Cholinergic deficiency and inflammation in cognitive dysfunction

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Chapter 7

Pre-operative inflammatory markers and the risk of postoperative delirium in elderly patients

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R. Vreeswijk
W.A. van Gool
P. Eikelenboom

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ABSTRACT

Pathophysiological mechanisms leading to delirium are not clear. Age is a known risk factor and hypothesised to be accompanied by a low-grade inflammatory state. Previous studies have shown an association between delirium and circulating proinflammatory markers in acutely ill and postoperative patients. In light of the ageing/inflammation theory, we investigated the association of these markers with delirium in not acutely ill, elderly patients.

In a prospective nested case-control study levels of C-reactive protein (CRP), interleukin 6 (IL-6), insulin growth factor 1 (IGF-1) were measured pre-operatively in elderly patients admitted for hip-surgery. These levels were compared between patients who later developed a post-operative delirium and patients who did not. Patients were matched for age and disease severity.

Eighteen patients who developed delirium post-operatively were matched with 50 controls. Median APACHE-scores were below 16 in both groups. Pre-operative serum concentrations of CRP, IL-6 and IGF-1 did not differ between groups. IL-6 levels were associated with a measure of cognitive impairment.

In the present study no relationship was found between levels of pre-operative circulating pro-inflammatory markers and post-operative delirium in elderly patients, who were free from acute or severe disease.
INTRODUCTION

Delirium is a transient global disorder of cognition and attention. About 15-35% of the patients with age above 65 develop delirium during hospital admission. The pathophysiological mechanisms which lead to the eventual clinical picture of delirium remain to be elucidated. There is an emerging interest in serum inflammatory proteins and their possible association with the development of delirium. There may be at least two ways in which inflammatory markers are involved in delirium. First, prevalent delirium is associated with altered levels of cytokines. Pro-inflammatory cytokines are raised for example in infectious states or after operations and these conditions are frequently accompanied by delirium. In elderly patients postoperative raised serum levels of C-reactive protein (CRP) and interleukin 6 (IL-6) were associated with confusion. Elevated levels of IL-6 and low levels of insulin growth factor 1 (IGF-1) have been observed in prevalent delirium in acutely ill patients admitted to a medical ward. High doses of cytokines (mainly interferons) are known to induce delirium. In this respect cytokines can be considered as markers of disease state as reviewed by Marcantonio.

However, alterations in cytokine levels have also been proposed as markers of a trait that is associated with an increased risk of delirium; i.e. raised premorbid cytokine levels have been suggested to predispose for the development of delirium. The rationale behind this is based on the concept of immunosenescence. Ageing is accompanied by a low-grade inflammatory state caused by an increased activity of the innate immunity. These circulating inflammatory proteins are probably associated with the development of chronic disease, cardiovascular events and cognitive decline. In two small studies conducted in elderly patients admitted to acute medical wards high levels of CRP and low levels of IGF-1 have been found as risk factors for incident delirium.

If this latter hypothesis is true, and age-related alterations in cytokine profiles do pose a risk to develop delirium in older patients, this should hold also in patients who are not acutely ill. The majority of studies analysing correlations between inflammatory markers and delirium have been performed in acutely admitted patients at medical wards. These patients are prone to have confounding factors due to a variety of underlying illnesses. Therefore we aimed to investigate the association of cytokine levels and incident delirium in a homogeneous and well-defined population. In the present study cytokine concentrations were measured preoperatively in elderly patients who were admitted for hip-surgery and not otherwise acutely ill.
METHODS

Patients

This study was a prospective nested case-control study. Study subjects were participants of a large randomised placebo-controlled trial evaluating the efficacy of haloperidol prophylaxis for elderly hip-fracture patients at risk for delirium which was conducted at a teaching hospital in Alkmaar, The Netherlands. This study population has been described in detail elsewhere and comprises patients aged 70 and older admitted for acute or elective hip surgery. Blood was drawn pre-operatively within 12 hours of admission and stored at -80°C. Patients were operated within 72 hours after admission. Patients were eligible for the present study if the blood sample was still available in storage. Patients were not allowed to use cholinesterase inhibitors or levodopa treatment. Medication and comorbidity at admission were recorded. Patients who had developed a delirium within 5 days after surgery were matched with a control-group consisting of patients from the same study who had not developed a delirium. Patients were matched for age (+/- 1 year) and disease severity as measured by the Acute Physiology Age and Chronic Health Examination, APACHE-II score (+/- 2 points). Age and severe illness are known independent risk factors for the development of delirium. Next to that, age and severe illness are accompanied by altered levels of cytokines on their own account. Patients were not matched for cognitive dysfunction although this is a risk factor for delirium. Cognitive decline can also be associated with elevated cytokine levels in light of the immunosenescence theory but in this case as a result of the proinflammarory state. Data were therefore stratified by Mini Mental State Examination (MMSE) score ≥ 24 or ≤ 24. The matching procedure was carried out independently of the investigators by use of a statistical programme. All patients in this study consented to laboratory blood tests.

Clinical trial data were available for all patients. Included in the analysis for the present study were age, sex, cognition as measured by the MMSE (scale 0-30), disease severity as measured by the APACHE-II (scale 0-70, in which severe illness was considered if the score exceeded 16) and type of admission (acute or elective) all determined pre-operatively. Daily assessments with the MMSE, Delirium Rating Scale (DRS-R-98) and the Digit span test were used to diagnose delirium using the DSM-IV and the Confusion Assessment Method (CAM) criteria.

Laboratory assessment

IGF-1 was determined by immunometric assay (Immulite 2500, DPC, Los Angeles, CA, USA). Human Interleukin-6 was measured by sandwich enzyme
immunoassay (PeliKine, Sanquin, Amsterdam, The Netherlands). The intra- and inter-assay coefficients of variation for IL-6 are 4.6% and 7.5% respectively, as established in our laboratories.

CRP was measured with a highly sensitive in-house sandwich enzyme immunoassay, using rabbit anti-human CRP immunoglobulin and peroxidase-conjugated rabbit anti-human CRP immunoglobulin as a catching and detecting antibody (Dako, Copenhagen, Denmark), with O-Phenylenediamine (Sigma Chemical Co., St. Louis, USA) as substrate with intra- and inter-assay coefficients of variation of 3.9% and 6.6%, respectively. Analyses were carried out at Endocrine and Clinical chemistry laboratories of the Free University Medical Centre, Amsterdam.

Statistical analysis
Statistical analysis was performed using SPSS 12.0 statistical software for Windows. Mann-Whitney U test and Chi-square test were used to assess differences in baseline characteristics and serum concentrations of inflammatory markers between patients with and without delirium. Associations between baseline characteristics and serum concentrations were analysed by calculating Spearman’s rank coefficient. A value of $P < 0.05$ was considered statistically significant.

RESULTS
Of the first 178 of the 430 participants of the randomised trial no blood samples were stored. Of the next consecutive 252 participants samples of blood were available for the present study. All patients were assessed for the symptoms of delirium. The presence of post-operative delirium was established in 20 patients using the DSM-IV and the CAM-criteria as described above. Median time from surgery to the onset of delirium was 1.5 days.

Of two patients the blood sample was too little to properly run the assays. Eighteen patients with delirium were matched for age and baseline APACHE-II-score with 50 suitable controls. This relatively small number was due to a relative higher age in the group of patients with a delirium, which forced us to exclude many younger patients from the group of patients without a delirium, in order to achieve appropriate matching. Baseline characteristics are displayed in Table 1. This group of 68 patients was comparable to the initial population of 430 patients in terms of age (79.3 vs. 79.1), MMSE (25 vs. 24.7), APACHE (13.8 vs. 13.4) and mode of admission (21% vs. 26% acute admissions). A selection bias is not likely based on this figures. Only sex distribution differed, 45 % male in the final population versus 25 % male in the initial group.
Chapter 7

Table 1. Baseline characteristics

<table>
<thead>
<tr>
<th></th>
<th>delirium (n=18)</th>
<th>non-delirium (n=50)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age</td>
<td>80 (71-91)</td>
<td>78.5 (71 - 88)</td>
</tr>
<tr>
<td>Sex ratio, M:F</td>
<td>8:10</td>
<td>13:37</td>
</tr>
<tr>
<td>MMSE</td>
<td>23.5 (15-28)</td>
<td>26.5 (13-30)</td>
</tr>
<tr>
<td>APACHE</td>
<td>14.5 (10-20)</td>
<td>13 (9-20)</td>
</tr>
<tr>
<td>Admission</td>
<td>acute : elective 6 : 12</td>
<td>8 : 42</td>
</tr>
</tbody>
</table>

There was a significant difference in MMSE-score between groups in which patients with delirium had a lower median MMSE-score before the onset of delirium (Mann Whitney U, \( p = 0.015 \)). This is a known risk factor for the development of delirium. The proportion of acute admissions was higher in patients who developed a delirium (33% vs. 16%). Acute admissions were cases of hip fractures, elective admissions were those patients who needed hip replacement for other reasons such as severe osteoarthrosis. The difference was not significant (\( \chi^2 = 2.432, \text{df} 1, p = 0.119 \)). Median APACHE-scores were below 16 in both groups indicating only mild illness severity. Active comorbidity was present in 6 patients who developed a delirium (4 pulmonary disease, 1 psychiatric disorder and 1 endocrinological disorder) and in 7 patients without delirium (4 pulmonary disease, 1 intestinal disease, 1 endocrinological disorder and 1 neurological complaint). All use of medication was recorded and no differences were found between both groups, especially not in terms of medication that could elicit delirium (data not shown).

Levels of CRP, IL-6 and IGF-1 in both groups were compared using the Mann-Whitney U test for non-parametric data. There was no significant difference in baseline serum concentrations between patients that developed a post-operative delirium and those who did not for either of these inflammatory proteins (Table 2). If the same analysis was performed with only the data from the elective surgery patients again no significant differences in cytokine-levels were detected (data not shown).
Table 3. Levels of serum cytokines in patients with delirium vs. patients with no delirium

<table>
<thead>
<tr>
<th></th>
<th>delirium (n=18)</th>
<th>non-delirium (n=50)</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>CRP (mg/L)</td>
<td>5.3 (0.3 – 32.1) *</td>
<td>3.0 (0.2 – 104.5)</td>
<td>0.523</td>
</tr>
<tr>
<td>IL-6 (pg/ml)</td>
<td>3.6 (1.5 – 185.1)</td>
<td>3.0 (1.0 – 37.2)</td>
<td>0.121</td>
</tr>
<tr>
<td>IGF-1 (nmol/L)</td>
<td>14.4 (6.3 – 31.9) **</td>
<td>12.9 (5.9 – 28.9)</td>
<td>0.675</td>
</tr>
<tr>
<td>Albumine (g/L)</td>
<td>41.2 (26.5 – 45.8) **</td>
<td>40.0 (30.9 – 52.9)</td>
<td>0.994</td>
</tr>
</tbody>
</table>

* data are displayed as median and range
** 3 cases missing (1 delirium; 2 non-delirium)

A moderate inverse correlation was found between MMSE and IL-6 serum concentration (Spearman’s r -0.316, p = 0.009) indicating that lower MMSE-scores were associated with higher IL-6 serum levels. CRP and IGF-1 were not correlated with MMSE. Stratifying for MMSE, taking a cut-off at 24 points did not reveal a different outcome (data not shown).

Albumin concentrations were comparable in both groups and medians were within normal limits. This adds to the notion already reflected in the APACHE-scores that patients were not seriously ill at time of admission.

**DISCUSSION**

In the present study we investigated the possible association between pre-operative serum inflammatory markers and the occurrence of post-operative delirium in an elderly population admitted for hip-surgery. Baseline CRP, IL-6 and IGF-1 serum levels did not differ between patients who developed a delirium and patients who did not. The MMSE was lower in patients who developed a delirium which was not unexpected, since cognitive decline is a known risk factor for the development of delirium and we did not match our groups for MMSE-score. Although more cases of delirium were expected in patients who were acutely admitted, mode of admission did not differ statistically significantly between groups. Previous studies did show associations between delirium and the same inflammatory markers that were examined in this study4,6,10,15,16. High IL-6 and CRP levels and low IGF-1 levels have been observed in incident delirium. Based on these observations, CRP and IGF-1 levels have been proposed to be risk-factors for the development of delirium15,16. However, these studies were conducted in small
numbers of post-operative patients or in elderly patients acutely admitted to a medical ward. It could be argued that in these settings there are many factors (for example infections) that could contribute to both delirium and altered immune status.

If cytokines are indeed independent risk factors for delirium one would expect that patients who develop delirium have different pre-morbid levels of pro-inflammatory markers from patients who do not develop a delirium.

In our study blood was drawn at baseline, i.e. before the precipitating event of hip-surgery. According to the APACHE-score and normal albumin levels these patients were not severely ill at that time, apart from cognitive impairments. Moreover, 65% of the subjects were electively admitted to the hospital. This implies that other factors, than increased age alone, that may have caused a change in cytokine levels were presumably scarce. In this relatively ‘healthy’ elderly population no positive association between cytokine levels and delirium was found. These findings implicate that in a population of elderly patients relatively unaffected by various comorbid conditions, circulating cytokines and acute phase response proteins are not strong risk factors for the development of delirium. Since we could study only a limited number of patients this study is prone to a type II error; we may have missed a minute increase of delirium risk as a consequence of increased inflammatory markers.

The fact that we fail to find a distinct association between the studied markers and incident delirium casts doubts on the status of these markers as valid predictors of delirium. Cytokines profiles are non-specific and are subjected to many different physiological influences. Cytokine levels can rise after an insult and may contribute to the development of delirium and be a marker of state in this respect. Sequential measurements of cytokine levels in serum before and at several time points after an insult could shed more light on this issue. The exact role of inflammatory markers in the pathogenesis of delirium remains to be elucidated, but apparently they are not suitable as markers of an increased delirium risk.

Within the framework of the extensive literature on immunosenescence a more indirect link between cytokines and delirium can hypothesised as has been done by others. During ageing, alterations in levels of circulating cytokines and other inflammatory markers occur. A low grade inflammatory state is associated with age-related pathology such as atherosclerosis, Alzheimer’s disease and diabetes mellitus. Increased concentrations of pro-inflammatory cytokines are believed to contribute to cognitive decline and to predict frailty and mortality. Consistent with this we did find a correlation between cognition and IL-6 as described before in population-based studies. Alterations in the immune system due to aging also lead to a pro-inflammatory profile in the aged brain.
Heightened neuroinflammatory response and a modified cytokine milieu in the brain may potentially lead to neurobehavioral changes and even delirium. In animal studies these consequences were even more distinct with certain pre-existing conditions such as high age or chronic neurodegeneration. Taken together the current literature and the findings of the present study, it is more likely that in elderly patients, increased risk for delirium should be attributed to the presence of cerebral changes rather than directly to increased levels of circulating pro-inflammatory markers before the onset of delirium.

Conclusion
We did not find an association between pre-operatively measured CRP, IL-6 and IGF-1 and the incidence of delirium after hip-surgery. Adamis et al also reported a lack of association between CRP and various cytokines and the incidence of delirium in elderly hospitalised patients. Although this observation was explained by these authors as an effect of suppression of some aspects of the immune system in frail elders, one could also argue that maybe there is no direct relationship between delirium and pre-morbid circulating pro-inflammatory proteins. Further research in larger cohorts of elderly patients is warranted to exclude the possibility that changes in cytokine levels below the detection levels of our relatively small study do increase delirium risk and also replication of the present findings in different settings may add to the external validity of our conclusions. Future studies should probably not only focus on serum-markers as determinants of delirium, but also investigate the role of immunological alterations in the CNS itself.

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