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Higher CSF Interleukin-6 and CSF Interleukin-8 in current depression in older women. Results from a population-based sample

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Abstract

Objective: The literature regarding cerebrospinal fluid (CSF) cytokines in geriatric depression is sparse. The aim of this study was to examine associations between CSF interleukin-6 (IL-6), interleukin-8 (IL-8) and depression in a population-based sample of older women who were followed for 17 years.

Methods: 86 non-demented women aged 70-84 years who participated in the Prospective Population Study of Women in Gothenburg, Sweden took part in a lumbar puncture in 1992-3. CSF IL-6 and CSF IL-8 were measured. Psychiatric symptoms were rated with the Comprehensive Psychopathological Rating Scale at baseline and at three subsequent face-to-face examinations. Depression (major or minor) was diagnosed in accordance with DSM-IV/DSM-IV research criteria.

Results: At baseline**,** women with ongoing major (n=10) or minor depression (n=9) had higher levels of CSF IL-6 (p= 0.008) and CSF IL-8 (p=0.007) compared with those without depression (n=67). Higher CSF IL-8 was related to higher MADRS score (p=0.003). New cases of depression were observed in 9 women during follow-ups. No associations between CSF cytokine levels and future depression could be shown in women without depression at baseline.

Conclusion: Higher levels of CSF IL-6 and IL-8 were associated with current depression in this population-based sample. CSF IL-6 and CSF IL-8 may play a role in depression in late life.

1. Introduction

In recent years it has been hypothesized that changes in proinflammatory cytokines might play an important role in pathophysiological processes related to depression (Miller et al 2009). While most studies have demonstrated higher serum levels of IL-6 in depressed persons compared to non-depressed (Hiles et al 2012), findings regarding peripheral IL-8 levels are mixed (Dowlati et al 2010). Regarding geriatric depression, there are several studies showing higher serum IL-6 and IL-8 levels in patients with major or minor depression (Baune et al 2012, Brambilla & Maggioni 1998, Lu et al 2013, Stewart et al 2009). The literature on cytokine levels in the cerebrospinal fluid (CSF) is more limited. Studies involve relatively small clinical samples; results are disparate (Carpenter et al 2004, Levine et al 1999, Lindqvist et al 2009, Martinez et al 2011, Sasayama et al 2013). We could identify only one clinical study that focused on a geriatric sample (Stubner et al 1999). Findings showed *lower* CSF IL-6 levels in depressed patients compared to healthy controls. CSF IL-8 was not examined in that study.

To our knowledge there are to date no studies examining CSF proinflammatory cytokines and depression in population-based samples. The aim of the present study was to examine a possible association between CSF proinflammatory cytokines and depression in older women. The reader should take note that this paper replaces our retracted paper (Kern et al 2013) in which an error had occurred when merging laboratory and survey data.

2. Methods

2.1 Subjects

The study sample was derived from the Prospective Population Study of Women (PPSW), a population-based survey in Gothenburg, Sweden including both those living in private households and in residential care. The study began in 1968-1969 and included a representative sample of 1462 women born on certain dates in 1908, 1914, 1918 and 1922. The original sample has been described in detail previously (Gudmundsson et al 2007).

In 1992-1993, 837 surviving women were invited to participate in a psychiatric examination, which for the purpose of the current study will be referred to as the baseline examination. Among 590 who agreed to take part, 88 (aged 70-84 years) consented to undergo a lumbar puncture (LP). Two of these who were diagnosed with dementia at the time of the LP were excluded from the current analyses, leaving 86 women born in 1908 (n=2), 1914 (n=7), 1918 (n=33) and 1922 (n= 43). The mean age of the participants at baseline in 1992-1993 was 72.5 years (SD 3.3). As previously reported (Gudmundsson et al 2007), there were no differences between women with (n=86) and without LP (n=504) with regard to age, psychiatric illnesses and several somatic conditions. Further, no differences could be shown regarding Mini Mental State Examination scores (p= 0.438), and Eysenck Personality Inventory ratings of the personality traits neuroticism (p= 0.666) and extroversion (p= 0.941).
Follow-up psychiatric examinations were conducted in 2000, 2005 and 2009. Response rates for surviving women were as follows: 2000, 87% (62 out of 71); 2005, 84% (42 out of 49); 2009, 74% (20 of 27). The study was approved by the Ethics Committee for Medical Research at the University of Gothenburg.

2.2 Psychiatric Examination and Diagnostics

The baseline psychiatric examination was semi-structured and performed by psychiatrists in 1992-1993. Follow-up exams were carried out by psychiatric nurses. Major depression was diagnosed in accordance with DSM-IV and minor depression was diagnosed according to DSM- IV research criteria. Depression symptom burden was measured with the Montgomery-Åsberg Depression Rating Scale (MADRS). The neuropsychiatric exam, used for exclusion purposes in this study, has been described in detail (Skoog et al 1993).

2.3 CSF Analyses

LPs were carried out in 1992-1993. CSF-samples (12 ml) were taken through the L3/L4 interspace. Samples were immediately centrifuged at 2000g for 10 minutes to eliminate cells and other insoluble materials, aliquoted in 1 ml portions, snap frozen at -80 ^{o}C, stored at that temperature and brought in an unbroken freeze chain to the laboratory for analyses. CSF levels of IL-6 and IL-8 were analyzed using the Human Pro-inflammatory II 4-Plex Assay Ultra-Sensitive Kit (Meso Scale Discovery, Gaithersburg, MD, USA). Intra-assay coefficients of variation were below 10% for all analytes.

2.4 Statistical analysis

Differences in cytokine levels were tested with the Mann-Whitney U test. Binary logistic regressions were used to explore how cytokine levels were related to depression. All models were adjusted for age, Body Mass Index (BMI) and smoking as possible confounders. MADRS score was used as a continuous variable to test for associations between cytokine

levels and depression severity. Statistical tests were carried out using SPSS for Windows (version17, SPSS Chicago, IL.). A two-tailed level of significance, $p < 0.05$ was used in all tests.

3. Theory

It has been hypothesized that cytokines can be secreted de novo in the brain by microglia, astrocytes and under some circumstances by neurons (Beumer et al 2012). Microglia are pivotal in immune surveillance and interpret and propagate inflammatory signals. Models of the aging brain show increased proinflammatory cytokines in the brain and increased expression of inflammatory receptors (Norden & Godbout 2012), a condition referred to as "priming". Active glia produce proinflammatory cytokines that can lead to sickness-behavior (Beumer et al 2012), linking cytokines to depression.

4. Results

4.1 Baseline findings

One fifth of the women had depression (Table 1). There was no age difference between women with (n=72.8) and without (n=72.4 years) ongoing depression (p=0.90).
Current depression was associated with higher CSF-levels of IL-6 and IL-8 (Table 2). As BMI and smoking status are confounders for CSF IL-6 (O'Connor et al 2009), we added these variables to the regression model. The association was no longer significant for CSF IL-6 (OR 1.22 CI [0.99-1.49] p=0.055). Higher IL-8-levels were associated with current depression in the adjusted model (OR 1.07 CI [1.02-1.12], p=0.005). Due to skewness in the distribution of

IL-6, regression models were also examined with CSF- quartiles. Higher CSF IL-6 quartiles were associated with depression at baseline in the adjusted models, as were higher CSF IL-8 quartiles (Table 2).

Ongoing antidepressant treatment was reported in five women and three of these fulfilled criteria for depression at the time of the baseline examination. After excluding the latter group, associations remained with CSF IL-6 (p= 0.049) and CSF IL-8 (p= 0.006) in the adjusted models. One of the participants was on treatment with systemic steroids and 8 women used antirheumatics/ anti-inflammatory medicine at baseline. Associations regarding CSF IL-8 (p= 0.002) remained, but not for CSF IL-6 (p= 0.055), after these women were excluded in the adjusted model.

Relationships between levels of CSF IL-6 and CSF IL-8 and dimensionally defined depression were also explored. While the adjusted model showed no correlation between CSF IL-6 levels and baseline MADRS score (r = 0.083, P = 0.450), higher CSF IL-8 levels were related to higher MADRS score (r = 0.237, p=0.003).

4.2 Prospective findings

Nine women without depression at baseline developed any depression during follow-up.

In a set of exploratory analyses, we examined the relationship between CSF cytokine levels in women without depression at baseline and development of new depression at any follow-up, but with only 9 cases of new depression the study was underpowered. No associations were seen between CSF IL-6 (p= 0.101) and IL-8 (p= 0.536) and future depression.

Dementia was diagnosed in 23 women at some point during follow-ups. No significant differences could be shown regarding baseline levels of CSF IL-6 (p=0.871) and IL-8 (p=0.259) between those who did and did not develop dementia in the fully adjusted models.

5. Discussion

To our knowledge, this is the first study to demonstrate an association between higher CSF IL-6 and CSF-IL 8 levels and ongoing depression in a population-based sample. The finding of elevated CSF IL-6 levels is in the anticipated direction in terms of what has been seen in younger clinical samples (Lindqvist et al 2009, Sasayama et al 2013).

We could show no relationship between CSF IL-6 levels and MADRS score. While this might reflect low study power, another interpretation could be that CSF IL-6 might constitute a marker of increased depression *vulnerability*, rather than a marker of depression severity. It must be remembered that this is a population-based study. Previous community-derived reports regarding peripheral cytokines suggest that low symptom load may reduce the influence of depression on cytokine concentrations (Howren et al 2009). It is probable that this applies to CSF cytokines as well.

In our study we showed a correlation between higher CSF IL-8 levels and current depression. CSF IL-8 is important in neuroprotection and innate immunity. While previous peripheral IL-8 studies have shown elevated levels in depressed patients (Dowlati et al 2010), there are only two CSF IL-8 studies and both focus on younger / middle-aged suicide attempters (Isung et al 2012, Lindqvist et al 2009). In our study higher CSF IL-8 was related to higher MADRS score. This, taken together with results of a recent study on lung cancer patients that showed that IL-8 genetic variations were related to depression severity (Reyes-Gibby et al 2013) , suggests that CSF IL-8 might be a marker for depression severity.

We could show no relationship between cytokine levels and future depression in women who were not depressed at baseline. Our sample size was small and thus underpowered. Further,

213 immune changes are dynamic and event-driven making it less possible to capture prospective
214 associations.
215
216 It is important to stress that this is a survivor sample; participants were older than those who
217 took part in the above-cited clinical study that found lower CSF-IL 6 in geriatric depression
218 patients (Stubner et al 1999). Aging-related neurodegeneration may influence results. CSF
219 biomarkers may be altered already 5 to 10 years before dementia onset (Buchhave et al 2012).
220 In the current study we could not show an association between baseline CSF cytokine levels
221 and future dementia.Our study involved women only and single-gender CSF studies are
222 lacking for comparison. It is clear, however that hormonal changes throughout female life
223 have an impact on inflammation levels (Vogelzangs et al 2012).
224
225 Among the strengths of this study are the population-based sample, the comprehensive
226 examinations and the prospective design with repeated follow-ups over nearly 20 years. The
227 women were well-characterized, enabling us to take into consideration possible confounders
228 including antidepressant treatment, BMI and smoking. Further, the temperature chain was
229 unbroken for the analysed aliquots. While the long-term stability of the analytes is unknown,
230 mean CSF IL-6 concentrations were similar to those previously reported in a clinical study
231 that used the same technique (Lindqvist et al 2009). While it is possible that changes in
232 cytokine levels occurred during long-term storage, this would have reduced the likelihood of
233 finding significant differences between women with and without depression.
234
235 While the number of participants with CSF data is relatively large compared to previous
236 studies, the number of cases with major depression was limited due to the population-based
237 study design. This necessitated the merging of major and minor depression cases for the

analyses. Another limitation is the fact that affective psychopathology was assessed at four time points only. Some women may have had depressive episodes prior to baseline, and others may have had depression episodes between examination waves. LPs were performed at baseline only, and we could thus not test for change in CSF cytokines over time. While women with and without LP were similar regarding a number of characteristics, those who opted to take part in this examination may have been healthier, which might limit representativity to the underlying population. However, this type of selection bias would be expected to decrease the likelihood of significant findings. Finally, this is a population study focusing on women aged 70-84 years at baseline and results cannot be extrapolated to clinical samples or to younger/male populations.

In conclusion, this paper adds to the scanty literature on CSF cytokines and depression in older people. Higher levels of CSF IL-6 and CSF IL-8 were associated with current but not future depression in this population-based sample of older women. Findings indicate a role for proinflammatory cytokines in late-life depression, but possible pathogenic mechanisms require further clarification.

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Table 1. Baseline characteristics in a population-based sample of non-demented older women (N=86). The Prospective Population Study of Women.

		MADRS score
	N (%)	Mean (SD)
No depression	67 (77.9%)	4.0 (4.8)
Any depression	19 (22.1%)	17.5 (9.5)
Major	10 (11.6%)	24.8 (6.3)
Minor	9 (10.5%)	9.4 (4.2)
Antidepressant use	5 (5.8%)	14.6 (13.8)

Table 2. CSF cytokine levels in relation to depression (major or minor) in a population-based sample of older women (N=86). The Prospective Population Study of Women.

Depression status at baseline				
	No depression (n=67)	**Depression (n=19)**	**Mann Whitney U-test[a]**	
	median (range), (pg/ml)	**median (range), (pg/ml)**	***P* value**	**MWU**
IL-6	1.7 (0.7-14.8)	2.1 (0.6-20.3)	0.008	381.5
IL-8	34.9 (17.5-64.2)	41.5 (29.3-78.7)	0.007	378.5
	N (%)	**N (%)**	**Logistic regression results[b]**	
IL-6 quartiles				
I	19 (90.5%)	2 (9.5%)		
II	19 (86.4%)	3 (13.6%)	OR 1.92 [1.11-3.31] p=0.019	
III	17 (77.3%)	5 (22.7%)		
IV	12 (57.1%)	9 (42.9%)		
IL-8 quartiles				
I	19 (90.5%)	2 (9.5%)		
II	20 (87.0%)	3 (13.0%)	OR 1.81 [1.08-3.04] p=0.025	
III	15 (71.4%)	6 (28.6%)		
IV	13 (61.9%)	8 (38.1%)		

a. Test between women with and without depression at baseline.

b. Multivariate binary logistic regressions examined the effect of quartiles of cytokine levels on depression status including age, Body Mass Index, and smoking as covariates.