

Raise in MCP-1 is associated with delirium in elderly hip fracture patients

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Abstract:

Background: delirium is a common condition associated with poor outcome among older people. Despite its common occurrence, the pathophysiology of delirium remains poorly understood.

Objective: to investigate the relationship between levels of cytokines, including monocyte chemoattractant protein (MCP-1), C-reactive protein (CRP), tumor necrosis factor- α (TNF α), interleukin-6 (IL-6), interleukin-10 (IL-10), and insulin-like growth factor 1 (IGF-1), and the development of delirium in a sample of hip fracture patients.

Design: prospective study conducted on hip fracture patients.

Measures: patients were screened for delirium on a daily basis using the Confusion Assessment Method (CAM). Levels of cytokines were measured at time of arrival, and, if possible, also postoperatively.

Results: 19 patients were included in the study, median age was 83 years. 12 of the patients were screened for delirium preoperatively. Levels of IGF-1 measured preoperatively were statistically significantly lower in patients with preoperative delirium (n=5) compared to patients without preoperative delirium (n=7) (median 40.0 ng/ml vs. 75.0 ng/ml, $p=0.04$). From 13 patients blood samples were also collected postoperatively. Among these, the number of patients with a raise in MCP-1 was statistically significantly higher in the group with delirium in the postoperative phase compared to the no-delirium group (5/6 vs. 1/7, $p=.03$).

Conclusions: patients with preoperative delirium had lower levels of IGF-1, whereas delirium in the postoperative phase was associated with a raise in MCP-1. Our results indicate that the inflammatory response might be of importance in the development of delirium.

Background

Delirium, defined as an acute and fluctuating change in consciousness and cognition, is a common condition associated with poor outcome among older people [1]. The highest prevalence rates have been found in intensive care units [2], among medical in-patients [3], and among hip fracture patients [4]. Despite its common occurrence, the pathophysiology of delirium remains poorly understood [5]. A number of mechanisms have been proposed, and there is now substantial evidence suggesting a role of inflammation in the pathogenesis [6].

Inflammatory biomarkers measured in the circulation can give insight into the pathophysiology of delirium. Interpretation of the findings may however be challenging due to confounding risk factors, the fluctuating nature of delirium and the possibility of the blood-brain barrier segregating disease processes in the brain from the bloodstream [7, 8]. Some researchers have found an association between the development of delirium and elevated circulating levels of the proinflammatory cytokines interleukin-6 (IL-6) and interleukin-8 (IL-8) [9, 10], while others have not [11, 12]. Clinical studies have reported an association between elevated levels of C-reactive protein (CRP) and development of delirium in older acute medical inpatients [13], hip fracture patients [14] and mechanically ventilated patients [15], whereas a prospective case-control study of hip fracture patients did not find such a relationship [16]. Delirium has also been associated with low levels of the neuroprotective cytokine insulin-like growth factor-1 (IGF-1) in some studies [11, 17], but not in others [16, 18]. To our knowledge, only one previous study has explored the association between delirium and monocyte chemoattractant protein-1 (MCP-1) [19].

The aims of the current study were to investigate the relationship between levels of cytokines, including MCP-1, CRP, Tumor necrosis factor alfa (TNFa), IL-6, IL-10, and IGF-1, measured in serum preoperatively and postoperatively, and the development of delirium in a population of elderly hip fracture patients.

Methods:

Study design

This is a substudy of a prospective study designed to evaluate risk factors for preoperative and postoperative delirium in elderly hip fracture patients [20]. Patients acutely admitted for a hip fracture (femoral neck fracture, intertrochanteric fracture, or subtrochanteric fracture) aged ≥ 65 , who spoke Norwegian, had no severe aphasia, head injury or terminal illness, and were admitted for at least 48 hours, were eligible for inclusion. Patients admitted to Oslo University Hospital (Ulleval clinic) and Diakonhjemmet Hospital, Oslo, Norway, in the period from May through December 2006, providing a written informed consent, were included in the current study.

The study was undertaken in accordance with the Declaration of Helsinki and approved by the Regional Committee for Ethics in Medical Research and the Data Protection Authorities. The patients were observed for a minimum of 48 hours and a maximum of 5 days after the operation. Information on pre-fracture cognitive status and functioning in activities of daily living was collected retrospectively.

Measurements and procedures

Patients were screened for delirium within 48 hours of admission, thereafter on a daily basis (weekdays) until discharge or the fifth postoperative day. Delirium was diagnosed using the Confusion Assessment Method (CAM) criteria for delirium [21]: Change in mental status with acute onset and/or fluctuating course, inattention, and disorganized thinking or altered level of consciousness.

Pre-fracture cognitive function was determined using the short form of the Informant Questionnaire on Cognitive Decline in the Elderly (IQCODE), and patients with IQCODE scores 3.44 or greater were considered as probably suffering from pre-fracture cognitive decline [22]. The patients' overall physical health was assessed according to the American Society of Anesthesiologists (ASA) score. The Barthel Index was scored by a close caregiver to give an indication of pre-fracture functioning in activities of daily living, and a score of 19 or 20 out of 20 demonstrated functional independency.

Blood samples were collected for routine preoperative and postoperative testing respectively, and additional blood was drawn for serum preparation by centrifugation at 3500 rpm for 10 minutes and stored at -70°C. CRP was determined by an enzyme-linked immunosorbent assay (DRG Instruments GmbH, Germany) (detection limit 0.1 mg/L). TNF α , IL-6, IL-10, IGF-1 and MCP-1 were all measured by ELISA methods with commercially available kits (R&D Systems Europe, Abingdon, Oxon, UK). To minimize run-to-run variability, serial samples from the same individuals were analyzed in the same microtiter run. In our laboratory the

inter-assay coefficients of variation were for CRP < 5 %, TNFa 8.5 %, IL-6 10.5 %, IL-10 6.2 %, IGF-1 8.0 %, MCP-1 9.2 %.

Statistical analyses

Mann-Whitney U test was conducted to compare levels of inflammatory markers in patients with and without delirium. In patients where blood samples were collected both pre- and postoperatively, change in levels of inflammatory markers (raise versus steady state or fall) was compared between those who did and did not develop delirium, using the Chi-square test. Every raise in biomarker concentration (above zero) was defined as a raise. We chose to dichotomize the variables and use the chi-square test as the distributions were not homoscedastic, and the conditions for using the Mann-Whitney U test thus not fulfilled [23].

All analyses were performed with SPSS 18.0 software (Chicago, IL, USA).

Results

Preoperative blood samples were collected from 19 patients with median (interquartile range [IQR]) age 83 (79-91) years. 14 (74%) were women, 3 (16%) had ASA score III, IV or V, 5/16 (31%) had an IQCODE score > 3.44, and 5/16 (31%) had a premorbid Barthel Index score ≤ 18. All had at least three days of postoperative delirium assessments.

12 of the patients were screened for delirium preoperatively and 5 of them were found to be delirious at that time. The median concentration of IGF-1 measured preoperatively was statistically significantly lower in patients with preoperative delirium (n=5) compared to patients without preoperative delirium, Table 1.

In 13 of the 19 patients screened for delirium postoperatively, blood samples were collected both preoperatively and postoperatively. Of these, 6 were delirious in the postoperative phase, regardless of time of onset. The change in biomarker concentrations as well as the number of patients with a raise in the measured biomarkers is shown in Table 2. Among those with increasing concentrations in the biomarkers from the preoperative to the postoperative measurement, the median (range) raise in MCP-1 concentration was 210 pg/ml (81-408), in IGF-1 14.5 ng/ml (2.0-31.0), in IL-6 6.3 pg/ml (0.6-43.9), in IL-10 3.1 pg/ml (0.7-11.4), in TNFa 1.0 pg/ml (0.1-3.9), and in CRP 23.7 mg/ml (3.6-37.6). The number of patients with a raise in MCP-1 was significantly higher in the delirium group compared to the no-delirium group (5/6 vs. 1/7, $p=0.03$).

Discussion

Our main finding was that a raise in monocyte chemoattractant protein (MCP-1) was associated with the prevalence of delirium postoperatively. MCP-1 is a chemokine that attracts leukocytes of the monocyte lineage [24]. Within the CNS, MCP-1 is produced mainly by astrocytes and resident microglia [25], and MCP-1 overexpression has been associated with neuroinflammatory conditions like multiple sclerosis, stroke and Alzheimer's disease [26]. MCP-1 is constitutively expressed by both glial cells and neurons, suggesting a possible neuromodulating function [27] and has been shown to increase cell excitability and dopamine release in dopaminergic neurons in rat substantia nigra [28]. Since both neuroinflammation and neurotransmitter disturbances, especially acetylcholine deficiency and dopamine excess, have been proposed as pathophysiological mechanisms in delirium [5], a role of MCP-1 in the

pathogenesis of delirium is possible. To our knowledge, the role of MCP-1 in delirium has not been studied, with the exception of one recent study performed on ICU patients, showing a significant association between MCP-1 and delirium in patients with systemic inflammatory response syndrome (SIRS) [19]. In accordance with previous studies on IGF-1 and delirium [11, 17], we identified an association between lower levels of IGF-1 and the development of delirium, supporting the hypothesis that low levels of this universally cytoprotective cytokine with neurotrophic properties may be of importance to the development of delirium [29].

Strengths of our study are its prospective design with comprehensive serial clinical evaluations, and the high sensitivity and specificity and low inter-assay coefficients of variation of the laboratory assays. Main limitations include the low number of patients, the fact that the time of collecting the blood samples was not standardized according to the time of surgery, and that the analyses are not accounting for diurnal or other physiological fluctuations in peripheral blood. The sample size may well have given an insufficient power as to detect a significant difference in the change in biomarkers between patients with and without delirium (Table 2). Since our knowledge on delirium pathophysiology is rather scarce, we assume that the results from our limited material may be of interest, and subject to confirmation in larger studies.

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Author contribution:

Kjersti Skrede: responsibility for content, conception and design, analysis and interpretation of data, and drafting of the manuscript.

Vibeke Juliebø: responsibility for content, conception and design, practical procedures, acquisition of data, supervision, analysis and interpretation of data, supervision, and drafting of the manuscript.

Ingebjørg Seljeflot: responsibility for content, analysis of the blood samples, interpretation of data, and drafting of the manuscript.

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Table 1.
Association between preoperative inflammatory markers and preoperative delirium,
n=12.

	Preoperative delirium, n=5	No preoperative delirium, n=7	p-value *
IGF-1 ng/ml, median	40.0	75.0	.04
MCP-1pg/ml, median	274.0	334.0	.29
IL-6 pg/ml, median	19.6	8.9	.12
CRP mg/ml, median	17.4	6.0	.22
IL-10 pg/ml, median	4.1	5.7	.57
TNF pg/ml, median	2.1	4.3	.68

↑ Mann-Whitney test

Table 2.

Association between raise in inflammatory markers and delirium during the stay in patients with both preoperative and postoperative blood samples, n=13.

Change from preoperative to postoperative measurement, median			Number of patients with a raise in the biomarker			
	Delirium, n=6	No delirium, n=7		Delirium, n=6	No delirium, n=7	p-value [†]
IGF-1, ng/ml	8.5	2.0	Raise in IGF-1, n	4	4	1.00
MCP-1, pg/ml	209.5	-43.0	Raise in MCP-1, n	5	1	.03
IL-6, pg/ml	-.4	7.3	Raise in IL-6, n	3	7	.07
CRP, mg/ml	9.7	28.7	Raise in CRP, n	4	7	.19
IL-10, pg/ml	1.2	2.8	Raise in IL-10, n	3	6	.27
TNF, pg/ml	-.01	1.0	Raise in TNF, n	3	5	.59

* Mann-Whitney test

† Chi-square test

IGF=Insuline-like growth factor; MCP=Monocyte chemoattractant protein; IL=Interleukin; CRP=C-reactive protein; TNF=Tumor necrosis factor