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ORIGINAL PAPER

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Inverse association of cortisol serum levels with T-tau, P-tau 181 and P-tau 231 peptide levels and T-tau/Aβ 1–42 ratios in CSF in patients with mild Alzheimer's disease dementia

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Abstract Hypercortisolemia and increased levels of hyperphosphorylated tau proteins in cerebrospinal fluid (CSF) are common features with pathogenic relevance in Alzheimer's disease (AD). Experimental studies point to an influence of cortisol on Aβ and tau pathology in AD. Association of both parameters have not yet been described in a sample of AD patients. In the present study, serum levels of cortisol were determined in 26 patients with mild AD dementia and 20 age-matched healthy elderly controls by ELISA. In addition, we measured in AD patients CSF levels of cortisol, total tau (T-tau), tau phosphorylated at threonine 181 (P-tau 181), tau protein phosphorylated at threonine 231 (P-tau 231) and beta-Amyloid (Aβ) 1–42 and determined T-tau/Aβ 1–42 ratios in CSF. We found in AD patients significantly increased cortisol serum levels (551.4 ± 146.1 nmol/l; $P = 0.002$) as compared to healthy controls (435.3 ± 83.9 nmol/l). In AD patients, cortisol serum levels were significantly inversely correlated with T-tau ($r = -0.496$; $P = 0.01$), P-tau 181 ($r = -0.558$; $P = 0.003$) and P-tau 231 (−0.500; $P = 0.009$) protein levels and T-tau/Aβ 1–42 ratios ($r = -0.450$; $P = 0.021$) in CSF. In addition, cortisol serum levels showed a trend of positive correlation with Aβ 1–42 CSF levels ($r = 0.386$; $P = 0.052$). However, no significant correlations of cortisol serum with CSF levels as well as cortisol CSF levels with CSF biomarkers could be detected in AD patients. In conclusion, our results show that increased cortisol serum but not CSF levels are associated with minor signs of AD pathology in CSF, indicating a putative neuroprotective effect of moderately elevated cortisol serum levels in patients with mild AD dementia.

Key words Alzheimer's disease · cortisol · total tau · phospho-tau 181 · phospho-tau 231 · beta-Amyloid 1–42

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Introduction

Alzheimer's disease (AD) is the most common type of dementia in the elderly, which currently affects about 26 million people worldwide. Cerebral accumulation of beta-amyloid (Aβ) 1–42 in amyloid plaques and hyperphosphorylated tau protein in neurofibrillary tangles are hallmarks of AD pathology. According to recent data, there are also changes in cerebral neurogenesis in AD patients [20]. Nevertheless, the exact mechanisms leading to AD pathology are still not completely understood.

Hyperactivity of the hypothalamus-pituitary-adrenal (HPA) axis is a well-described feature in AD, resulting in increased cortisol levels in blood and cerebrospinal fluid (CSF) [8, 21, 24, 25]. However, the role of HPA-axis disturbance in AD is still subject of controversy. Elevated cortisol levels could be the cause or a consequence of the damage of cerebral structures involved in the HPA-axis regulation (i.e. hippocampus) [17]. Measurement of cortisol levels in AD patients seems to be of prognostic relevance. AD patients show increased cortisol plasma levels with the highest cortisol levels in the most severely

demented patients [8]. In addition, increased plasma cortisol levels have been associated with more rapid disease progression in AD subjects [7]. In a recent study, increased cortisol CSF levels have been demonstrated in AD patients but not in patients with mild cognitive impairment, suggesting that the increase of CSF cortisol is related to progressing AD pathology [24].

Aβ 1–42 and protein levels of total tau (T-tau) and of its hyperphosphorylated isoforms P-tau 181 and P-tau 231 in CSF are useful biomarkers for diagnosis of AD. T-tau, P-tau 181 and P-tau 231 protein levels are significantly elevated, whereas Aβ 1–42 is markedly reduced in the CSF of AD patients [14, 26]. The CSF T-tau/Aβ 1–42 ratio has also been demonstrated to be useful for differentiating AD from normal ageing with significantly increased CSF T-tau/Aβ 1–42 ratios in AD patients as compared with healthy controls [19]. In addition, pathological concentrations of Aβ 1–42 and tau protein at baseline have predictive value for the development of AD in patients with mild cognitive impairment [15]. A recent study demonstrated, that CSF T-tau/Aβ 1–42 ratio has predictive value for cognitive decline in non-demented older adults with higher ratios in subjects with higher risk to develop AD [11]. Conflicting data exist whether CSF Aβ 1–42, T-tau and P-tau may also play a role as surrogate markers for cerebral Aβ and tau pathology. CSF P-tau 231 protein levels have been demonstrated to correlate with neocortical neurofibrillary pathology in AD [5], which itself is associated with dementia severity in AD [3]. In contrast, CSF levels of Aβ 1–42, T-tau and P-tau 181 were not associated with tangle or plaque burden in autopsy-confirmed AD patients [6, 10].

Experimental studies point to an influence of cortisol on Aβ and tau pathology in AD. Acute stress increases interstitial fluid Aβ via corticotrophin-releasing factor and neuronal activity [18], and glucocorticoids have been demonstrated to increase Aβ and tau pathology in a mouse model of AD [13]. Thus, in the present study we measured cortisol levels, both in serum and CSF, in patients with mild AD dementia, and evaluated their correlation with biomarker levels of AD in CSF to assess the association of cortisol with AD pathology. We hypothesized that higher cortisol levels may be associated with more signs of AD pathology in CSF.

Materials and methods

Subjects

Twenty-six AD outpatients from our Memory clinic and 20 age-matched healthy elderly controls were included in the study (Table 1). All AD patients met the diagnostic criteria of probable AD according to ICD-10 and DSM-IV 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) [23]. The severity of dementia was assessed by MMSE [12]. The control subjects were without any organic brain disorders and had to reach a MMSE score ≥27.

Table 1 Demographic, clinical and CSF parameters of AD patients and the control group

Variables	AD patients (n = 26)	Control group (n = 20)	P-value
Male/female (n)	10/16	13/7	0.136[a]
Age (years), [mean ± SD]	70.9 ± 8.1	70.0 ± 11.8	0.765[b]
MMSE, (mean ± SD)	23.5 ± 1.6	28.5 ± 1.5	<0.0001[b]

MMSE mini-mental state examination
[a]Chi-square test
[b]Two-tailed t test

The AD patients and the control group underwent a physical, neurological, and psychiatric examination. In all AD patients we examined CSF. In addition, an electroencephalography and a computed tomography or magnetic resonance imaging (MRI) were also performed, to validate the diagnosis of AD. Routine laboratory tests included analysis of vitamin B12, folic acid and thyroxine levels, to exclude other causes of dementia. Patients or control subjects with current or a history of depression or psychosis, with major physical illness, alcohol or substance abuse or use of psychoactive medications were excluded from the study. No patient or control subject received acetylcholinesterase inhibitors (AChE), antidepressants, nonsteroidal antiphlogistics, corticosteroids or statins. The regional ethical committee approved the study and written informed consent was obtained from each individual.

Sample collection

Serum samples were collected by venipuncture after inserting a cannula between 08:00 and 09:00 a.m. in the fasting state and following a patient rest of at least 20 min and then immediately placed in ice for 20 min. After centrifugation plasma was stored at −80°C until analysis.

Lumbar puncture (LP) was performed directly after serum collection. All CSF samples with more than 500 erythrocytes per µl were excluded, thus ensuring a clean CSF sample. The CSF samples were then centrifuged 2,000×g for 10 min to eliminate cells and other insoluble material. Aliquots of 2 ml were stored at −80°C until biochemical analyses were performed.

Measurement of cortisol in serum and CSF

Cortisol concentrations were assayed in duplicate using a commercially available chemiluminescence ELISA (detection range: 5.5–2,069 nmol/l, Siemens, Germany) according to the manufacturer's instructions. The intra- and interassay variabilities of the test were <10%.

Measurement of T-tau, P-tau 181, P-tau 231 and Aβ 1–42 levels in CSF

The determination of the CSF concentrations of T-tau, P-tau 181, P-tau 231 and Aβ 1–42 was performed by commercial ELISAs (ELISA kits from Innogenetics) according to the manufacturer's instructions.The intra- and interassay variabilities for all ELISAs were <10%.

Data analysis

The groups were compared with chi-square test in case of categorical variables or with two-tailed t test in case of quantitative variables. All biomarker data were normally distributed according

to Kolmogorov–Smirnov test. Pearson correlations between variables were determined. In a next step, age, gender and dementia severity using MMSE scores were controlled with partial correlation. The data are presented as the mean ± SD. Significance for the results was set at $P < 0.05$. All statistical analyses were carried out using the statistical analysis software package SPSS 14.0® (Munich, Germany).

Results

AD patients and healthy controls were comparable regarding age and gender, showing no significant differences (Table 1). AD patients suffered from mild AD.

We found in AD patients significantly increased cortisol serum levels (551.4 ± 146.1 nmol/l; $P = 0.002$) as compared to healthy controls (435.3 ± 83.9 nmol/l).

In AD patients, mean CSF T-tau level was 450.7 ± 240.3 pg/ml, P-tau 181 level 87.3 ± 25.7 pg/ml, P-tau 231 level 79.8 ± 42.1 pg/ml, Aβ 1–42 level 324.7 ± 160.0 pg/ml and tau/Aβ 1–42 ratio 1.7 ± 1.2. Cortisol serum levels were significantly inversely correlated with T-tau ($r = -0.496$; $P = 0.01$), (Fig. 1a) P-tau 181 ($r = -0.558$; $P = 0.003$) (Fig. 1b) and P-tau 231 (−0.500; $P = 0.009$) (Fig. 1c) protein levels and T-tau/Aβ 1–42 ratios ($r = -0.450$; $P = 0.021$) in CSF (Fig. 1e). In addition, cortisol serum levels showed a trend of positive correlation with Aβ 1–42 CSF levels ($r = 0.386$; $P = 0.052$) (Fig. 1d).

After controlling for age, gender and for dementia severity using MMSE scores with partial correlation, cortisol serum levels in AD patients remained significantly inversely correlated with T-tau ($r = -0.471$; $P = 0.013$), P-tau 181 ($r = -0.602$; $P = 0.002$) and P-tau 231 (−0.559; $P = 0.005$) protein levels and T-tau/Aβ 1–42 ratios ($r = -0.415$; $P = 0.031$) in CSF and cortisol serum levels showed a trend of positive correlation with Aβ 1–42 CSF levels ($r = 0.334$; $P = 0.088$).

Mean Cortisol CSF level in AD patients was ±34.4 ± 6.8 nmol/l. Cortisol CSF levels showed no significant correlations with any CSF biomarker (T-tau: $r = -0.045$; $P = 0.826$; P-tau 181: $r = 0-0.089$; $P = 0.667$; P-tau 231: $r = -0.015$; $P = 0.942$; T-tau/Aβ 1–42 ratio: $r = 0.123$; $P = 0.549$; Aβ 1–42: $r = -0.138$; $P = 0.501$) or with cortisol serum levels ($r = 0.139$; $P = 0.497$).

Discussion

To our knowledge, this is the first study investigating the association between cortisol serum and CSF levels with CSF levels of T-tau, P-tau 181, P-tau 231 and Aβ 1–42 in patients with mild AD dementia. Our finding of increased cortisol serum levels in AD patients is in line with the results of previous studies [8, 25]. As a new result, we found in AD patients an inverse correlation between cortisol serum levels and CSF levels of T-tau, P-tau 181, P-tau 231 and T-tau/Aβ 1–42 ratio in CSF as well as a trend of a positive correlation with Aβ 1–42 CSF levels.

In the present study, against our hypothesis we found that higher cortisol serum levels were associated with minor signs of AD pathology in CSF. However, the increase of cortisol serum levels in the present study was rather small compared to earlier examinations [8, 21, 25]. According to data in literature, glucocorticoids have the potential to affect the development, survival and death of neurons. Nevertheless, these data reflect paradoxical features of glucocorticoids, as they may be critically involved in both neurodegenerative and neuroprotective processes [2]. Several studies, i.e. those by Green et al. [13] (glucocorticoids increase the Aβ and tau pathology in a mouse model of AD) and by Csernansky et al. [7] (initially higher serum cortisol in the pre-dementia clinical stage of AD predicts a more rapid cognitive decline) provide evidence for harmful effects of glucocorticoids. However, the promotion of neurodegeneration by corticosterone seems to be dose-dependent: In an in vivo model experiment corticosteroids affected neurotoxic cell death in a U-shaped manner. Both lack of corticosterone and very high levels of corticosterone enhanced neuronal cell death, whereas moderately elevated corticosterone levels reduced the neurotoxic effects of N-methyl d-aspartate (NMDA) and Aβ [1]. These data and our findings point towards a dose-dependent role of cortisol in neurodegeneration with a neuroprotective potential of moderately increased cortisol serum levels at least in patients with mild AD dementia. Besides neuroprotection, alternative explanations for our observations could be a confound by dementia severity or the existence of a mild depression in the patients, which could lead to both, increased cortisol serum levels and decreased cognition despite identical levels of underlying pathology.

Several mechanisms are discussed to be involved in the neuroprotective effects of glucocorticoids including a decrease in after-hyperpolarization of neurons, increased synthesis and release of neurotrophic factors (e.g. nerve growth factor [NGF], basic fibroblast growth factor [bFGF]) and lipocortin-1, feedback regulation of Ca^{2+} currents in neurons and induction of antioxidant enzymes [2]. In addition, the anti-inflammatory effects of cortisol may diminish an initially harmful inflammatory reaction in AD [4, 9, 16, 22, 27]. Both brain-intrinsic and humoral inflammatory processes occur in AD. Epidemiological findings and data from studies of animal models of AD suggest that anti-inflammatory drugs can delay disease onset and may decelerate the progression of AD [16]. Thus, targeting inflammatory processes may be helpful to prevent and treat AD.

We did not find significant correlations of cortisol serum and CSF levels as well as cortisol CSF levels and

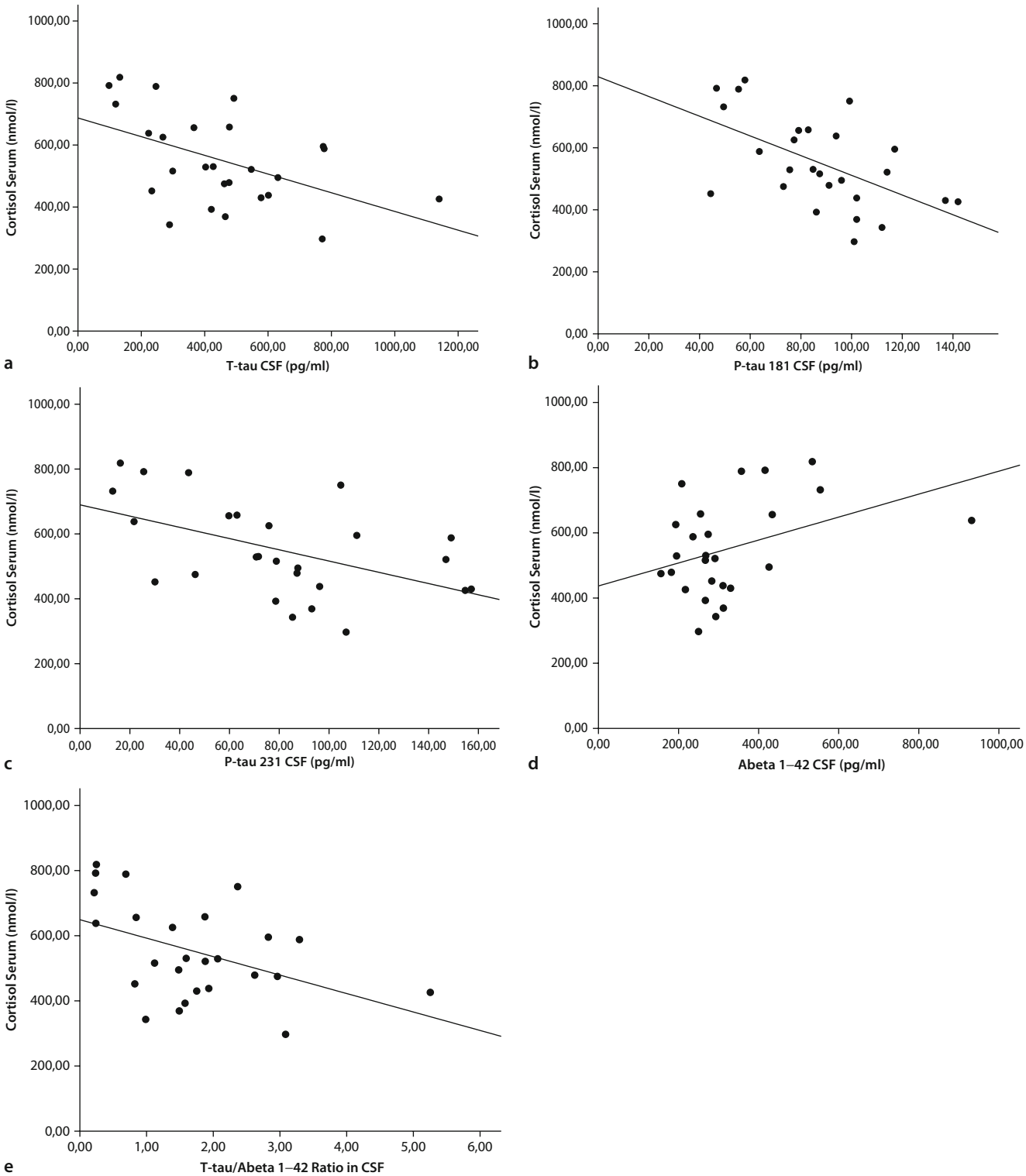

Fig. 1 **a**–**e** Correlations between cortisol serum and T-tau ((**a**) $r = -0.496$; $P = 0.01$), P-tau 181 ((**b**) $r = -0.558$; $P = 0.003$), P-tau 231 ((c) $r = -0.500$; $P = 0.009$), Aβ 1–42 (**d** $r = 0.386$; $P = 0.052$) CSF concentrations and T-tau/Aβ 1–42 ratios ((**e**); $r = -0.450$; $P = 0.021$) in CSF in 26 patients with Alzheimer's disease (Pearson test)

CSF biomarkers in AD patients. Thus, in AD patients levels of cortisol in serum and CSF appear to be independently and influence of cortisol on AD pathology is not reflected in an association of CSF cortisol and AD biomarker levels. Possibly, cortisol levels in the CSF compartment are not as relevant for the putative regulatory mechanism of glucocorticoids in AD as the cortisol levels in serum.

Certain limitations in the present study need to be considered when interpreting the results. First, we examined a small sample size. Second, we examined only patients with mild AD dementia. A larger sample also including moderate and severe dementia patients could allow to estimate cortisol changes related to the different clinical stages of the disease. Third, it remains unclear, whether increased (or decreased) serum cortisol levels contribute to or represent a consequence of the pathological changes in AD. Hence, increased cortisol serum levels may only reflect dysregulation of the HPA-axis resulting from the cerebral damage and dysfunction of structures involved in the HPA-axis.

Conclusions

In conclusion, our results show that higher cortisol serum but not CSF levels are associated with minor signs of AD pathology in CSF, indicating a putative neuroprotective effect of moderately elevated cortisol serum levels in patients with mild AD dementia. Further studies with larger numbers of patients are required, to elucidate the meaning and prognostic value of cortisol serum levels in different AD stages.

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References

1. Abraham I, Harkany T, Horvath KM, Veenema AH, Penke B, Nyakas C, Luiten PGM (2000) Chronic corticosterone administration dose-depentently modulates A$\beta_{(1-42)}$- and NMDA-induced neurodegeneration in rat magnocellular nucleus basalis. J Neuroendocrinol 12:486–494
2. Abraham IM, Harkany T, Horvath KM, Luiten PGM (2001) Action of glucocorticoids on survival of nerve cells: promoting neurodegeneration or neuroprotection? J Neuroendocrinol 13:749–760
3. Bierer LM, Hof PR, Purohit DP, Carlin L, Schmeidler J, Davis KL, Perl DP (1995) Neocortical neurofibrillary tangles correlate with dementia severity in Alzheimer's disease. Arch Neurol 52(1):81–88
4. Bjelakovic G, Beninati S, Pavlovic D, Kocic G, Jevtovic T, Kamenov B, Saranac LJ, Bjelakovic B, Stojanovic I, Basic J (2007) Glucocorticoids and oxidative stress. J Basic Clin Physiol Pharmacol 18(2):115–127
5. Buerger K, Ewers M, Pirttila T, Zinkowski R, Alafuzoff I, Teipel SJ, DeBernardis J, Kerkman D, McCulloch C, Soininen H, Hampel H (2006) CSF hyperphosphorylated tau protein correlates with neocortical neurofibrillary pathology in Alzheimer's disease. Brain 129(Pt 11):3035–3041
6. Buerger K, Alafuzoff I, Ewers M, Pirttilä T, Zinkowski R, Hampel H (2007) No correlation between CSF tau protein phosphorylated at threonine 181 with neocortical neurofibrillary pathology in Alzheimer's disease. Brain 130(Pt 10):e82, 1–2
7. Csernansky JG, Dong H, Fagan AM, Wang L, Xiong C, Holtzman DM, Morris JC (2006) Plasma cortisol and progression of dementia in subjects with Alzheimer-type dementia. Am J Psych 163:2164–2169
8. Davis KL, Davis BM, Greenwald BS, Mohs RC, Mathe AA, Johns CA, Horvath TP (1986) Cortisol and Alzheimer's disease, 1: basal studies. Am J Psych 143(3):300–305
9. Eikelenboom P, Veerhuis R, Scheper W, Rozemuller AJM, van Gool WA, Hoozemans JJM (2006) The significance of neuroinflammation in understanding Alzheimer's disease. J Neural Transm 113:1685–1695
10. Engelborghs S, Sleegers K, Cras P, Brouwers N, Serneels S, De Leenheir E, Martin JJ, Vanmechelen E, Van Broeckhoven C, De Deyn PP (2007) No association of CSF biomarkers with APOEepsilon4, plaque and tangle burden in definite Alzheimer's disease. Brain 130:2320–2326
11. Fagan AM, Roe CM, Xiong C, Mintun MA, Morris JC, Holtzman DM (2007) Cerebrospinal fluid tau/beta-amyloid(42) ratio as a prediction of cognitive decline in nondemented older adults. Arch Neurol 64(3):343–349
12. Folstein M, Folstein SE, McHugh PR (1975) "Mini-mental-state". A practical method for grading the cognitive state of patients for the clinician. J Psych Res 12:189–198
13. Green KN, Billings LM, Roozendaal B, McGaugh JL, La Ferla FM (2006) Glucocorticoids increase amyloid-beta and tau pathology in a mouse model of Alzheimer's disease. J Neurosci 26(35):9047–9056
14. Hampel H, Buerger K, Zinkowski R, Teipel SJ, Goernitz A, Andreasen N, Sjoegren M, DeBernardis J, Kerkman D, Ishiguro K, Ohno H, Vanmechelen E, Vanderstichele H, McCulloch C, Moller HJ, Davies P, Blennow K (2004) Measurement of phosphorylated tau epitopes in the differential diagnosis of Alzheimer disease: a comparative cerebrospinal fluid study. Arch Gen Psych 61(1):95–102
15. Hansson O, Zetterberg H, Buchhave P, Londos E, Blennow K, Minthon L (2006) Association between CSF biomarkers and incipient Alzheimer's disease in patients with mild cognitive impairment: a follow-up study. Lancet Neurol 5:228–234
16. Heneka MT, O'Banion MK (2007) Inflammatory processes in Alzheimer's disease. J Neuroimmunol 184:69–91
17. Höschl C, Hajek T (2001) Hippocampal damage mediated by corticosteroids—a neuropsychiatric research challenge. Eur Arch Psychiatry Clin Neurosci 251(Suppl 2):1181–1188
18. Kang JE, Cirrit JR, Don H, Csernansky JG, Holtzman DM (2007) Acute stress increases interstitial fluid amyloid-β via corticotrophin-releasing factor and neuronal activity. Proc Nat Acad Sci 104(25):10673–10678
19. Kapaki E, Paraskevas GP, Zalonis I, Zournas C (2003) CSF tau protein and beta-amyloid (1–42) in Alzheimer's disease diagnosis: discrimination from normal ageing and other dementias in the Greek population. Eur J Neurol 10(2):119–128
20. Kuhn HG, Cooper-Kuhn CM, Boekhoorn K, Lucassen PJ (2007) Changes in neurogenesis in dementia and Alzheimer mouse models: are they functionally relevant? Eur Arch Psychiatry Clin Neurosci 257(5):281–289
21. Martignoni E, Petraglia F, Costa A, Bono G, Genazzani AR, Nappi G (1990) Dementia of the Alzheimer type and hypothalamus-pituitary-adrenocortical axis: changes in cerebrospinal fluid corticotrophin releasing factor and plasma cortisol levels. Acta Neurol Scand 81(5):452–456
22. McColl A, Michlewska S, Dransfield I, Rossi AG (2007) Effects of glucocorticoids on apoptosis and clearance of apoptotic cells. Scientific WorldJournal 7:1165–1181
23. Mc Khann G, Drachman D, Folstein M, Katzman R, Price D, Stadlan EM (1984) Clinical diagnosis of Alzheimer's disease: report of the NINCDS-ADRDA Work Group under the auspices of Department of Health and Human Services Task Force on Alzheimer's disease. Neurol 34:939–944
24. Popp J, Schaper K, Kölsch H, Cvetanovska G, Rommel F, Klingmüller D, Dodel R, Wüllner U, Jessen F (2007) CSF cortisol in Alzheimer's disease and mild cognitive impairment. Neurobiol Aging doi:10.1016/j.neurobiolaging.2007.07.007
25. Rasmuson S, Nasman B, Carlstrom K, Olsson T (2002) Increased levels of adrenocortical and gonadal hormones in mild to moderate Alzheimer's disease. Dement Geriatr Cogn Disord 13(2):74–79

26. Sunderland T, Linker G, Mirza N, Putnam KT, Friedman DL, Kimmel LH, Bergeson J, Manetti GJ, Zimmermann M, Tang B, Bartko JJ, Cohen RM (2003) Decreased beta-amyloid 1–42 and increased tau levels in cerebrospinal fluid of patients with Alzheimer disease. J Am Med Assoc 289(16):2094–2103

27. Wyss-Coray T (2006) Inflammation in Alzheimer disease: driving force, bystander or beneficial response? Nat Med 12(9):1005–1015