Phone: +43-512-504-23712; Fax: +43-512-504-23713

E-mail: christian.humpel@i-med.ac.at

Abstract:

Alzheimer's disease (AD) is a severe chronic neurodegenerative disorder of the brain. A probable diagnosis of AD can be obtained by cerebrospinal fluid levels of 3 biomarkers: beta-amyloid (1-42), total tau and phospho-tau-181. Researchers are interested in finding additional biomarkers in CSF to improve the specificity and sensitivity of diagnosis, including also other forms of dementia, such as mild cognitive impairment (MCI). In addition, less invasive diagnostic methods using blood or blood-derived cells are being investigated. This mini-review (*in concert with the other reviews of this special issue*) summarizes the usefulness of growth factors and cytokines/chemokines as putative surrogate biomarkers for diagnosing AD and MCI in CSF and blood. Briefly, the expression levels of growth factors and cytokines/chemokines are very heterogenous, indicating the pathological diversity of these diseases. At present, no single growth factor or cytokine alone stands out as a useful biomarker for diagnosing AD or MCI. However, the combined "*patients profile signature*" of several selected growth factors and/or cytokines/chemokines may allow to diagnose AD and MCI with higher selectivity and specificity.

Key words: cerebrospinal fluid, blood, plasma, Alzheimer, diagnosis.

The life expectancy of humans has markedly increased within the last 100 years. As age is the main risk factor for Alzheimer's disease (AD), the number of patients suffering from AD, and mixed forms of dementia will increase within the next 50 years. It is expected that there will be about 80 million AD patients worldwide in 2050. The establishment of reliable diagnostic surrogate markers for AD and measures to monitor disease progression is essential in order to evaluate novel treatments to counteract and/or delay symptoms in AD and to allow initiation of therapeutic interventions as early as possible. A valid and easily accessible diagnostic procedure should be the basis for the treatment.

Diagnosis of Alzheimer's disease and other forms of dementia

AD is morphologically characterized by extracellular beta-amyloid plaque deposition, intraneuronal tau pathology, neuronal cell death, vascular dysfunction and inflammatory processes. Diagnosis requires both clinical assessment of the disease and post mortem detection of beta-amyloid plaques and tau-pathology. A probable diagnosis of AD can be established with a confidence of >90%, based on clinical criteria, including medical history, physical examination, laboratory tests, neuroimaging and neuropsychological evaluation. Accurate, early diagnosis of AD is still difficult because early symptoms of the disease are shared by a variety of disorders, reflecting common neuropathological features. The diagnosis of mixed forms of dementia, such as vascular dementia, frontotemporal lobe dementia or Lewy body dementia, is even more difficult.

The search for biomarkers becomes extremely important, due the large expected increase in dementia and AD patients within the next 50 years. It will be necessary to find "*biomarkers of aging*" and "*biomarkers of disease*". While a "*biomarker of aging*" may allow to follow up individuals over several years, a "*biomarker of disease*" will directly reflect the stage of the disease process (for details on diagnosis see Sprott as well as Cedazo-Minguez and Bengt Winblad; both this special issue of EXG). Briefly, we need to distinguish between biomarkers (1) that add to diagnostic certainty in the setting of a clinical evaluation, (2) those that can be used in lieu of a clinical evaluation, and (3) those that can be used to track disease progression at a specific stage. Although the

same biomarker could in theory be used in all these settings, most do not perform evenly across them. In addition, some biomarkers are more reasonably thought about as "*risk factors*" rather than "*true disease*" markers. In order for a diagnostic biomarker to be useful, certain criteria need to be met (Humpel and Marksteiner, 2009). (1) The biomarker should reflect some basic pathophysiological processes, and detect a fundamental feature of the disease with high sensitivity and specificity. (2) The biomarker should be specific for the disease compared with related disorders. (3) The biomarker should not reflect symptomatology of disorder. (4) The biomarker can be measured repeatedly over time and should be reproducible. (5) The biomarker should be measured in noninvasive easy-to-perform tests. (6) The biomarker should not cause harm to the individual being assessed. (7) The biomarker should be reliable in many testing environments/labs. (8) Validation by pathological diagnosis as gold standard should verify the set up of a biomarker in a specific disease. This mini-review (*in concert with the other reviews in this special issue*) will give an overview on the measurement of growth factors and cytokines/chemokines in CSF and blood. It will be shown, that all mentioned factors show a very high heterogeneity between the disease groups, and so far none of these biomarkers will meet all criteria for being a good biomarker.

Growth factors in Alzheimers disease

One characteristic feature of AD pathology is death of basal forebrain cholinergic neurons and vascular damage. Nerve growth factor (NGF) is the most potent growth factor able to counteract cell death of cholinergic neurons in vitro and in vivo (Thoenen and Barde 1980; Levi-Montalcini 1987). While NGF dysfunction has been suggested in the development of AD, NGF knockout mice have not shown clear cognitive deficits. Nevertheless, NGF has been considered a candidate for treating AD and purified NGF was infused in some AD patients (Olson et al. 1992). And recently genetically engineered skin cells secreting NGF were transplanted into the nBM of Alzheimer patients by Tuszynski and colleagues (2005). Interestingly, NGF is up-regulated in brains (Fahnestock et al. 2001) and CSF (Hock et al. 2000) of AD patients, while the high-affinity NGF receptor trkA is down-regulated (Mufson et al. 2004). Furthermore, vascular endothelial growth factor (VEGF) is increased (Fukumura et al. 2001; Tarkowski et al. 2002), resulting in enhanced microvascular density in developing AD. Other growth factors may also contribute to AD pathology

and/or be dysregulated. Platelet-derived growth factor (PDGF) has been found to up-regulate amyloid-precursor-protein in hippocampus by inducing secretases (Gianni et al. 2003; Zambrano et al. 2004; Lim et al. 2007). Insulin-like growth factor-I (IGF-I) regulates beta-amyloid levels and displays protective effects against beta-amyloid toxicity (Carro et al. 2002; Aguado-Llera et al. 2005). Fibroblast growth factor-2 (FGF-2) shares binding sites with beta-amyloid fibrils on heparan sulfate from cerebral cortex (Lindahl et al. 1999) and plays a role in beta-amyloid toxicity (Cantara et al. 2005). Finally, members of the transforming growth factor- β (TGF- β) family interact with beta-amyloid, contributing to its toxicity or constituting a risk for cerebral beta-amyloid angiopathy (Hashimoto et al. 2005 and 2006).

Inflammation and Alzheimer's disease

Inflammation may constitute an important trigger of neurodegeneration during aging (“Inflammaging”) (Franceschi et al. 2001) and anti-inflammatory drugs may delay AD (Perry et al. 1995; Moore and O’Banion 2002). Chronic release of pro-inflammatory cytokines, such as interleukin-1 β or tumor necrosis factor- α suggests roles in inflammation, pathology, and neuronal dysfunction associated with AD (Perry et al. 1995; Grammas and Ovasse 2002). These inflammatory processes include activation of microglia and subsequent neuroinflammatory processes (Gonzalez-Scarano and Baltuch 1999). However, it is not clear if inflammation is merely a possibly aggravating result of beta-amyloid dysregulation (Moore and O’Banion 2002) or a primary cause of AD. Perivascular small vessel inflammation is another key feature of AD, and it is well known that endothelial cells respond strongly to inflammatory stimuli (Moser, et al. 2004), especially involving production of reactive oxygen species (Iadecola 2004).

A. GROWTH FACTORS

Growth factors are proteins, which support the survival of cells of the central and peripheral nervous system, play a role in the development of the brain, stimulate axonal growth and regulate the growth of different kinds of cells in the brain and periphery. In many cases the same growth factor and corresponding receptor signaling system may thus serve a number of different functions in the body,

Nerve growth factor (NGF) is the most potent factor providing trophic support of cholinergic neurons in the basal forebrain. These cholinergic neurons degenerate early in AD, possibly due to compromised retrograde transport of NGF to the cell bodies of origin in the basal nucleus of Meynert and septum. NGF has been found to be increased in CSF of AD patients (Table 1A; Blasko et al., 2006; Hock et al., 2000; Marksteiner et al., 2008). Interestingly, the increase of NGF was specific for AD compared to healthy controls and was dependent on the extent of neurodegeneration as expressed by the phospho-tau181/beta-amyloid ratio (1-42) (Blasko et al., 2006). Although NGF data alone did not reveal a significant difference, the comparison of NGF in AD patients having a phospho-tau181/beta-amyloid ratio >10 with healthy control subjects (ratio <6) revealed a significant difference (Blasko et al., 2006). This might suggest that NGF accumulates in neurodegeneration only at a certain stage of the disease.

Several other growth factors have been measured in CSF or plasma, however, the data are very divergent between the different groups (Table 1A).

- *Hepatocyte growth factor (HGF)* is a paracrine cellular growth, motility and morphogenic factor and plays a major role in embryonic organ development, in adult organ regeneration and in wound healing.
- *Vascular endothelial growth factor (VEGF)* is an important growth factor, regulating angiogenesis in the nervous system.
- *Brain-derived neurotrophic factor (BDNF; Barde, 1990)* belongs to the family of neurotrophins. It serves very general plastic functions in the adult brain and is strictly upregulated in response to locally increased neuronal activity (Galloway et al., 2008). Of note, it has been reported that women have higher plasma BDNF levels than men, and that decreased plasma BDNF levels have been suggested to correlate with impaired memory and general cognitive function in aging women but not men (Komulainen et al., 2008).
- *Glial cell line-derived neurotrophic factor (GDNF)* stimulates the survival of several neurons, such as e.g. dopaminergic neurons and motoneurons.

- *Fibroblast growth factor-2 (FGF-2, bFGF)* belongs to a family of heparin-binding growth factors involved in angiogenesis, wound healing, and embryonic development.
- *Transforming growth factor-beta-1 (TGF β -1)* plays a role in cellular proliferation and differentiation of a broad range of cells
- *Insulin-like growth factor (IGFs)* are polypeptides with a marked sequence homology to insulin, playing an important role in cell-cell communication. Insulin-like growth factor binding protein-6 (IGFBP-6) controls the distribution, function and activity of insulin-like growth factors.
- *Epidermal growth factor (EGF)* stimulates the proliferation of various epidermal and epithelial cells, inhibits gastric acid secretion and is involved in wound healing.
- *Angiopoietin (ANG-2)* binds to the endothelial cell specific receptor Tie2 and promotes angiogenesis, sprouting and tube formation and the formation of new blood vessels.
- *Platelet-derived growth factor-BB (PDGF-BB)* is mitogenic for cells of mesenchymal origin.
- *Progranulin* is a glycoprotein growth factor involved in the regulation of multiple processes including tumorigenesis, wound repair, development and inflammation.

In summary, consensus is lacking regarding possible changes of the levels of several different growth factor in CSF and plasma in AD. However, NGF levels in CSF seem to be consistently increased in at least certain stages of AD. The consistency of NGF changes adds weight to a role of NGF in AD pathology, particularly when compared to the variable results seen with most other studied growth factors.

B: CYTOKINES AND CHEMOKINES

Cytokines comprise a large diverse family of proteins, peptides or glycoproteins that are used extensively in cellular communication. Historically, the term cytokine was

used to refer to immune modulating agents. Cytokines are now classified as lymphokines, interleukines and chemokines, based on their function and action on cellular targets. The term interleukin was initially used for cytokines targeting leukocytes. **Chemokines** are a family of small cytokines mediating chemoattraction (chemotaxis) between cells. Some chemokines are considered pro-inflammatory and can be induced during an immune response to recruit cells of the immune system to a site of infection, while others are considered homeostatic and are involved in controlling the migration of cells during normal processes of tissue maintenance or development. Interleukin-8 (CXCL8) is the only chemokine originally named an interleukin.

Nomenclature of chemokines: The **C-C chemokines** have two adjacent cysteines near their amino terminus. An example of a C-C chemokine is monocyte chemoattractant protein-1 (MCP-1 or CCL2) or CCL5 (RANTES). The two N-terminal cysteines of **C-X-C chemokines** are separated by one amino acid. An example of a C-X-C chemokine is interleukin-8 (IL-8, CXCL8). Other groups of chemokines are the **C chemokines** (e.g. lymphotactins) and the **CX3C chemokines** (e.g. fractalkine).

Cytokines

Different cytokines have been measured in CSF, plasma or serum (see Table 1B), such as interleukin-1 α and -1 β , interleukin-6 (IL-6), -10 (IL-10), -11 (IL-11), -18 (IL-18) and tumor-necrosis factor alpha (TNF- α , or TNFSF2) or macrophage migration inhibitory factor (MIF). As shown in Table 1B, the measurements are very divergent between the different groups. However, the combination of several chemokines in plasma (e.g. MIP1 δ , MIP4 and RANTES; Marksteiner et al., 2009) may allow to diagnose AD and MCI (see Table 2).

Colony-stimulating factors (CoSF)

Three members belong to the CoSF and have been measured in CSF and plasma (table 1B):

- Granulocyte colony-stimulating factor (G-CSF, or CSF-3) stimulates the bone marrow to produce granulocytes and stem cells and stimulates survival,

proliferation, differentiation and function of mature and neutrophil precursors and white blood cells.

- Macrophage colony stimulating factor (M-CSF, CSF-1) regulates proliferation, differentiation and survival of blood monocytes, tissue macrophages and progenitor cells.
- Interleukin-3 (IL-3 or multipotential colony-cstimulating factor, Multi-CSF) is a hematopoietic growth factor that promotes the survival, differentiation and proliferation of megakaryocyte, granulocyte-macrophage, erythroid, eosinophil, basophil and mast cell progenitors and enhances thrombopoiesis, phagocytosis and cellular cytotoxicity and plays a role in immune defense.

Chemokines

Several chemokines have been measured in CSF or plasma, however, measurements are very divergent between the different groups (Table 1B): CCL2 (monocyte chemoattractant protein-1, MCP-1), CCL5 (RANTES), CCL7 (MCP-3), CCL15 (MIP-1- δ , macrophage inflammatory protein-1 δ , MIP-5), CCL18 (MIP-4, macrophage inflammatory protein-4 or PARC) and CXCL8 (IL-8, interleukin-8). These chemokines are chemotactic or chemoattractant for monocytes and T-cells and other cell types and play an active role in recruiting cells into inflammatory sites.

Culture of PBMCs and stimulated release

To increase the specificity and sensitivity for biomarker search, isolated blood cells can be used to explore differential release of cytokines in AD compared to controls. The stimulation of cultured of peripheral blood mononuclear cells (PBMC) has been performed, and especially lipopolysaccharide (LPS) or phytohaemagglutinin (PHA) or also beta-amyloid seem to be very potent. In LPS stimulated PBMCs enhanced release of different cytokines (e.g. IL-2, TNF- α) has been reported in AD patients (Tan et al., 2007; Bergman et al., 2002; Kassner et al., 2008; Guerreiro et al., 2007; Kaplin et al., 2008), a decrease of IL-1 β and IL-10 in severely demented patients (Sala et al., 2003; Bergman et al., 2002), while no effect on IL-6 release was found (Magaki et al., 2006). In PHA-stimulated cultures of PBMC from patients with MCI a

markedly enhanced secretion of IL-6 and a less pronounced enhanced secretion of IL-10 and IL-8, but not of TNF α or IL-1 β , was found (Magaki et al., 2006). A significant decrease in IL-10 production was found in PBMCs after stimulation with beta-amyloid (Speciale et al., 2007).

To further explore differential release of cytokines/chemokines/growth factors from PBMCs we isolated in our lab PBMCs from 9 controls, 37 AD, 20 MCI and 11 depressive patients (Table 3). After stimulation with 4 μ g/ml phytohemagglutinin-L (PHA) for 24hr in culture, the levels of biomarkers (interleukins-1 α , -3, -11, EGF, G-CSF and MCP-3) were measured in the supernatants by Searchlight multiplex ELISA (Marksteiner et al., 2009) or commercial single ELISA (IL-6, BioLegend). Statistical analysis was performed by one way ANOVA with a subsequent Dunnet posthoc test where $p < 0.05$ was significant. Our novel data show that no changes were observed for IL-1 α , IL-6, G-CSF and MCP-3 release. However, IL-3 and EGF release were significantly enhanced in MCI but not in the other groups, while IL-11 was significantly decreased in MCI but not in other groups (Table 3). When calculating the ratio EGF/IL-11 MCI patients could be differentiated from the other groups with high significance (Table 3). Our data suggest that the PHA-induced release of EGF and IL-11 from PBMCs may allow to diagnose MCI with high significance.

Summary

In summary, CSF and plasma levels of cytokines and chemokines are very heterogenous in different studies, and changes point to inflammatory reactions in AD. The striking differences between published studies may partly be due to true variations during the course of AD, for instance with respect to inflammatory and immune events. Needless to say, other explanations, including methodological differences between groups are also possible. Thus the mean levels of an individual biomarker may vary between serum, EDTA-plasma, citrate plasma and heparin plasma by a factor 20. Different commercial ELISA systems may lead to different levels of the biomarkers, depending on the sensitivity and specificity of the used antibodies. An antibody may detect full length forms or spliced forms or precursor forms. Moreover, some biomarkers may show enhanced binding capacities to plasma/serum proteins, and only a pre-treatment may release the soluble forms. The stability of the biomarkers may vary and freezing and thawing may influence the

biochemical biomarker properties. Platelets serve as one storage compartment for several of the measured proteins and differences in platelet stability or perturbation may cause markedly different results. Finally, the ethnicity of patients may have an effect on protein levels as well. Thus, in summary, controlled standardized procedures to measure biomarkers in blood must be established in order to better compare data from different centers. While more studies are needed to clarify these issues, a combined pattern of several cytokines/chemokines and/or their ratios may become of value in the diagnosis of AD and MCI using blood samples.

CONCLUSIONS

The expression levels of growth factors and cytokines/chemokines in CSF and blood of AD patients are very heterogeneous, indicating pathological diversity of AD. In CSF only NGF seems to be significantly enhanced, probably reflecting reactions to the ongoing neurodegenerative processes in AD. Inflammatory cytokines and chemokines are differentially regulated, pointing to inflammatory events in AD. At present, no single growth factor or cytokine stands out as a useful biomarker for diagnosing AD or MCI. However, the combined "*patients profile signature*" of selected growth factors (e.g. EGF) and cytokines/chemokines (e.g. IL-11, RANTES, MIP-1 δ , MIP4) may provide a diagnostic tool with high selectivity and specificity in AD and MCI by the use of blood samples.

Acknowledgements: This study was supported by the Austrian Science Funds (L429-B05). We want to thank Prof. Josef Marksteiner (Klagenfurt, Austria) and Prof. Elisabeth Weiss (Innsbruck, Austria) for blood collection and diagnosis and help with the study.

ACCEPTED MANUSCRIPT

REFERENCES

- Aguado-Llera D, Arilla-Ferreiro E, Campos-Barros A, Puebla-Jimenez L, Barrios V. 2005. Protective effects of insulin-like growth factor-I on the somatostatinergic system in the temporal cortex of beta-amyloid-treated rats. *J Neurochem.* 92:607–615.
- Angelopoulos P, Agouridaki H, Vaiopoulos H, Siskou E, Doutsou K, Costa V, Baloyiannis SI. Cytokines in Alzheimer's disease and vascular dementia. *Int J Neurosci.* 2008 Dec;118(12):1659-72.
- Baranowska-Bik A, Bik W, Wolinska-Witort E, Martynska L, Chmielowska M, Barcikowska M, Baranowska B. Plasma beta amyloid and cytokine profile in women with Alzheimer's disease. *Neuro Endocrinol Lett.* 2008 Feb;29(1):75-9.
- Barde, Y.A. (1990) The nerve growth factor family, *Progr. Growth Factor Res.* 2: 237-248.
- Bergman M, Salman H, Beloosesky Y, Djaldetti M, Bessler H. Are peripheral blood cells from patients with Alzheimer disease more sensitive to apoptotic stimuli? *Alzheimer Dis Assoc Disord.* 2002 Jul-Sep;16(3):156-60.
- Bermejo P, Martin-Aragon S, Benedi J, Susin C, Felici E, Gil P, Ribera JM, Villar AM. Differences of peripheral inflammatory markers between mild cognitive impairment and Alzheimer's disease. *Immunol Lett.* 2008 May 15;117(2):198-202.
- Blasko I, Lederer W, Oberbauer H, Walch T, Kemmler G, Hinterhuber H, Marksteiner J, Humpel C. Measurement of thirteen biological markers in CSF of patients with Alzheimer's disease and other dementias. *Dementia and Geriatric Cognitive Disorders* 2006; 21: 9-15
- Bruunsgaard H, Andersen-Ranberg K, Jeune B, Pedersen AN, Skinhoj P, Pedersen BK. A high plasma concentration of TNF-alpha is associated with dementia in centenarians. *J Gerontol A Biol Sci Med Sci.* 1999 Jul;54(7):M357-64.
- Cantara S, Ziche M, Donnini S. 2005. Opposite effects of beta amyloid on endothelial cell survival: role of fibroblast growth factor2 (FGF-2). *Pharmacol Rep.* 57:138–143.
- Carro E, Trejo JL, Gomez-Isla T, LeRoith D, Torres-Aleman I. 2002. Serum insulin-like growth factor I regulates brain amyloid-beta levels. *Nat Med.* 8:1390–1397.
- De Servi B, La Porta CA, Bontempelli M, Comolli R. Decrease of TGF-beta1 plasma levels and increase of nitric oxide synthase activity in leukocytes as potential biomarkers of Alzheimer's disease. *Exp Gerontol.* 2002 Jun;37(6):813-21.
- Dik MG, Jonker C, Hack CE, Smit JH, Comijs HC, Eikelenboom P. Serum inflammatory proteins and cognitive decline in older persons. *Neurology.* 2005 Apr 26;64(8):1371-7.
- Fahnstock M, Michalski B, Xu B, Coughlin MD. 2001. The precursor pro-nerve growth factor is the predominant form of nerve growth factor in brain and is increased in Alzheimer's disease. *Mol Cell Neurosci.* 18:210–220.

- Franceschi C, Valensin S, Bonafe M et al. 2000. The network and the remodeling theories of aging, historical background and new perspectives. *Exp Gerontol.* 35:879–896.
- Fukumura D, Xu L, Chen Y, Gohongi T, Seed B, Jain RK. 2001. Hypoxia and acidosis independently up-regulate vascular endothelial growth factor transcription in brain tumor in vivo. *Cancer Res.* 61:6020–6024.
- Galloway EM, Woo NH, Lu B (2008) Persistent neural activity in the prefrontal cortex: a mechanism by which BDNF regulates working memory? *Prog Brain Res.* 169:251-66.
- Galimberti D, Venturelli E, Fenoglio C, Guidi I, Villa C, Bergamaschini L, Cortini F, Scalabrini D, Baron P, Vergani C, Bresolin N, Scarpini E. Intrathecal levels of IL-6, IL-11 and LIF in Alzheimer's disease and frontotemporal lobar degeneration. *J Neurol.* 2008 Apr;255(4):539-44.
- Galimberti D, Fenoglio C, Lovati C, Venturelli E, Guidi I, Corra B, Scalabrini D, Clerici F, Mariani C, Bresolin N, Scarpini E. Serum MCP-1 levels are increased in mild cognitive impairment and mild Alzheimer's disease. *Neurobiol Aging.* 2006 Dec;27(12):1763-8. Epub 2005 Nov 22.
- Ghidoni R, Benussi L, Glionna M, Franzoni M, Binetti G. Low plasma progranulin levels predict progranulin mutations in frontotemporal lobar degeneration. *Neurology.* 2008 Oct 14;71(16):1235-9. Epub 2008 Sep 3.
- Gianni D, Zambrano N, Bimonte M et al. 2003. Platelet-derived growth factor induces the beta-gamma-secretase-mediated cleavage of Alzheimer's amyloid precursor protein through a Src-Rac-dependent pathway. *J Biol Chem.* 278:9290–9297.
- Gonzalez-Scarano F, Baltuch G. 1999. Microglia as mediators of inflammatory and degenerative diseases. *Annu Rev Neurosci.* 22:219–240.
- Grammas P, Ovase R. 2002. Cerebrovascular transforming growth factor-beta contributes to inflammation in the Alzheimer's disease brain. *Am J Pathol.* 160:1583–1587.
- Grundstrom E, Lindholm D, Johansson A, Blennow K, Askmark H (2000) GDNF but not BDNF is increased in cerebrospinal fluid in amyotrophic lateral sclerosis. *Neuroreport* 11:1781-1783.
- Guerreiro RJ, Santana I, Bras JM, Santiago B, Paiva A, Oliveira C. Peripheral inflammatory cytokines as biomarkers in Alzheimer's disease and mild cognitive impairment. *Neurodegener Dis.* 2007;4(6):406-12.
- Guerreiro RJ, Santana I, Bras JM, Santiago B, Paiva A, Oliveira C. Peripheral inflammatory cytokines as biomarkers in Alzheimer's disease and mild cognitive impairment. *Neurodegenerative Dis* 2007; 4: 406-412
- Hashimoto Y, Chiba T, Yamada M et al. 2005. Transforming growth factor beta2 is a neuronal death-inducing ligand for amyloid-beta precursor protein. *Mol Cell Biol.* 25:9304–9317.

- Hashimoto Y, Nawa M, Chiba T, Aiso S, Nishimoto I, Matsuoka M. 2006. Transforming growth factor beta2 autocrinally mediates neuronal cell death induced by amyloid-beta. *J Neurosci Res.* 83:1039–1047.
- Hock C, Heese K, Müller-Spahn F et al. 2000. Increased CSF levels of nerve growth factor in patients with Alzheimer's disease. *Neurology.* 54:2009–2011.
- Hock C, Heese K, Müller-Spahn F, Huber P, Riesen W, Nitsch RM, Otten U. Increased CSF levels of nerve growth factor in patients with Alzheimer's disease. *Neurology* 2000; 54: 2009-2011
- Humpel C, J. Marksteiner J. Peripheral biomarkers in dementia and Alzheimers disease. In: "The Handbook of Neuropsychiatric Biomarkers, Endophenotypes and Genes, Volume III: Metabolic and Peripheral Biomarkers", Michael S. Ritsner (Editor), Springer, 2009; ISBN: 978-1-4020-9837-6.
- Iadecola C. 2004. Neurovascular regulation in the normal brain and in Alzheimer's disease. *Nat Rev Neurosci.* 5:347–360.
- Kaplin A, Carroll KA, Cheng J, Allie R, Lyketsos CG, Calabresi P, Rosenberg PB. IL-6 release by LPS-stimulated peripheral blood mononuclear cells as a potential biomarker in Alzheimer's disease. *Int Psychogeriatr.* 2009 Apr;21(2):413-4. Epub 2008 Dec 1.
- Kassner SS, Bonaterra GA, Kaiser E, Hildebrandt W, Metz J, Schröder J, Kinscherf R. Novel systemic markers for patients with Alzheimer disease? - a pilot study. *Curr Alzheimer Res.* 2008 Aug;5(4):358-66.
- Komulainen P, Pedersen M, Hänninen T, Bruunsgaard H, Lakka TA, Kivipelto M, Hassinen M, Rauramaa TH, Pedersen BK, Rauramaa R. BDNF is a novel marker of cognitive function in ageing women: the DR's EXTRA Study. *Neurobiol Learn Mem.* 2008 Nov;90(4):596-603.
- Kong QL, Zhang JM, Zhang ZX, Ge PJ, Xu YJ, Mi RS, Zhao YH, Sui YP, He W. [Serum levels of macrophage colony stimulating factor in the patients with Alzheimer's disease] *Zhongguo Yi Xue Ke Xue Yuan Xue Bao.* 2002 Jun;24(3):298-301. Chinese.
- Levi-Montalcini R. 1987. The nerve growth factor, thirty-five years later. *Biosci Rep.* 7:681–699.
- Lim JS, Cho H, Hong HS, Kwon H, Mook-Jung I, Kwon YK. 2007. Upregulation of amyloid precursor protein by platelet-derived growth factor in hippocampal precursor cells. *Neuroreport.* 6:1225–1229
- Lindberg C, Chromek M, Ahrengart L, Brauner A, Schultzberg M, Garlind A. Soluble interleukin-1 receptor type II, IL-18 and caspase-1 in mild cognitive impairment and severe Alzheimer's disease. *Neurochem Int.* 2005 Jun;46(7):551-7.
- Lindahl B, Westling C, Gimenez-Gallego G, Lindahl U, Salmivirta M. 1999. Common binding sites for beta-amyloid fibrils and fibroblast growth factor-2 in heparan sulfate from human cerebral cortex. *J Biol Chem.* 274:30631–30635.

- Lue LF, Ryde I R, Brigham EF, Yang LB, Hampel H, Murphy GM Jr, Brachova L, Yan SD, Walker DG, Shen Y, Rogers J. Inflammatory repertoire of Alzheimer's disease and nondemented elderly microglia in vitro. *Glia* 2001; 35: 72-79
- Magaki S, Mueller C, Dickson C, Kirsch W. Increased production of inflammatory cytokines in mild cognitive impairment. *Exp Gerontol.* 2006; 42: 233-240
- Malaguarnera L, Motta M, Di Rosa M, Anzaldi M, Malaguarnera M. Interleukin-18 and transforming growth factor-beta 1 plasma levels in Alzheimer's disease and vascular dementia. *Neuropathology.* 2006 Aug;26(4):307-12.
- Marksteiner J., Georg Kemmler, Elisabeth M. Weiss, Gabriele Knaus, Celine Ullrich, Sergej Mechteriakov, Harald Oberbauer, Simone Auffinger, Josef Hinterhölzl, Hartmann Hinterhuber and Christian Humpel. Five out of 16 plasma signaling proteins are enhanced in plasma of patients with mild cognitive impairment and Alzheimer's disease. *Neurobiol Aging.* 2009 Apr 21. [Epub ahead of print]
- Marksteiner J, M. Pirchl, C. Ullrich, H. Oberbauer, I. Blasko, W. Lederer, H. Hinterhuber and C. Humpel. Analysis of cerebrospinal fluid of Alzheimer patients: biomarkers and toxic properties. *Pharmacology* 82 (2008) 214-220.
- Mocali A, Cedrola S, Della Malva N, Bontempelli M, Mitidieri VA, Bavazzano A, Comolli R, Paoletti F, La Porta CA. Increased plasma levels of soluble CD40, together with the decrease of TGF beta1, as possible differential markers of Alzheimer disease. *Exp Gerontol.* 2004 Oct;39(10):1555-61.
- Moore AH, O'Banion MK. 2002. Neuroinflammation and anti-inflammatory therapy for Alzheimer's disease. *Adv Drug Deliv Rev.* 54:1627-1656.
- Moser K, Reindl M, Blasig I, Humpel C. 2004. Brain capillary endothelial cells proliferate in response to NGF, express NGF receptors and secrete NGF after inflammation. *Brain Res.* 1017:53-60.
- Mufson EJ, Ma SY, Dills J et al. 2002. Loss of basal forebrain p75^{NTR} immunoreactivity in subjects with mild cognitive impairment and Alzheimer's disease. *J Comp Neurol.* 443:136-153.
- Olson L, Nordberg A, von Holst H, Bäckman L, Ebendal T, Alafuzoff I, Amberla K, Hartvig P, Herlitz A, Lilja A, Lundqvist H, Långström B, Meyerson B, Persson A, Viitanen M, Winblad B, Seiger Å. Nerve growth factor affects 11C-nicotine binding, blood flow, EEG, and verbal episodic memory in an Alzheimer patient. *J Neural Transm* 4, 79-95, 1992
- Perry VH, Bell MD, Brown HC, Matyszak MK. 1995. Inflammation in the nervous system. *Curr Opin Neurobiol.* 5:636-641.
- Popp J, Bacher M, Kölsch H, Noelker C, Deuster O, Dodel R, Jessen F, 2008. Macrophage migration inhibitory factor in mild cognitive impairment and Alzheimer's disease. *J Psychiatr Res.* 43: 749-753.

- Ray S, Britschgi M, Herbert C, Takeda-Uchimura Y, Boxer A, Blennow K, Friedman L F, Galasko D R, Jutel M, Karydas A, *et al.* (2007). Classification and prediction of clinical Alzheimer's diagnosis based on plasma signaling proteins. *Nat Medicine* 13, 1359-1362.
- Rodriguez-Rodriguez E, Sanchez-Juan P, Mateo I, Llorca J, Infante J, Garcia-Gorostiaga I, Berciano J, Combarros O. Serum levels and genetic variation of TGF-beta1 are not associated with Alzheimer's disease. *Acta Neurol Scand.* 2007 Dec;116(6):409-12.
- Sala G, Galimberti G, Canevari C, Raggi ME, Isella V, Facheris M, Appolloniol, Ferrarese C. Peripheral cytokine release in Alzheimer patients: correlation with disease severity. *Neurobiol Aging.* 2003 Nov;24(7):909-14.
- Schuitemaker A, Dik MG, Veerhuis R, Scheltens P, Schoonenboom NS, Hack CE, Blankenstein MA, Jonker C. Inflammatory markers in AD and MCI patients with different biomarker profiles. *Neurobiol Aging.* 2008 Mar 29.
- Speciale L, Calabrese E, Saresella M, Tinelli C, Mariani C, Sanvito L, Longhi R, Ferrante P. Lymphocyte subset patterns and cytokine production in Alzheimer's disease patients. *Neurobiol Aging.* 2007 Aug;28(8):1163-9. Epub 2006 Jun 30.
- Tan ZS, Beiser AS, Vasan RS, Roubenoff R, Dinarello CA, Harris TB, Benjamin EJ, Au R, Kiel DP, Wolf PA, Seshadri S. Inflammatory markers and the risk of Alzheimer disease: the Framingham Study. *Neurology.* 2007 May 29;68(22):1902-8.
- Tarkowski E, Issa R, Sjögren M, *et al.* 2002. Increased intrathecal levels of the angiogenic factors VEGF and TGF-beta in Alzheimer's disease and vascular dementia. *Neurobiol Aging.* 23:237-243.
- Thoenen H, Barde Y-A. 1980. Physiology of nerve growth factor. *Physiol Rev.* 60:1284-1335.
- Tsuboi, Y., Kakimoto, K., Nakajima, M., Akatsu, H., Yamamoto, T., Ogawa, K., Ohnishi, T., Daikuhara, Y., Yamada, T. Increased hepatocyte growth factor level in cerebrospinal fluid in Alzheimer's disease. *Acta Neurol. Scand.* 2003, 107:81-86
- Tuszynski MH, Thal L, Pay M, Salmon DP, Sang UH, Bakay R, Patel P, Blesch A, Vahlsing ML, Ho G, Tong G, Potkin S, Fallon J, Hansen L, Mufson EJ, Kordower JH, Gall C, Conner, J., 2005. A phase I clinical trial of nerve growth factor gene therapy for Alzheimer disease, *Nature Medicine* 11: 551-555.
- Zambrano N, Gianni D, Bruni P, Passaro F, Telese F, Russo T. 2004. Fe65 is not involved in the platelet-derived growth factor-induced processing of Alzheimer's amyloid precursor protein, which activates its caspase-directed cleavage. *J Biol Chem.* 279:16161-16169
- Zetterberg H, Wahlund L-O, Blennow K. Cerebrospinal fluid markers for prediction of Alzheimer's disease, *Neurosci. Lett.* 2003; 352: 67-69
- Zuliani G, Cavalieri M, Galvani M, Passaro A, Munari MR, Bosi C, Zurlo A, Fellin R. Markers of endothelial dysfunction in older subjects with late onset Alzheimer's disease or vascular dementia. *J Neurol Sci.* 2008 Sep 15;272(1-2):164-70.

Table 1A: Growth factors in cerebrospinal fluid and blood in dementia

Biomarker	increased	decreased	unchanged	References
Nerve growth factor	CSF (AD)			Blasko et al., 2006; Hock et al., 2000; Marksteiner et al., 2008
Hepatocyte growth factor	CSF (AD)		CSF (AD)	Tsuboi et al., 2003; Blasko et al., 2006
Vascular endothelial growth factor	CSF (AD)		CSF (AD)	Tsuboi et al., 2003; Blasko et al., 2006
Brain-derived neurotrophic factor	P (women)		CSF (AD)	Komulainen et al., 2008
Glial cell line-derived neurotrophic factor	P (AD)	P (AD)	CSF (AD)	Blasko et al., 2006; Ray et al., 2007; Marksteiner et al., 2009
Fibroblast growth factor-2			CSF (AD)	Blasko et al., 2006; Zetterberg et al., 2003
Transforming growth factor-1 β	CSF+P (AD)	S (AD)	CSF+S (AD)	Blasko et al., 2006; Zetterberg et al., 2003; Malaguarnera et al., 2006; Rodriguez et al., 2007; Mocali et al., 2004; de Servi et al., 2002
Insulin-like growth factor binding protein	P (AD)			Ray et al., 2007
Epidermal growth factor	P (AD)	P (AD)		Ray et al., 2007; Marksteiner et al., 2009
Angiopoietin-2	P (AD)		P (AD)	Ray et al., 2007; Marksteiner et al., 2009
Platelet-derived growth factor-BB		P (AD)	P (AD)	Ray et al., 2007; Marksteiner et al., 2009
Progranulin		CSF+P (FTLD)		Ghidoni et al., 2009

Abbreviations: AD, Alzheimers disease; CSF, cerebrospinal fluid; FTLD, frontotemporal lobe dementia; P, plasma; S, serum. Note that sometimes the same biomarkers has been found to be increased or decreased or unchanged reflecting the heterogeneity of the disease.

Table 1B: Cytokines and chemokines in cerebrospinal fluid and blood in dementia

Biomarker	increased	decreased	unchanged	References
Interleukin-1 α		P (AD)	P (AD, MCI)	Ray et al., 2007; Marksteiner et al., 2009
Interleukin-1 β	S (AD)		P (AD, vD)	Angelopoulos et al., 2008; Kong et al., 2002
Interleukin-6	CSF (AD,MCI)		P (AD, FTLD, vD)	Schuitemaker et al., 2008; Galimberti et al., 2008; Angelopoulos et al., 2008; Dik et al., 2005; Bergman et al., 2002; Kog et al., 2002; Bermejo et al., 2008; Baranowska-Bik et al., 2008; Zuliani et al., 2007
Interleukin-10			P (AD, vD)	Angelopoulos et al., 2008
Interleukin-11	CSF+P (AD, FTLD)		P (AD, MCI)	Galimberti et al., 2008; Ray et al., 2007; Marksteiner et al., 2009
Interleukin-18	P (AD,vD)		P(AD)	Lindberg et al., 2005; Malaguarnera et al., 2006
Tumor necrosis factor- α	P (Ce)	P (AD)	P+S (AD, vD)	Bergman et al., 2002; Kong et al., 2002; Angelopoulos et al., 2008; Ray et al., 2007; Marksteiner et al., 2009; Bruunsgaard et al., 1999
Granulocyte-colony stimulating factor = CSF-3		P (AD)	P (AD)	Ray et al., 2007; Marksteiner et al., 2009
Macrophage-colony stimulating factor = CSF-1	P (AD)	P (AD)	P (AD)	Ray et al., 2007; Marksteiner et al., 2009; Kong et al., 2009
Interleukin-3 = Multi-colony stimulating factor		P (AD)	P (AD)	Ray et al., 2007; Marksteiner et al., 2009
CCL2 = Monocyte chemoattractant protein-1	S (AD, MCI)		CSF (AD)	Blasko et al., 2006; Galimberti et al., 2006
CCL5 = RANTES	P (AD)	P (AD)		Ray et al., 2007; Marksteiner et al., 2009
CCL7 = Monocyte chemoattractant protein -3		P (AD)	P (AD)	Ray et al., 2007; Marksteiner et al., 2009
CCL15 = Macrophage inflammatory protein-1 δ	P (AD)	P (AD)		Ray et al., 2007; Marksteiner et al., 2009
CCL18 = Macrophage inflammatory protein-4	P (AD, MCI)		P (AD)	Ray et al., 2007; Marksteiner et al., 2009
CXCL8 = Interleukin-8	P (AD)		P (AD)	Ray et al., 2007; Marksteiner et al., 2009
Macrophage migration inhibitory factor (MIF)	CSF (MCI, AD)			Popp et al., 2009

Abbreviations: AD, Alzheimers disease; CSF, cerebrospinal fluid; Ce, centenarians; ; FTLD, frontotemporal lobe dementia; MCI, mild cognitive impairment; P, plasma; S, serum; vD, vascular dementia. Note that

sometimes the same biomarkers has been found to be increased or decreased or unchanged reflecting the heterogeneity of the disease.

ACCEPTED MANUSCRIPT

Table 2: Plasma derived biomarkers

	control	MCI	AD
n	19	44	96
MIP1 δ	3,890 \pm 510	4,784 \pm 334 ns	5,212 \pm 260 *
MIP4	65,900 \pm 9,300	90,942 \pm 7,275 *	81,716 \pm 5,720 ns
RANTES	9,823 \pm 2,418	20,824 \pm 4,581 *	23,280 \pm 3,724 *

Values are taken from Marksteiner et al., Neurobiology of Aging, 2009 in press; values are given as mean \pm SEM pg/ml, as measured by Multiplex Searchlight ELISAs. MCI, mild cognitive impairment; AD Alzheimer's disease. *p<0.05, ns not significant. MIP, macrophage inflammatory protein, RANTES, regulated upon activation, normal T cell expressed and secreted.

Table 3: Stimulated release of cytokines and growth factors from peripheral blood mononuclear cells of patients with mild cognitive impairment (MCI) or Alzheimer's disease (AD) or depression compared to healthy controls.

	Control	MCI	AD	Depression
Age	68±4	71±1	76±1	70±2
MSSE	28.4±0.3	27.0±0.4	18.6±0.1 ***	28.0±0.2
n	9	20	37	11
IL-1 α	11.3±4.1	13.3±1.5	9.0±0.8	11.2±2.6
IL-3	0.13±0.09	0.42±0.05 *	0.18±0.03	0.15±0.08
IL-6	1.38±0.40	1.02±0.14	1.01±0.12	0.91±0.17
IL-11	5.14±1.15	1.90±0.14 **	3.93±0.45	3.95±1.02
EGF	1.22±0.28	1.91±0.15 *	1.17±0.09	0.94±0.13
G-CSF	124.7±29	97.5±11	88.7±8	100.5±15
MCP-3	0.64±0.24	0.47±0.04	0.52±0.06	0.52±0.08
Ratio EGF/IL-11	0.31±0.08	1.14±0.14 ***	0.43±0.04	0.37±0.07

EDTA blood was collected from patients, centrifuged on a Biocoll gradient, the PBMCs were isolated and an aliquot cultured in medium with 4 μ g/ml phytohemagglutinin-L for 24hr, and then the levels of biomarkers were measured in the supernatants by Searchlight multiplex ELISA or commercial single ELISA (IL-6). Values are given as mean \pm SEM ng/ml per 1 mg cells per 24 hr. Statistical analysis was performed by one way ANOVA with a subsequent Dunnet posthoc test (* p <0.05; ** p <0.01; *** p <0.001). EGF, epidermal growth factor; G-CSF, granulocyte-colony stimulating factor; IL, interleukins, MCP, monocyte chemoattractant protein.