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Negative results

# Five out of 16 plasma signaling proteins are enhanced in plasma of patients with mild cognitive impairment and Alzheimer's disease☆

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**Abstract**

Alzheimer's disease (AD) is a progressive neurodegenerative disorder with characteristic neuropathological changes of the brain. Great efforts have been undertaken to determine the progression of the disease and to monitor therapeutic interventions. Especially, the analysis of blood plasma had yielded incongruent results. Recently, Ray et al. (Nat. Med. 13, 2007, 1359f) identified changes of 18 signaling proteins leading to an accuracy of 90% in the diagnosis of AD. The aim of the present study was to examine 16 of these signaling proteins by quantitative Searchlight multiplex ELISA in order to determine their sensitivity and specificity in our plasma samples from AD, mild cognitive impairment (MCI), depression with and without cognitive impairment and healthy subjects. Quantitative analysis revealed an increased concentration in Biocoll isolated plasma of 5 out of these 16 proteins in MCI and AD patients compared to healthy subjects: EGF, GDNF and MIP1δ (in AD), MIP4 (in MCI) and RANTES (in MCI and AD). ROC analysis predicted a sensitivity of 65–75% and a specificity of 52–63% when comparing healthy controls versus MCI or AD. Depression without any significant cognitive deficits did not cause any significant changes. Depressed patients with significant cognitive impairment were not different from MCI patients. In conclusion, we detected a number of altered proteins that may be related to a disease specific pathophysiology. However, the overall expression pattern of plasma proteins could not be established as a biomarker to differentiate MCI from AD or from depression.


*Keywords:* Diagnosis; Blood; Plasma; Biomarker; Alzheimer; Mild cognitive impairment; Dementia; Multiplex ELISA

Patients were recruited from an outpatient clinic located at the Department of Psychiatry in Innsbruck, Austria. The study was approved by the local ethical committee. Healthy controls, patients with mild cognitive impairment or Alzheimer's disease and patients with depression with or without cognitive impairment were diagnosed according to international standards. Blood was collected, centrifuged on a Biocoll gradient and the plasma was frozen. The detection of 16 biomarkers was performed using the Thermo Scientific SearchLight Protein Array Technology (THP Medical Products, Vienna). Statistical analysis was performed by One Way ANOVA and receiver-operating characteristic (ROC) analysis.

Healthy controls ($n = 19$) had an age of $72.1 \pm 1.3$ years and a MMSE of $28.4 \pm 0.3$. The mean (±SEM) plasma control levels of the 16 measured biomarkers were (in pg/ml): ANG-2: $2168 \pm 534$; EGF: $2.4 \pm 0.5$; G-CSF: $7.8 \pm 1.4$; GDNF: $10.8 \pm 2.1$; ICAM1: $242{,}370 \pm 28{,}580$; IL1α: $3.2 \pm 0.4$; IL3: $8.1 \pm 0.7$; IL8 (CXCL8): $2.3 \pm 0.5$; IL11: $16.6 \pm 1.9$; MCP3 (CCL7): $3.3 \pm 0.6$; M-CSF: $1680 \pm 416$; MIP1δ (CCL15): $3890 \pm 510$; MIP4 (CCL18): $65{,}900 \pm 9300$; PDGF-BB: $228 \pm 65$; RANTES (CCL5): $9823 \pm 2418$ and TNFα: $9.5 \pm 0.7$. No statistically signif-

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Table 1
Plasma biomarkers in mild cognitive impairment (MCI), Alzheimer's disease (AD), depression with or without cognitive impairment (CI).

| | Mild cognitive impairment | Alzheimer's disease | Depression + CI | Depression |
|---|---|---|---|---|
| *n* | 44 | 96 | 26 | 16 |
| Age | 73.5 ± 1.2 | 77.0 ± 0.8* | 71.0 ± 1.6 | 69.1 ± 1.6 ns |
| MMSE | 27.1 ± 0.2 | 18.8 ± 0.6* | 24.8 ± 0.6 | 29.3 ± 0.1 ns |

| | Mild cognitive impairment | | | | Alzheimer's disease | | | | | Depression + CI | | | Depression | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | Mean | Median | *p*1 | *p*2 | Mean | Median | *p*1 | *p*2 | *p*3 | Mean | Median | *p*3 | Mean | Median | *p*1 |
| EGF | 106 ± 12 | 75 | ns | ns | 181 ± 16 | 81 | **0.015** | **0.021** | ns | 68 ± 14 | 67 | ns | 179 ± 32 | 122 | ns |
| G-CSF | 186 ± 30 | 166 | ns | ns | 167 ± 18 | 125 | ns | ns | ns | 181 ± 27 | 138 | ns | 67 ± 16 | 69 | ns |
| GDNF | 134 ± 12 | 203 | ns | ns | 239 ± 14 | 221 | **0.044** | **0.013** | ns | 305 ± 36 | 295 | ns | 99 ± 26 | 68 | ns |
| ICAM1 | 121 ± 4 | 134 | 0.054 | **0.011** | 111 ± 4 | 122 | ns | ns | ns | 108 ± 8 | 110 | ns | 94 ± 9 | 136 | ns |
| IL1α | 105 ± 5 | 113 | ns | ns | 108 ± 5 | 112 | ns | ns | ns | 112 ± 11 | 105 | ns | 97 ± 13 | 123 | ns |
| IL3 | 94 ± 6 | 106 | ns | ns | 94 ± 4 | 90 | ns | ns | ns | 91 ± 6 | 93 | ns | 129 ± 19 | 139 | ns |
| IL8 | 266 ± 45 | 205 | ns | ns | 257 ± 26 | 226 | ns | ns | ns | 138 ± 24 | 195 | ns | 246 ± 34 | 150 | ns |
| IL11 | 78 ± 4 | 94 | ns | ns | 88 ± 4 | 95 | ns | ns | ns | 97 ± 13 | 110 | ns | 138 ± 31 | 111 | ns |
| MCP3 | 116 ± 11 | 165 | ns | ns | 143 ± 15 | 157 | ns | ns | ns | 126 ± 13 | 144 | ns | 77 ± 20 | 67 | ns |
| M-CSF | 113 ± 18 | 107 | ns | ns | 86 ± 7 | 101 | ns | ns | ns | 84 ± 12 | 101 | ns | 81 ± 22 | 113 | ns |
| MIP1δ | 123 ± 7 | 128 | ns | **0.035** | 134 ± 5 | 145 | **0.032** | **0.002** | ns | 128 ± 8 | 149 | ns | 105 ± 17 | 101 | ns |
| MIP4 | 138 ± 8 | 169 | **0.016** | **0.012** | 124 ± 7 | 127 | ns | ns | ns | 179 ± 31 | 136 | ns | 118 ± 18 | 134 | ns |
| PDGF-BB | 142 ± 16 | 139 | ns | ns | 129 ± 10 | 138 | ns | ns | ns | 104 ± 17 | 95 | ns | 96 ± 23 | 152 | ns |
| RANTES | 212 ± 22 | 131 | **0.013** | **0.011** | 237 ± 16 | 148 | **0.026** | **0.022** | ns | 183 ± 23 | 161 | ns | 88 ± 22 | 110 | ns |
| TNFα | 150 ± 7 | 140 | ns | **0.007** | 158 ± 7 | 145 | ns | **0.004** | ns | 188 ± 11 | 173 | ns | 81 ± 11 | 86 | ns |

Plasma biomarkers were analyzed by Searchlight Multiplex ELISA as described in Methods. Values are presented as % of controls. Statistical analysis: *p*1, versus healthy control ($n = 19$); *p*2, versus mixed controls and depression ($n = 35$); *p*3, versus MCI. Statistical significance $p < 0.05$ (* or bold values).

icant difference was observed in any of the 16 measured plasma biomarkers between non-depressive healthy controls and depressive patients without cognitive impairment ($n = 16$) without any other neurological or psychiatric disorder. In MCI two proteins out of those 16 tested biomarkers were significantly increased compared to healthy controls: MIP4 and RANTES (Table 1). Out of 16 measured biomarkers 4 were significantly elevated in AD compared to healthy controls: EGF, GDNF, MIP1δ and RANTES (Table 1). When healthy controls and depressive patients without cognitive impairment were pooled in addition also ICAM1 and MIP1δ were enhanced in MCI and TNFα in MCI and AD (Table 1). Depressive patients with cognitive impairment were not different from MCI patients (Table 1). ROC analysis yielded a sensitivity of 75% and a specificity of 63% to discriminate healthy controls from MCI patients. Discrimination between healthy controls and AD subjects was somewhat lower showing a sensitivity of 66% and a specificity of 53%. No useful discrimination between MCI and AD patients was seen (60% sensitivity and 59% specificity).

In the present study, we detected significant increases in the expression levels in 5 out of 16 proteins in AD and MCI: EGF, GDNF, MIP1δ, MIP4 and RANTES. The protein expression profile revealed that certain proteins were significantly increased in MCI and AD. In general, neither MCI patients, AD patients or patients suffering from depression with cognitive impairment exhibited a protein expression profile that was specific and sensitive enough to be used as diagnostic biomarkers.

## Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.neurobiolaging.2009.03.011.