Delirium in the elderly: biomarkers and outcomes

Witlox, J.

Citation for published version (APA):

General rights
It is not permitted to download or to forward/distribute the text or part of it without the consent of the author(s) and/or copyright holder(s), other than for strictly personal, individual use, unless the work is under an open content license (like Creative Commons).

Disclaimer/Complaints regulations
If you believe that digital publication of certain material infringes any of your rights or (privacy) interests, please let the Library know, stating your reasons. In case of a legitimate complaint, the Library will make the material inaccessible and/or remove it from the website. Please Ask the Library: http://uba.uva.nl/en/contact, or a letter to: Library of the University of Amsterdam, Secretariat, Singel 425, 1012 WP Amsterdam, The Netherlands. You will be contacted as soon as possible.
Chapter 3

Cytokines in Cerebrospinal Fluid and Risk of Postoperative Delirium

Joost Witlox
Leo. Koenderman
Kees J. Kalisvaart
Jos F.M. de Jonghe
Wilco de Jager
Mireille F.M. van Stijn
Alexander P.J. Houdijk
Han S. Traast
Simone Gurlit
Alasdair M.J. MacLullich
Piet Eikelenboom
Willem A. van Gool

Submitted
CHAPTER 3

ABSTRACT

Background: Delirium has been hypothesized to result from a neuroinflammatory response. Ageing and neurodegenerative disease predispose to delirium and are associated with increased activity of the innate immune system resulting in an imbalance between pro- and anti-inflammatory mediators in the brain. Based on these changes in the regulation of central inflammatory processes we examined if hip fracture patients that develop postoperative delirium, show altered levels of inflammatory mediators in cerebrospinal fluid (CSF) prior to surgery.

Methods: Patients were 75 years and older and admitted for surgical repair of acute hip fracture. CSF samples of 74 patients at risk of delirium were collected preoperatively. We compared preoperative CSF levels of pro- and anti-inflammatory cytokines and their ratios between patients with and without postoperative delirium and examined the association between CSF cytokine levels and delirium severity. Mann-Whitney-U tests or Student t-tests were used for between group comparisons and the Spearman correlation coefficient was used for correlation analyses.

Results: Twenty-seven (36.5%) patients developed postoperative delirium. We found that higher preoperative ratios of the pro-inflammatory cytokine interleukin-8 (IL-8) and the anti-inflammatory cytokine IL-10, were positively associated with the incidence and the severity of postoperative delirium, especially in the first two days post-surgery. Analyzed separately none of the cytokines showed any relationship with incidence or severity of postoperative delirium.

Conclusions: Our findings suggest that a more pronounced preoperative pro-inflammatory state of the brain may be associated with the development of postoperative delirium, particularly in the first days after surgery. A role for neuroinflammation in the development of delirium may offer new possibilities for prevention of this debilitating neuropsychiatric syndrome.
BACKGROUND

Delirium is a serious and common complication in older hospitalized patients that is independently associated with an increased risk of death, institutionalization, and dementia after discharge. Although the pathogenesis of delirium is poorly understood current evidence suggests that neuroinflammation may be important. Important precipitating risk factors for delirium such as infections, trauma, and surgery are accompanied by increased systemic production of both pro- and anti-inflammatory cytokines. The brain monitors the peripheral innate immune response by several means and this immune-to-brain communication can ultimately lead to activation of microglia, the innate immune cells of the CNS. Once activated, microglia secrete a range of bioactive substances that further modulate immunological actions but can also disrupt homeostasis and compromise neuronal and synaptic function thereby potentially inducing delirium in vulnerable individuals. Advanced age and dementia are the main predisposing risk factors for delirium. Ageing and neurodegenerative disease are associated with increased activity of the CNS innate immune system resulting in an imbalance between pro- and anti-inflammatory cytokines. Under these circumstances microglia display a more reactive phenotype and release excessive quantities of pro-inflammatory cytokines in the brain after peripheral stimulation. In addition, the blood-brain barrier (BBB) shows structural and functional changes with ageing and dementia that may inappropriately increase the strength of inflammatory signalling from the periphery. Moreover, the pro-inflammatory response may be less well-regulated due to reduced cholinergic and glucocorticoid control. Elderly hip fracture patients are a highly vulnerable population with a high prevalence of cognitive impairment and a high incidence of postoperative delirium. Prior to development of postoperative delirium hip fracture patients undergo two events that elicit a systemic inflammatory response, that is the fracture, then the surgery. Given the exaggerated CNS response to peripheral immune stimulation with ageing and neurodegeneration it can be hypothesized that hip fracture patients who develop postoperative delirium already show altered CNS cytokine profiles before the operation, that is, stimulated by the fracture. The aim of the present study was to investigate the association between preoperative cytokine levels and incidence and severity of postoperative delirium in elderly hip fracture patients. It is important to emphasize that blood levels of cytokines may not adequately mirror CNS inflammatory processes. Therefore, we determined cytokine levels in cerebrospinal fluid (CSF). One small preliminary study found increased CSF interleukin-8 levels in patients with peri-operative delirium but this study did not specifically determined if preoperative CSF cytokine levels are
associated with incidence of postoperative delirium. Because the state of inflammation does not depend on the net effect of individual cytokines but on the balance between pro- and anti-inflammatory mediators, cytokines should be studied in the context of other cytokines in order to adequately characterize inflammatory signals. Therefore, we calculated the ratio between pro- and anti-inflammatory cytokines as a measure of the overall inflammatory status in the CSF.

We examined whether postoperative delirium in elderly hip fracture patients would be associated with an increased pro-inflammatory profile in the CNS before the operation, indexed by higher levels of pro-inflammatory cytokines, lower levels of anti-inflammatory cytokines, and higher ratios between pro- and anti-inflammatory cytokines in the CSF. Because immune-to-brain communication is expected to be strongest when circulating levels of cytokines peak we also examined whether the relationship between CSF cytokine levels and delirium would be strongest in patients that develop delirium in the early postoperative period.

METHODS

Ethical considerations
This study was conducted in accordance with the Declaration of Helsinki and the guidelines on Good Clinical Practice. Approval of the regional research ethics committee was obtained. All patients gave written informed consent.

Study design and objectives
Patients were participants in a clinical trial that compared the effectiveness of taurine versus placebo in reducing morbidity and one-year mortality in elderly hip fracture patients (the results of this trial will be described elsewhere). Evaluating the relationship between risk factors and delirium was a pre-specified secondary aim of this trial. Risk factors for delirium were assessed preoperatively. Presence and severity of delirium were assessed daily. All patients were undergoing surgical repair of a hip fracture. CSF samples were collected after cannulation for the introduction of spinal anaesthesia but prior to the administration of any anesthetic. We compared preoperative CSF cytokine levels and baseline risk factors between patients who did or did not develop postoperative delirium and investigated the association between preoperative CSF cytokine levels and delirium severity. Even though patients with a low delirium severity score would generally not be regarded as categorically delirious the analysis of delirium severity included all patients. We determined the following pro-inflammatory cytokines; tumor necrosis factor-α (TNF-α), interleukin 1β (IL-1β),
IL-6, IL-8, monocyte chemotactic protein-1 (MCP-1), and the anti-inflammatory cytokine IL-10. We also calculated the ratio of IL-6/IL-10 and IL-8/IL-10 to measure overall inflammatory status in the CSF. To examine if the association between CSF cytokine levels and delirium is strongest when immune-to-brain communication peaks we repeated our analyses in a sample that included only non-delirious controls and patients who developed delirium on the first or second postoperative day. Since all participants were at high risk for delirium (i.e. age 75 years or older, and acute hospital admission) patients received routine care with prophylactic treatment of 0.5 mg haloperidol, three times daily, from time of admission until postoperative day three, unless contraindications regarding its use were present.

Participants
The study was conducted in a series of consecutively admitted elderly hip fracture patients to a teaching hospital in Alkmaar, the Netherlands. Eligibility was checked for all patients 75 years and older admitted for primary surgical repair of hip fracture. Patients were not eligible if they had no acute trauma, received total hip prosthesis for surgical repair of their hip fracture, had a pathological fracture, were not capable (e.g. dementia in the medical case notes, aphasia, coma) or not willing to provide informed consent, or had contraindications regarding the administration of taurine (i.e. renal failure defined as a creatinine clearing less than 30 ml/min). Written informed consent was obtained after eligibility was checked and the trial had been explained. From March 2008 to March 2009 122 hip fracture patients fulfilled criteria for participation and provided consent (figure 1).

Baseline assessment
Baseline assessment was completed within 12 hours after admission and before surgery. This comprised delirium assessment, patient and proxy interviews and questionnaires, and inspection of the medical record to assess risk factors for delirium. Preoperative cognitive functioning was assessed with the Mini Mental State Examination (MMSE) on a scale of 0 (poor) to 30 (good) with scores lower than 24 indicating cognitive impairment. Prefracture cognitive decline was estimated with the short version of the Informant Questionnaire on Cognitive Decline in the Elderly (IQCODE-N) which is scored by a close relative or caregiver and measures pre-existent cognitive decline over the past 10 years on a scale of 16 (improvement) to 70 (decline). A score higher than 57 (i.e. a mean score higher than 3.6) indicates cognitive decline. Visual acuity and impairment was assessed with the standardized Snellen test for visual impairment. Visual impairment was defined as binocular near vision, after correction, worse than 20/70. The medical record was reviewed to determine the preoperative Acute Physiology Age and Chronic Health Examination
The APACHE II score measures severity of acute illness on a scale of 0 (no acute health problems) to 70 (severe acute health problems). The Geriatric Depression Scale (GDS) was administered as a 15 item self-rating scale for depression with higher scores indicating depression. The Barthel Index (BI) was used to determine prefracture functioning in activities of daily living and is scored by a close relative or caregiver on a scale from 0 (dependence) to 20 (independence). Prefracture instrumental activities of daily living were assessed with the Lawton Figure 1. Flow chart. CSF= cerebrospinal fluid
Instrumental Activities of Daily Living (Lawton IADL) scale, range 8 (no disability) to 31 (severe disability). Biomedical factors included the number and type of medical co-morbidities and medications before admission to hospital and the American Society of Anesthesiologists (ASA) physical status classification system, range 1 (normal health patient) to 5 (moribund patient). Demographic factors included age, gender, home situation, and low or high educational level. For the IQCODE-N, BI and Lawton IADL proxies were asked to describe the patient’s condition a week before the fracture as to determine function unbiased by the event of hip fracture itself or any acute or sub-acute event leading to hip fracture.

**Cerebrospinal fluid**

CSF samples were collected by the anesthesiologist when patients underwent spinal anesthesia for surgical repair of hip fracture. Lumbar punctures were performed with a 25-gauge needle between the L3-L4 or L4-L5 intervertebral space. Of each patient 13 ml of CSF was collected in polypropylene tubes and transported to the laboratory within two hours. The CSF sample was centrifuged at 1800 g for 10 minutes at 4°C and aliquotted into polypropylene tubes that were stored at -80 °C. After all CSF samples had been collected the samples were sent in one batch on dry ice with express delivery to the laboratory of the Centre for Cellular and Molecular Intervention of the University Medical Centre Utrecht. Upon arrival the status of the CSF samples was checked and they were stored for a few weeks at -80°C until analysis. Cytokine concentrations were determined with a multiplex immunoassay using antibody pairs from different commercial sources. All CSF analyses were performed at the same time and the investigators that performed the cytokine measurements were blinded to the clinical status of the patients. The lower limit of quantitation for the different proteins were as following: TNF-α, 1.2 pg/ml; IL-1β, 1.5 pg/ml; IL-6, 2.4 pg/ml; IL-8, 5.3 pg/ml; IL-10, 2.3 pg/ml; MCP-1, 1.2 pg/ml. The intra- and inter-assay coefficients of variation for TNF-α, IL-1β, IL-6, IL-8, IL-10, and MCP-1 ranged between 5.9% and 9.6%, and 7.7% and 16.6% respectively.

**Outcome**

The main outcome was postoperative delirium. Delirium was defined according to the Confusion Assessment Method (CAM), which consist of acute onset and fluctuating course of cognitive function, inattention, and either disorganized thinking, and/or altered level of consciousness. Delirium severity was measured using the Delirium Rating Scale Revised-98 (DRS-R-98), a 16-item rating scale with thirteen severity items (and 3 diagnostic items) and a range of 0 (no severity) to 39 (maximum severity). Presence and severity of delirium were assessed within 12 hours after admission and before surgery and continued daily until the fifth postoperative day.
or discharge. In case of a positive CAM score assessments were continued at least until the CAM was negative for three consecutive days or discharge. Severity of postoperative delirium was defined as the highest DRS-R-98 score. The CAM and DRS-R-98 rating were based on brief formal cognitive testing with the MMSE, patient and hospital staff interviews, and scrutiny of the medical and nursing records.

Statistical analysis
Statistical calculations were performed using SPSS for Windows, version 14 (SPSS; Inc. Chicago, IL). Quantitative variables are presented as mean (standard deviation (SD)) or median (interquartile range (IQR)). Categorical variables were analysed using Chi-Square or Fisher Exact tests. Continuous variables were tested with Mann-Whitney-U tests or Student t-tests depending on sample size, distribution, and skewness of data. The assumption of normality was tested with the Kolmogrov-Smirnov test. Because CSF cytokine variables were significantly skewed Mann-Whitney U tests were employed for pair-wise comparisons of these variables. The Spearman correlation coefficient was used for correlation analyses. Statistical significance was set at $P < .05$.

RESULTS
A total of 122 out of 257 consecutive hip fracture patients were included in this study (figure 1). Twenty-six patients received general anaesthesia and in 18 cases logistical limitations did not allow CSF collection (e.g. polypropylene tubes were unavailable or emergency situation in operating room). Because we specifically investigated whether preoperative CSF cytokine levels were associated with postoperative delirium another four patients were excluded; one patient without postoperative delirium assessments, and three patients with preoperative delirium.

Twenty-seven (36.5%) of the remaining 74 patients in our analyses developed postoperative delirium, 81% within two days after surgery. The characteristics of patients with and without delirium are shown in table 1. Patients with postoperative delirium showed more signs of cognitive impairment, both at baseline (MMSE) and before admission (IQCODE-N), and were more dependent in ADL and IADL functioning. Furthermore, patients with postoperative delirium were more severely ill before the operation as measured with the ASA classification system and used more medications at home. Other baseline risk factors were not significantly different between patients who did or did not develop delirium after the operation. IL-1β was detected in only five out of 74 (6.8%) CSF samples, four of which belonged to patients that developed delirium. TNF-α levels could be detected in the CSF of
seventeen patients of which eight developed delirium postoperatively. Because of the limited number of measurable IL-1β and TNF-α values no further statistical analyses were performed on these variables. IL-6 levels could be detected in 37 out of 74 (50.0%) samples. In contrast, IL-8 (73/74; 98.6%), IL-10 (73/74; 98.6%), and
MCP-1 (73/74; 98.6%) levels was detected in almost all CSF samples. Patients with postoperative delirium were not more likely to have detectable levels of CSF IL-6 than patients without postoperative delirium ($\chi^2(1) = .06, P = .81$).

Preoperative levels of CSF IL-6, IL-8, IL-10, MCP-1, and the ratios of IL-6 and IL-8 with IL-10 are compared in table 2. Analyzed separately CSF cytokine levels did not differ significantly between patients with and without postoperative delirium. Also, we found no relationship between separate CSF cytokine variables and severity of postoperative delirium (IL-6; Spearman’s rho = -.01, $P = .94$, IL-8; Spearman’s rho = .08, $P = .49$, IL-10; Spearman’s rho = -.10, $P = .42$, and MCP-1; Spearman’s rho = -.03, $P = .80$).

<table>
<thead>
<tr>
<th></th>
<th>Delirium</th>
<th>No delirium</th>
<th>$P$-Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>CSF IL-6 (pg/ml)</td>
<td>17.7 (11.2-32.6)</td>
<td>17.8 (12.6-26.8)</td>
<td>.82</td>
</tr>
<tr>
<td>CSF MCP-1 (pg/ml)</td>
<td>1223.0 (675.0-2319.0)</td>
<td>1266.0 (628.3-2014.3)</td>
<td>.96</td>
</tr>
<tr>
<td>CSF IL-8 (pg/ml)</td>
<td>140.4 (71.3-258.5)</td>
<td>87.0 (50.4-224.6)</td>
<td>.23</td>
</tr>
<tr>
<td>CSF IL-10 (pg/ml)</td>
<td>19.4 (9.0-44.3)</td>
<td>18.3 (11.8-43.3)</td>
<td>.57</td>
</tr>
<tr>
<td>CSF IL-6/IL-10 ratio (pg/ml)</td>
<td>.55 (.35-1.37)</td>
<td>.45 (.25-.90)</td>
<td>.34</td>
</tr>
<tr>
<td>CSF IL-8/IL-10 ratio (pg/ml)</td>
<td>6.8 (4.5-10.7)</td>
<td>4.8 (3.7-6.6)</td>
<td>.055</td>
</tr>
</tbody>
</table>

Abbreviations: CSF, cerebrospinal fluid; IL-6, interleukin 6; MCP-1, monocyte chemotactic protein 1; IL-8, interleukin 8; IL-10 interleukin 10. Values are expressed as median (IQR).

In contrast, we did find a strong trend towards higher ratios between the pro-inflammatory cytokine IL-8 and the anti-inflammatory cytokine IL-10 in patients with postoperative delirium compared with non-delirious controls ($P = .055$). Moreover, a significant correlation was found between the IL-8/IL-10 quotient and severity of delirium (Spearman’s rho = .24, $P = .04$). When the same analyses were performed only including controls and patients that developed delirium shortly after surgery (i.e. on the first or second postoperative day) both the difference in the IL-8/IL-10 ratio between patients with and without delirium (7.4 pg/ml [IQR 4.6-11.3 pg/ml] vs. 4.8 pg/ml [IQR 3.7-6.6 pg/ml]; $P = .02$) (figure 2) and the strength of the association between the IL-8/IL-10 ratio and severity of delirium increased (Spearman’s rho = .32, $P = .009$). For the other CSF cytokine variables no significant changes in the relationship with delirium incidence or severity occurred (data not shown). The main findings of our study are summarized in table 3a and b.

Since patients participated in a clinical trial comparing taurine with placebo we examined if levels of CSF cytokines and their ratios differed depending on treatment allocation. We found no significant differences between the intervention and placebo group for any of the CSF cytokine variables or their ratios. Moreover, the incidence
nor severity of postoperative delirium differed between the treatment and placebo group.

Figure 2. Box plot of CSF interleukin-8/interleukin-10 (IL-8/IL-10) ratios by group based on 22 patients with delirium and 46 patient without delirium ($P=.02$). Boxes represent the median, the 25th and the 75th percentiles, bars indicate the range of data distribution. Three patients in the delirium group, and 3 patients in the control group had an IL-8/IL-10 ratio outside the range of the depicted scale are not visualized in the box plot.

In additional analyses we explored the relationship between age, cognitive function, and CSF cytokine variables. No significant correlations were found between age, baseline cognitive function (MMSE), pre-existent cognitive impairment (IQCODE-N), and levels of CSF cytokines or their ratios. Stratification of the MMSE and IQCODE-N according to the abovementioned cut-off scores did not reveal different outcomes (data not shown). As levels of cytokines tend to fluctuate over time we investigated if time from admission to surgery influenced CSF cytokine levels. Mean time from admission to surgery was 18 hrs (SD ± 10.2). The time between admission and surgery was not associated with levels of CSF cytokines or their ratios. Furthermore, neither the incidence nor the severity of delirium was significantly influenced by the time patients waited for hip surgery.
CHAPTER 3

Table 3a. Association between CSF IL-8/IL-10 ratio and incidence of postoperative delirium

<table>
<thead>
<tr>
<th></th>
<th>No. of patients with delirium</th>
<th>No. of patients without delirium</th>
<th>CSF IL-8/IL-10 (pg/ml)</th>
<th>CSF IL-8/IL-10 (pg/ml)</th>
<th>P-Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Any delirium during hospital admission</td>
<td>26</td>
<td>46</td>
<td>6.8 (4.5-10.7)</td>
<td>4.8 (3.7-6.6)</td>
<td>.055</td>
</tr>
<tr>
<td>Delirium onset POD1 or POD2</td>
<td>22</td>
<td>46</td>
<td>7.4 (4.6-11.3)</td>
<td>4.8 (3.7-6.6)</td>
<td>.02</td>
</tr>
</tbody>
</table>

Table 3b. Association between CSF IL-8/IL-10 ratio and severity of postoperative delirium

<table>
<thead>
<tr>
<th></th>
<th>No. of patients with delirium</th>
<th>No. of patients without delirium</th>
<th>Spearman’s rho</th>
<th>P-Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Any delirium during hospital admission</td>
<td>26</td>
<td>46</td>
<td>.24</td>
<td>.04</td>
</tr>
<tr>
<td>Delirium onset POD1 or POD2</td>
<td>22</td>
<td>46</td>
<td>.32</td>
<td>.009</td>
</tr>
</tbody>
</table>

Abbreviations: CSF, cerebrospinal fluid; POD, postoperative day; IL-8, interleukin 8; IL-10, interleukin 10. Values are expressed as median (IQR).

DISCUSSION

Delirium after surgery has been hypothesized to result from a neuroinflammatory response. This study investigated the association between preoperative levels of inflammatory cytokines in CSF and incidence and severity of postoperative delirium in elderly hip fracture patients. Based on alterations in the regulation of central inflammatory processes in patients at risk for delirium we hypothesized that a more intense neuroinflammatory response following hip fracture would be associated with delirium after the operation. We also hypothesized that this relationship would be strongest when delirium develops shortly after surgery, that is at a time when circulating levels of cytokines peak and immune-to-brain communication is expected to be strongest. Indeed, our findings suggest that higher CSF IL-8/IL-10 ratios before the operation are associated with delirium after the operation, particularly in the early postoperative period.

Inflammation is a highly complex and dynamic process during which many different cytokines and numerous other effectors interact. Rather than levels of any one specific cytokine alone the complex balance between pro- and anti-inflammatory cytokines determines if the inflammatory response is adaptive or harmful. The
importance of cytokine homeostasis is exemplified by our finding that elevated IL-8/IL-10 ratios, and not individual cytokine levels, are associated with the development of postoperative delirium. Previous studies have shown that in elderly hip fracture patients blood levels of inflammatory cytokines are associated with postoperative delirium. However, delirium is the result of impaired brain function and blood levels of cytokines can be a poor reflection of intracerebral inflammatory processes. Indeed, previous studies have demonstrated that CSF cytokine levels, especially IL-8, can far exceed concentrations in serum after hip fracture and vascular aortic surgery. These findings demonstrate a lack of correlation between cytokine levels in CSF and serum but also suggest that the production of inflammatory cytokines within the compartment of the CNS can be induced by peripheral stimulation. A comparison of our CSF cytokine levels with that of elective hip surgery patients also suggests a neuroinflammatory response following hip fracture. Compared with elderly patients that undergo elective hip surgery levels of the pro-inflammatory cytokine IL-8 are increased 2-4 fold in the CSF of our hip fracture patients. One earlier preliminary study examined CSF biomarkers of inflammation in relation to delirium and showed that hip fracture patients with peri-operative delirium had higher preoperative CSF IL-8 levels compared with non-delirious controls. Because both patients with preoperative and postoperative delirium at the time of CSF collection were included, this study could not determine whether higher CSF IL-8 should be regarded as a ‘risk factor’ for delirium or as a ‘marker’ of delirium. Our findings are based on a larger sample of patients all without delirium at the time of CSF sampling. Hence, our findings can be taken as evidence that a more pronounced pro-inflammatory state of the brain before surgery, expressed by an elevated CSF IL-8/IL-10 ratio, is a risk factor for incident delirium after surgery. The signalling pathways of TNF-α and IL-1β act synergistically to drive systemic production of IL-6, IL-8, and IL-10 after peripheral trauma and mouse models of delirium and postoperative cognitive dysfunction, a syndrome similar to postoperative delirium, have demonstrated that peripheral inflammatory mediators can profoundly affect CNS function. The precise mechanisms by which immune signals from the periphery are translated to the brain and result in intracerebral production of IL-8 and IL-10 are unknown but likely involve both neural and humoral pathways. In the brain IL-8 and IL-10 are produced by activated microglia, astrocytes, and endothelial cells. The cytokine IL-8 generally has pro-inflammatory actions and can modulate neurotransmission and synaptic function. Importantly, receptors for IL-8 are expressed by neurons. Also, through induction of neurotoxic substances and pro-apoptotic signalling IL-8 may mediate neuronal cell death. In contrast to IL-8, the main function of the anti-inflammatory cytokine...
IL-10 is to repress excessive inflammatory responses. Because microglia are both a source and target of IL-10 microglial production of IL-8 may be regulated in an autocrine manner by IL-10 release. Through this negative feedback mechanism IL-10 is also able to prevent neurodegeneration, presumably by limiting activation of microglia.

Hence, in hip fracture patients that develop postoperative delirium higher preoperative CSF IL-8 levels, relative to IL-10, may set the stage for further cytokine dysregulation, especially after subsequent inflammatory insults. Our finding that the association between the CSF IL-8/IL-10 ratio and delirium is particularly strong in the first days after surgery is consistent with such a scenario.

There are several issues that deserve further comment. The increased CSF IL-8/IL-10 ratio that may mediate the risk for delirium may be related to individual differences and/or to co-morbid conditions. We deliberately choose not to include adjustments for baseline differences in our analyses because in doing so the mediating effect of changes in cytokine profiles could have been obscured.

Although we employed a sensitive method to quantify cytokines, levels of TNF-α and IL-1β remained below the detection limit in most of our samples. This could be due to the sensitivity of the assay or be related to compartmentalisation of cytokines in the brain extracellular space, and/or to their short half-life.

All participants received routine care with low dose prophylactic haloperidol. Haloperidol has been reported to reduce both the severity of postoperative delirium and levels of pro-inflammatory cytokines, although at low doses of haloperidol the latter effect seems limited. Hence, if haloperidol had any effect on our findings it mitigated rather than increased the strength of the association between delirium severity and levels of CSF cytokines, although we cannot completely exclude a specific effect on cytokine ratios.

This study represents a combination of the intervention and control arm of a randomized controlled trial. Ideally this study would have been performed in an observational cohort. However, no differences were found in CSF biomarker levels or incidence or severity of delirium between the intervention and placebo group. Delirium assessments generally took place several hours before CSF samples were collected. In theory, during this window some patients could have developed preoperative delirium, and as consequence may have been falsely classified in the preoperative period. However, patients were monitored closely and if nursing staff registered changes in cognition or behaviour the patient was subjected to further examination. However, future studies should try to conduct delirium assessments and CSF measurements as close together as possible.

A role for neuroinflammation in the development of delirium may provide new opportunities for clinical intervention. The poor long-term prognosis of delirium...
in elderly patients¹ and the evidence for prevention over management of delirium underlines the importance of adequate preventive measures.⁵³ Interventions that ameliorate neuroinflammation could raise the reserve capacity of the brain and thereby the threshold for episodes of delirium. Controlled studies are necessary to validate a role of neuroinflammation in the pathogenesis of delirium and to investigate if delirium and its negative sequelae can be averted by targeting inflammatory mediators.

CONCLUSION

Our results suggest that hip fracture elicits a neuroinflammatory response in elderly patients and that a more pronounced preoperative pro-inflammatory state may be associated with delirium shortly after surgery. Because the brain is never exposed to only a single cytokine future studies should take the balance between multiple pro- and anti-inflammatory mediators into account. A possible role of the innate immune system in the development of delirium offers scope for prevention by targeting neuroinflammation.
REFERENCES


