Cytokines and IGF-I in delirious and non-delirious acutely ill older medical inpatients

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Abstract

Background: therapeutic use of cytokines can induce delirium, and delirium often occurs during infections associated with elevated levels of cytokines. This study examined the association of demographic, clinical and biological factors (IL-1α, IL-1β, IL-1RA, IL-6, TNF-α, IFN-γ, LIF, IGF-I, APOE genotype) with the presence and severity of delirium.

Methods: in an observational prospective longitudinal study, patients aged 70+ were recruited from an elderly medical unit and assessed every 3–4 days (maximum assessments 4). At each time, the scales MMSE, DRS, CAM, APACHE II were administered and blood was withdrawn to estimate the above biological factors. Mixed effects (PQL) and GEE were used to analyse the repeated measurements and investigate the associations at the individual and population average levels.

Results: a total of 205 observations on 67 individuals were analysed. Lower levels of IGF-I, and lower levels of circulating IL-1RA, are significantly (P < 0.05) associated with delirium, while the remaining of cytokines, severity of illness and possession of epsilon 4 allele had a non-significant effect. This has been shown by both statistical methods. Similarly lower levels of IGF-I, and high levels of IFN-γ, are statistically significantly (P < 0.05) associated with higher DRS scores (more severe delirium).

Conclusions: this study finds that (i) low levels of both neuroprotective factors (IGF-I, IL-1RA) are associated with delirium, (ii) high IFN-γ and low IGF-I have significant effects on delirium severity and (iii) otherwise the pro-inflammatory cytokines studied, APOE genotype and severity of illness do not appear to be associated, in older medically ill patients, with either delirium or severity of it.

Keywords: delirium, APOE, cytokines, interleukin-1, IGF-I, elderly

Introduction

Delirium is a complex neuro-psychiatric syndrome with high prevalence among elderly medical inpatients. We [1] and other investigators [2, 3] have shown that cognitive impairment, illness severity and pre-morbid disability are predictive of prevalent and incident delirium among ill older hospital inpatients. These factors suggest that a loss of brain reserve causes susceptibility to delirium which is then precipitated by the addition of biomedical and psychosocial factors implicit in acute illness. However, the patho-physiological processes involved in this precipitation are not yet clarified.

In a previous study, we reported that elevated C-reactive protein (CRP) levels at or soon after hospital admission appear to be highly predictive of both incident delirium and recovery from it, although no differences in the levels were found between those with prevalent delirium and those without [4]. It is possible that CRP may have a direct patho-physiological role but its most likely association with delirium is that it merely reflects the play of other factors, e.g. cytokines or insulin-like growth factor-I (IGF-I). It seemed to us reasonable, therefore, to investigate the hypothesis that a number of cytokines are relevant to delirium. A review of the literature suggested that the most likely candidates were IL-1α (interleukin 1α), IL-1β (interleukin 1β), IL-1RA (interleukin 1 receptor antagonist), IL-6 (interleukin 6), TNF-α (tumour necrosis factor-α), IFN-γ (interferon gamma) and LIF (leukaemia inhibitory factor) [5]. Also, as the APOE genotype plays a role in cognitive function, can affect the immune response [6] and has been associated with amyloid...
depensation [7], we hypothesised that APOE genotype may be a possible predisposing factor for delirium.

Studies of prevalent delirium may miss associations important at the start of medical illness; it is not yet possible to capture data on patients prior to admission, but we decided to explore the cytokine-delirium relationship across the entire hospital stay of a group of older medically ill people, in which there would be prevalent, incident, recovering, relapsing and continuous delirium. We investigated the relationship of serum cytokines, IGF-I, severity of illness, cognition, possession of APOE epsilon 4 genotype, gender and age on (i) the presence of delirium and (ii) on its severity.

**Methods**

The study was performed between July 2003 and April 2004. The recruited population were consecutive patients aged 70 years or more who were admitted to an Elderly Medical Unit of a University hospital or transferred to the unit within 3 days or less from their admission elsewhere in the hospital. Patients were excluded if they were in hospital more than 3 days, if they had taken part in the study on a previous admission, if they had known terminal illness, severe aphasia, severe sensory problems, were intubated or did not speak English.

**Clinical assessments—measurements scales**

At each assessment of the subjects, the following were carried out.

Mini-Mental State Examination (MMSE) was standardized to give a maximum score of 30 if patients could not complete sections e.g. due to visual impairment. To avoid learning effects, we changed some of the items in each assessment (e.g. recall items were different at each time point of assessment and reverse spelling was alternated between WORLD and WATCH).

The Confusion Assessment Method (CAM) was used for a dichotomous classification of delirium/no delirium. CAM is a screening instrument that detects delirium. It consists of nine operationalised DSM-IIIIR criteria. The four ‘cardinal’ criteria are acute onset and fluctuating course, inattention, disorganized thinking and altered level of consciousness. These contribute to an algorithm for diagnosis of delirium.

The Delirium Rating Scale (DRS) was used for an assessment of the severity of delirium. The DRS was developed according to the DSM-III criteria to measure the severity of delirium. It has a 10-item rating scale, and each item can be scored from 0 to a maximum of 2, 3 or 4 points depending on the item. The maximum score for the scale is 32 points. A total scale score of 12 or more is compatible with the diagnosis of delirium.

The Acute Physiology and Chronic Health Evaluation II (APACHE II) and its subscale Acute Physiology Score APS were used for measurement of severity of physical illness. It takes into account age, chronic illness and acute physiological disturbance. The latter is measured with its subscale called Acute Physiology Score (APS) which has 11 items and each one can be scored from 0 to 4 (4 is the worst). The range of the scale is from 0 to 71 and an increased score is closely correlated with a subsequent risk of death in hospital. The measurement of arterial pH was omitted to avoid arterial blood sampling of patients.

**Laboratory assessments**

Venous samples of blood were taken in the morning immediately after the clinical assessments (non-fasting). Levels of IL-1α, IL-1β, IL-1RA, IL-6, IFN-γ, TNFα, LIF and IGF-I were estimated with ELISA methods. In normal situations many of these cytokines are at very low levels or undetectable. Blood for assessment of APOE 4 was withdrawn at the first assessment in all subjects. For details of the laboratory analysis of cytokines and APOE, see [http://www.psychopathology.org.uk/procedures/delirium/](http://www.psychopathology.org.uk/procedures/delirium/)

**Number and time of assessments**

Each participant was assessed in the morning. The maximum possible number of assessments for each was 5. Assessments were carried out twice per week at approximately 3-day intervals for the first 2 weeks and, if the subject was still in hospital and alive, a final one was carried out 28 days after the first assessment was completed. Blood was collected at the first four assessments. The method has been described previously [8].

**Ethical matters**

Informed consent was obtained using a previously published method [9]. Consent was obtained separately for blood tests and APOE4 assessment. The study was approved by the Local Research Ethics Committee.

**Analysis**

For the analysis of factors associated with the presence or absence of delirium, two approaches were used [10]: the Penalized Quasi Likelihood method (PQL) and the Generalized Estimating Equations (GEE) method. Both methods take into account the fact that the observations within a subject are dependent, but they differ in that the GEE approach uses the population-averaged estimates in accounting for dependence between repeated measurements, whereas the PQL uses the estimates for difference between subjects conditional on the same random effect [11]. For both approaches, the SAS 9.1 software was used, the Proc GLIMMIX for the PQL approach and the Proc GEMOD for the GEE method.

For analysis of the severity of any delirium, a mixed linear model was used (SPSS v13 software) with DRS score as the dependent variable. A higher score results from more individual delirium symptoms being present or these symptoms being more severe. Even though patients with a low score would not be generally regarded as categorically delirious, we henceforth describe this analysis as ‘delirium severity’ for all patients.
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Table 1. Summary of the final fitted model with the PQL (GLIMMIX) procedure

<table>
<thead>
<tr>
<th>Effect</th>
<th>Estimate</th>
<th>Standard error</th>
<th>df</th>
<th>t value</th>
<th>P &gt;</th>
<th>t</th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>Intercept</td>
<td>7.48</td>
<td>2.26</td>
<td>65</td>
<td>3.31</td>
<td>0.001</td>
<td></td>
<td></td>
</tr>
<tr>
<td>IL-1RA</td>
<td>−0.00087</td>
<td>0.0004</td>
<td>99</td>
<td>−1.96</td>
<td>0.05</td>
<td></td>
<td></td>
</tr>
<tr>
<td>IGF-I</td>
<td>−0.025</td>
<td>0.012</td>
<td>99</td>
<td>−2.08</td>
<td>0.04</td>
<td></td>
<td></td>
</tr>
<tr>
<td>MMSE</td>
<td>−0.398</td>
<td>0.074</td>
<td>99</td>
<td>−5.39</td>
<td>&lt;0.0001</td>
<td>--</td>
<td>--</td>
</tr>
<tr>
<td>Occasion 0</td>
<td>0.64</td>
<td>1.15</td>
<td>99</td>
<td>0.56</td>
<td>0.58</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Occasion 1</td>
<td>0.80</td>
<td>1.14</td>
<td>99</td>
<td>0.71</td>
<td>0.48</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Occasion 2</td>
<td>−1.69</td>
<td>1.32</td>
<td>99</td>
<td>−1.28</td>
<td>0.20</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Occasion 3</td>
<td>0.</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

CAM = 0 delirium.
The sign (± or −) in the second column implies the direction of the relationship between the variable and delirium. For example, lower IGF-I was associated with greater chance of delirium at a significance level of P = 0.04 and lower MMSE scores were associated with a greater chance of delirium at the statistically significant level of P < 0.0001.

Results

Sample description

A total of 205 observations on 67 individuals across four different time points are included in these data. The pattern of the assessments was: 33 subjects (49.3%) had four assessments, 13 (19.4%) three assessments, 13 (19.4%) two assessments, and 8 (11.9%) one assessment. The mean age of the subjects was 84.2 years (SD 6.3 range 70–94), and of these 48 (71.6%) were female. The mean length of hospital stay was 18.6 days (SD 17.6, median 13, range 1–86), and 40 (59.7%) had a previous history of dementia. Almost all subjects had more than one acute or chronic medical condition. The most frequent primary diagnosis (considered by the research clinician to be the most important to explain the hospital admission) were urinary track infection (n = 20, 30%), falls (n = 7, 10.4%), lower respiratory tract infection chest infection (n = 10, 15.5%), with chronic obstructive pulmonary disease in three cases (4.5%), hypertension (n = 3, 4.5%) and cellulitis (n = 3, 4.5%).

Fifteen of the subjects (22%) had at least one epsilon 4 allele in their APOE gene while 42 (63%) did not, the remaining 10 (15%) being missing values (in two subjects blood was unobtainable and in eight the analyses failed).

In 63 (31%) observations, the CAM was positive (delirium), which was distributed across the assessments thus: 25 (12%) delirium observations at first assessment, 21 (10%) at the second, 10 (5%) at the third and 7 (3%) at the fourth. A total of 39 subjects (58.2%) did not develop delirium during the study, 25 (37.3%) had prevalent delirium (of whom 10 had delirium throughout) and 3 (4.5%) had incident delirium.

Missing values

Little's MCAR test showed that there was no systematic pattern of missing values (chi-square = 106.010, df = 111, P = 0.616).

Examination of the effects of explanatory variables on the presence or absence of delirium

Analysis of Repeated measurements

(a) PQL approach: for this analysis, the GLIMMIX procedure was used (SAS 9.1). The dependent variable was binary CAM status, the fixed effects were APS score, age, sex, possession or otherwise of an epsilon 4 allele, cytokines levels, IGF-I level and ‘occasion’ (factorial variable of the time order of assessments coded as 0 the first and 3 the last fourth assessment). Subjects were treated as random effects. Interactions of sex and possession of epsilon 4 allele with each one of the dimensional variables were also entered into the initial model, but as their effects were not significant (P < 0.05) they were dropped one by one from subsequent models, as were all variables with less significant effects until a parsimonious model was achieved. The final model is shown in Table 1.

This analysis suggests a significant statistical effect on delirium status of MMSE, IGF-I, and IL-1RA (marginal significance), while the remaining cytokines, severity of illness and possession of epsilon 4 allele had a non-significant effect and were dropped from the final model. Similarly, the time of the assessments (occasion) had no significant effect on delirious status but this needs to be interpreted with caution as the data are not balanced; the occasion effects include both between-subject and within-subject components as not all subjects had the same length of stay.
Cytokines and delirium

Table 2. Summary of the final fitted model with the GEE approach (AR(1) correlation)

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Estimate</th>
<th>Standard error</th>
<th>95% confidence interval</th>
<th>Z</th>
<th>P &gt;</th>
<th>Z</th>
</tr>
</thead>
<tbody>
<tr>
<td>Intercept</td>
<td>7.65</td>
<td>1.92</td>
<td>3.89</td>
<td>11.41</td>
<td>3.98</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>IFN-γ</td>
<td>0.007</td>
<td>0.015</td>
<td>−0.02</td>
<td>0.036</td>
<td>0.43</td>
<td>0.67</td>
</tr>
<tr>
<td>IL-1RA</td>
<td>−0.0008</td>
<td>0.0004</td>
<td>−0.001</td>
<td>−0.001</td>
<td>−2.15</td>
<td>0.032</td>
</tr>
<tr>
<td>IGF-I</td>
<td>−0.02</td>
<td>0.009</td>
<td>−0.041</td>
<td>−0.004</td>
<td>−2.39</td>
<td>0.017</td>
</tr>
<tr>
<td>MMSE</td>
<td>−0.41</td>
<td>0.07</td>
<td>−0.55</td>
<td>−0.26</td>
<td>−5.6</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>Occasion 0</td>
<td>0.33</td>
<td>0.09</td>
<td>−1.43</td>
<td>2.08</td>
<td>0.37</td>
<td>0.71</td>
</tr>
<tr>
<td>Occasion 1</td>
<td>0.40</td>
<td>0.09</td>
<td>−1.35</td>
<td>2.14</td>
<td>0.44</td>
<td>0.66</td>
</tr>
<tr>
<td>Occasion 2</td>
<td>−1.60</td>
<td>0.92</td>
<td>−3.39</td>
<td>0.20</td>
<td>−1.74</td>
<td>0.08</td>
</tr>
<tr>
<td>Occasion 3</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>–</td>
<td>–</td>
</tr>
</tbody>
</table>

PROC GENMOD is modelling the probability that CAM = ‘0’

Analysis Of GEE parameter estimates

CAM = 0 delirium.

The sign (+ or −) in the second column implies the direction of the relationship between the variable and delirium. For example, lower IGF-I was associated with greater chance of delirium at a significance level of P = 0.017 and lower MMSE scores were associated with a greater chance of delirium at the statistically significant level of P < 0.0001.

(b) GEE approach: using the same variables, a model with the GENMOD procedure of SAS 9.1 was built. To control for correlation between assessments, the autoregressive (first-order) ‘working’ correlation (correlation decreasing over time) was used. The final model using with the AR(1) correlation is shown in Table 2.

With the PQL approach (Table 1), it can be seen that MMSE, IGF-I and IL-1RA appear to have significant effects on delirium status.

Examination of the effects of explanatory variables on delirium severity

The dimensional variable DRS score was treated as the dependent variable. Once more, the same fixed and random variables (subjects) were included but with a mixed linear model. Different models were constructed with different correlation structures to minimise AIC (Akaike’s Information Criterion). The retained model is presented in Table 3 (fixed effects). For random effects, the identity covariance structure was used and for the repeated measurement of the subjects (occasion) the diagonal. The residuals did not depart from the assumption of normality. The model had an AIC equal to 693.74.

Although each occasion did not have significant effects on the delirium severity, the overall effect was significant (F = 3.101; df numerator: 3, df denominator: 36.541; P = 0.038). As shown in Table 3, MMSE score and IGF-I appear to have significant effects on delirium severity. High MMSE and high IGF-I are associated with low DRS scores, whilst high levels of IFN-γ are associated with high scores. The other variables in Table 3 have a small effect on delirium severity, and although their effects were not significant, they were retained in the model as the final model had a lower AIC.

Thus from the mixed linear analysis, high levels of IGF-I, high MMSE scores and low levels of IFN-γ are associated with low DRS scores.

Discussion

This longitudinal study confirms that categorical delirium status is associated with cognitive impairment as measured by the MMSE [3], and shows that delirium presence is also associated with low levels of IGF-I and low levels of the cytokine IL-1RA, but not independently associated with levels of any of the pro-inflammatory cytokines studied. A previous study [12] reported that low IGF-I levels were a risk factor for incident delirium. The present analysis confirms this but extends it to delirium at any stage during the hospital stay, and also suggests a similar but independent role for the cytokine IL-1RA.

Both cytokines IGF-I and IL-1RA have neuroprotective properties. IGF-I has a major role in the Central Nervous System (CNS) promoting development, neuronal survival, proliferation, differentiation and synaptogenesis [13]. Interestingly it has been proposed that neurons are directly
protected by IGF-I, and not via any indirect mechanism involving IGF-I stimulation or additional trophic support from glia [14]. In animals, improvement in behavioural and cognitive outcome was observed after administration of IGF-I in brain injuries [15] which has also been proposed as a treatment for Alzheimer’s Disease (AD) because of its involvement in tau phosphorylation and in the acetyl-choline pathways, which are also implicated in delirium [16].

IL-1RA blocks the actions of IL-1α and IL-1β which are both pro-inflammatory cytokines and have deleterious effects directly or through other pathways in the brain [17]. IL-1RA downregulates ischaemic and excitotoxic neural damage and inhibits the induction of the inflammatory reaction by amyloid β peptide [18, 19]. Some circumstantial evidence also supports a role for IL-1RA in delirium. Haloperidol, an antipsychotic medication, is recommended in some clinical guidelines for management of symptoms of delirium. One of the effects of haloperidol in vitro is the increase of IL-1RA production [20].

In rats, repeated administration of antipsychotics including haloperidol induces IL-1RA MRNA in a widespread area of the brain [21]. In vivo, low doses of haloperidol given prophylactically has been shown [22] to have a positive effect on reducing the severity or duration of delirium. Thus, the therapeutic effects of this medication in delirium may be via the increased production of IL-1RA.

In our analysis, none of the other pro-inflammatory cytokines examined in this study were associated with delirium status. This negative finding is surprising. Two recent studies found increased levels of IL-6 or other pro-inflammatory cytokines [23, 24] in delirious patients or in post-operative patients with complications. Another study [25] found differences between groups of cytokines in matched delirious and not delirious patients. These studies differ from ours in a variety of ways, including using levels only of those cytokines which were detectable, or by studying only the pro-inflammatory cytokines or in their statistical analyses.

The results of this study suggest a novel hypothesis in relation to the role of cytokines. High levels or persistence of pro-inflammatory cytokines may not be patho-physiologically necessary for the development of delirium if there are deficits in the immunoreactivity of the brain, i.e. low cerebral reserve. This would explain the observation that delirium occurs after relatively minor precipitating illnesses more commonly in children and older adults (but less often in between even with severe acute illness).

Our results suggest that low MMSE scores and low levels of IGF-I are also associated with higher DRS scores, presumably by similar mechanisms. A novel finding is that also high levels of IFN-γ are similarly associated with more severe delirium. The secretion of IFN-γ in the brain can induce cognitive impairment [26]. Also IFN-γ plays a significant role in a variety of neurobehavioural symptoms like anxiety, depression, psychosis, sleep and arousal (all of them common symptoms of delirium) and therapeutic use of IFN-γ can cause delirium [27]. It could be that rising DRS scores are reflective, not of increasing probability or severity of delirium but increasing behavioural symptoms in delirium, and this is why an association is found with DRS scores but not categorical delirium. However, it needs to be noted that IFN-γ can regulate IGF-I [28] and thus the beneficial effects of lower levels of IFN-γ might be partly manifested through higher IGF-I.

If these findings are confirmed, there may be implications for the treatment of delirium, perhaps with small doses of IGF-I and/or IL-1RA. They are also relevant to the possible disease-modifying impact of antipsychotic treatment in delirium.

**Limitations**

The study was a naturalistic observational longitudinal study and the analysis incorporated the sequence of observation.
However, not all agree that such longitudinal data can resolve cause–effect problems, especially when the study is purely observational [29]. Thus, in this study we discuss only the association of MMSE, IL-1RA, and IGF-I with delirium and do not describe them as ‘risk factors’ as no experimental manipulation of the above variables occurred. The study was only on patients requiring admission to hospital, and the findings cannot be generalised to other cohorts of patients who develop delirium elsewhere.

Conclusions

This study confirms previous findings that low levels of IGF-I are associated with incident delirium and extend it to all instances of delirium. Three new findings in older medically ill patients are as follows: (i) low levels of IL-1RA have a significant (statistical) effect on delirium status, (ii) high IFN-γ and low IGF-I have significant effects on delirium severity and (iii) the pro-inflammatory cytokines studied do not appear to be associated with either.

Key points

- This prospective observational study shows that low levels of both neuroprotective factors (IGF-I, IL-1RA) are associated with delirium.
- High circulating levels of IFN-γ and low IGF-I are significantly associated with greater severity of delirium.
- In older medically ill patients, the presence or severity of delirium does not appear to be associated with the severity of illness, the APOE genotype or levels of the pro-inflammatory cytokines studied.
- This study suggests that in the presence of deficits of the immunoreactivity of the brain, then high levels or persistence of pro-inflammatory cytokines may not be patho-physiologically necessary for the development of delirium.

Acknowledgements

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Conflicts of interest

The authors have reported no conflicts of interest.

References


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