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CSF beta-amyloid 1-42 concentration predicts delirium following elective arthroplasty surgery in an observational cohort study

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Short running head: CSF A β predicts postoperative delirium

Mini-Abstract

This observational cohort study of participants undergoing elective arthroplasty found an independent association between a cerebrospinal fluid biomarker of Alzheimer's Disease and subsequent delirium following surgery. This suggests that postoperative delirium may indicate incipient Alzheimer's disease and if replicated should influence postoperative follow-up practices.

Abstract

Objective: To test the hypothesis that *APOE* $\epsilon 4$ status and cerebrospinal fluid (CSF) A β 42, T-tau and P-tau would independently predict the risk of postoperative delirium.

Summary Background Data: Delirium following surgery is common and associated with adverse outcomes. Age and cognitive impairment are consistent risk factors for postoperative delirium.

Methods: This observational cohort study recruited 282 participants aged 65 years or older, without a diagnosis of dementia, admitted for primary elective hip or knee arthroplasty. Cognitive tests were undertaken preoperatively, blood and CSF were sampled at the time of spinal anaesthesia and participants were assessed daily postoperatively for delirium.

Results: Increasing age ($p=0.04$), preoperative comorbidity ($p=0.03$), type of surgery ($p=0.05$), intravenous opioid usage ($p=0.04$) and low CSF A β 42 ($p<0.01$) were independent predictors of postoperative delirium.

Conclusions: This study is the first to show an independent association between CSF A β 42 and delirium incidence in an elective surgical population, suggesting that postoperative delirium may indicate incipient Alzheimer's disease.

Introduction

Delirium is common following elective arthroplasty surgery, with an estimated incidence of 17% (1), and is associated with significant negative outcomes including dementia and institutionalisation (2). As greater numbers of older people, with higher levels of comorbidity, undergo surgery (3), numbers developing postoperative delirium are likely to rise.

Whilst age and pre-existent cognitive impairment are established risk factors for postoperative delirium (4, 5) there is currently no reliable method to predict preoperatively who will become delirious after surgery. Better prediction would inform our understanding of the pathophysiological basis for delirium which remains unclear. In time it may also inform consent and perioperative counselling processes as well as care and discharge planning.

Given the consistent association between cognitive impairment and postoperative delirium, risk factors for, and biomarkers of, Alzheimer's disease (AD) are being investigated as predictors of postoperative delirium. The apolipoprotein (*APOE*) ϵ 4 allele, the main genetic risk factor for AD, has been studied as a potential predictor of postoperative delirium with conflicting results (6-8).

Cerebrospinal fluid (CSF) amyloid β 42 ($A\beta$ 42), total tau (T-tau) and phospho-tau (P-tau) are established as biomarkers of the AD process, reflecting plaque pathology, neurodegeneration and neurofibrillary tangle pathology, respectively. Alterations in these biomarkers are seen prior to the clinical emergence of AD dementia (9). One study and one pilot study have suggested these biomarkers may be predictors of delirium in elective surgical populations (10, 11). Two other studies conducted in hip fracture populations yielded conflicting results (12, 13). A proven association between delirium and AD pathophysiology would necessitate review of follow-up practices.

Although delirium is associated with subsequent cognitive decline no clinical standards regarding follow-up are in place in the UK and Ireland at the current time.

This observational cohort study was designed to test the hypothesis that quantification of brain vulnerability, using *APOE* $\epsilon 4$ status and CSF A β 42, T-tau and P-tau would independently predict the risk of delirium following elective primary hip and knee arthroplasty in an older population.

Methods

Study population

Participants aged 65 years or older admitted for primary elective hip or knee arthroplasty to a single surgical centre and planned to undergo spinal anaesthesia with intrathecal diamorphine were eligible for inclusion. Other anaesthetic variations beyond this requirement were permitted.

Exclusion criteria included a pre-existing diagnosis of dementia or other neurodegenerative condition, visual or hearing impairment such that the participant was unable to undertake the neuropsychological assessments, illiteracy and stroke with residual deficit.

Those participants for whom sufficient CSF was available for analyses were included in this study.

Study methods

Written informed consent was obtained from all participants and the study was performed in accordance with local ethical committee procedures (REC reference: 10/NIR01/5; protocol number: 09069PP-OPMS). Demographic data and potential confounding variables, as considered in similar previous studies (10, 12, 13) (including age, gender, type of surgery, American Society of Anaesthesiologists physical (ASA) status (14), Charlson Comorbidity Index (CCI) (15), level of education, estimated IQ (16), functional status (17), depression score (18), smoking status and alcohol intake) were collected and included in analyses. Information regarding potential confounding operative and anaesthetic factors including surgical duration, intraoperative blood loss and hypotension and use of sedatives, opioids and general anaesthesia (19-21) was recorded from the medical charts where possible. Bispectral index monitoring was not routinely undertaken in our

centre during this study period. Mini Mental State Examination (MMSE) (22) was completed preoperatively. Participants were not assessed for delirium preoperatively.

Postoperatively participants were assessed for delirium once daily for the first three days by trained medical research staff (TM then ELC). The MMSE, including the spelling WORLD backwards test of attention, and the Confusion Assessment Method (CAM) (23) were completed on each occasion with symptoms or signs of delirium sought from nursing staff. Delirium was deemed to be present or absent based on the CAM criteria. An additional test of attention – months of the year backwards (MOTYB) was added during the study. In addition, where possible, medical and nursing notes were interrogated post-discharge by the researchers or a research assistant. In all cases where there were reports of possible delirium but the CAM criteria had not been fulfilled, cases were discussed between ELC and APP and a consensus reached as to whether they reflected delirium according to the Diagnostic and Statistical Manual of Mental Disorders (DSM) IV criteria (24).

Both TM and ELC completed the online Confusion Assessment Method training manual and coding guide (25). TM was further trained in the use of delirium assessments by experienced research nurses. ELC was further trained and observed by TM in the delirium assessments prior to undertaking recruitment. TM recruited the first 35 participants and ELC the remaining 247, each completing the delirium assessments for those participants.

Sample collection

Venous blood was sampled preoperatively into PAXgene Blood DNA tubes (PreAnalytiX; Qiagen/BD, Catalogue No. 761125). Spinal anaesthetics were carried out in the sitting or lateral positions using 25 gauge (diameter 0.53mm; length 90mm) Whitacre type spinal needles with graduated metal introducers (Vygon). Once CSF was obtained, a 5ml syringe (BD Plastipak; BD) was attached to the spinal needle and up to 5mls of CSF withdrawn by the anaesthetist. The CSF was immediately transferred to a 30ml sterile polypropylene universal container (Unisurge). All samples were immediately put on wet ice in an insulated container.

Sample processing

All samples were transported to the laboratory within 12 hours of collection. DNA samples were processed according to the manufacturer's instructions (TaqMan Single Nucleotide Polymorphism Genotyping Assay; Life Technologies; Catalogue No. 4351379). CSF samples were centrifuged at 4°C at 3000 rpm/1811g for 5 minutes with no brake and 500µl aliquots of the supernatant pipetted (Sarstedt, Germany, Catalogue No. 70.762) into 1.5ml polypropylene tubes (Sarstedt, Germany, Catalogue No. 72.69.001). By necessity, some aliquots were of a lower volume. All samples were stored at -80°C.

DNA collection and analysis

DNA was analysed using the TaqMan Single Nucleotide Polymorphism Genotyping Assay (Life Technologies; Catalogue No. 4351379) as per the manufacturer's instructions. *APOE* status was inferred from the genotype at each of the two alleles, rs7412 and rs429358 (26).

CSF biomarker analysis

CSF samples were transported to the Leonard Wolfson Biomarker Laboratory, University College London on dry ice. There A β 42, T-tau and P-tau were analysed by a single, trained, technician, blinded to the clinical data, using the INNOTEST β -amyloid (1-42), hTau Ag and Phospho-tau (181P) ELISA kits respectively (Fujirebio, Ghent, Belgium) run on a FLUOstar Omega BMG LABTECH instrument (chemiluminescent plate reader) according to the manufacturer's protocols. Samples were thawed at 21°C in an air-conditioned lab and vortexed prior to use. CSF biomarker values were determined using Omega software (version 5.10 R2) and a 4-parameter curve fit. Intra- and inter-assay CVs respectively were 2.0% and 3.9% for A β 42, 3.3% and 8.1% for T-tau and 1.6% and 5.1% for P-tau, according to International Organization for Standardisation standards (27). Longitudinal stability in the measurements is further monitored in the laboratory by participation in the Alzheimer's Association Quality Control program (28).

Statistical methods

The study was powered to detect a difference in the risk of postoperative delirium between the *APOE* ϵ 4 positive and negative groups based on an estimated *APOE* ϵ 4 carrier rate of 25.5% (29) and a rate of delirium in the *APOE* ϵ 4-positive and *APOE* ϵ 4-negative groups of 28% and 11%, respectively, in an elective non-cardiac surgery population (30). It was estimated that 316 participants would be required to enable detection of significant difference, at the 5% significance level, with 90% power.

Biomarker concentrations were considered as continuous and dichotomous variables (based on published cut-offs of $A\beta$ 42 <530ng/L, T-tau >350ng/L, P-tau >60ng/L and $A\beta$ 42/P-tau <6.5 (31)). Both $A\beta$ 42/P-tau and t-tau/ $A\beta$ 42 ratios were calculated (31, 32). Univariate analyses of baseline characteristics and anaesthetic factors between the delirium and no delirium groups utilised t-tests (incorporating Levene's test for equality of variances), Mann-Whitney U tests and chi-squared test with Yates' correction (or Fisher's exact test) as appropriate.

Where appropriate, non-normally distributed variables were logarithmically (log) transformed and analysed using t-tests. Group characteristics were presented as geometric means (with interquartile range (IQR) of raw values) where the geometric mean is the antilog of the arithmetic mean (the mean of the log transformed values).

Binary logistic regression, with postoperative delirium as the dependent variable and continuous CSF variables as the independent variables undertaken using an entry significance level of 10% ($P < 0.10$).

Analysis was performed using SPSS for Windows version 22 (IBM Inc., Armonk, NY, USA).

Given the range of MMSE scores and the possibility that the cohort included participants with undiagnosed dementia the analyses were rerun in those with a preoperative MMSE ≥ 24 . Methods and results are presented in accordance with STROBE guidance (33) where possible.

Results

Baseline characteristics of the entire cohort and the delirium and no delirium groups are outlined in Table 1. Ethnicity was not considered during recruitment but all participants were white reflecting the racial homogeneity of those presenting for surgery in our centre. Of the 315 participants recruited 282 had sufficient CSF for A β 42 and P-tau analyses with 279 of these also sufficient for T-tau analyses. The rate of delirium in the subgroup with sufficient CSF (40/282 [14%] vs 44/315 [14%]) was not significantly different to the cohort as a whole ($\chi^2=0.006$, $P=0.94$).

The number of *APOE* ϵ 4 alleles (0,1 or 2) was not associated with postoperative delirium. Table 1 also shows the univariate analyses significant at the 10% level (and therefore entered into the logistic regression model) and these are highlighted in **bold**. Age, knee vs hip surgery, ASA status, CCI, MMSE, CSF A β 42, CSF T-tau and CSF P-tau showed significant differences between the delirium and no delirium groups on univariate analyses. Gender, years of education, estimated IQ, Bristol Activities of Daily Living Scale score, Geriatric Depression Scale score, smoking status and units of alcohol per week were not significantly different between the delirium and no delirium groups.

The results of the sensitivity analysis, carried out using only the 239 participants with an MMSE \geq 24, are also shown in Table 1. ASA status, CCI, CSF T-tau and CSF P-Tau were not significantly different between the delirium and no delirium groups for these participants.

Univariate analyses of operative and anaesthetic factors, where the information was available, are outlined for the delirium and no delirium groups in Table SDC1. Only intraoperative intravenous opioid usage was significantly different between delirium and no delirium groups, and only for the whole cohort ($p=0.039$), this did not withstand the sensitivity analysis ($p=0.105$). Of those 27 participants receiving intraoperative intravenous opioids, 22 received alfentanil and 5 fentanyl.

Entering those variables predicting delirium in the univariate analysis into the binary logistic regression (Table 2), shows that age, CCI, type of surgery, intravenous opioid and CSF A β 42 are

independent predictors of postoperative delirium in the entire cohort. Thus the odds ratio of developing delirium postoperatively is increased by a factor of 1.1 for each additional year of age, 1.8 for each additional class of the CCI and 2.9 with the usage of intravenous opioids and decreased by a factor of 0.4 for hip vs knee surgery and 0.996 for each unit (ng/L) increase in CSF A β 42. Interaction effects were considered for these five variables and the interaction effect of CCI and intravenous opioids, only, was significant ($p=0.023$). Of note the interaction effect of age and CSF A β 42 did not significantly affect the model ($p=0.388$). This analysis was rerun in the MMSE ≥ 24 group. Fewer variables were entered (see Table 1 for those significant at the $p=0.1$ level) but the analysis yielded similar results (Table 2). CSF A β 42/ ρ tau and t tau/ CSF A β 42 ratios were too highly correlated with the individual concentrations to be included in the same model.

Discussion

This study finds CSF A β 42 concentrations to be the strongest independent predictor of postoperative delirium following elective arthroplasty in an older population without a prior diagnosis of dementia. Importantly, this association was independent of tau and suggests that cerebral β -amyloidosis *per se* may make the brain vulnerable to postoperative delirium.

Whilst this finding is in keeping with epidemiological literature suggesting an association between dementia pathophysiology and delirium it is the first time to our knowledge that it has been demonstrated in an elective surgical population. These findings are significant as they i) provide *in vivo* biomarker evidence that support epidemiological and animal work suggesting a link between Alzheimer pathophysiology and delirium ii) potentially shed light on the association between delirium and subsequent cognitive decline.

Decline in CSF A β 42 level is thought to be a very early feature of Alzheimer's disease, and to occur a decade or more prior to the onset of any cognitive symptoms. Deposition of A β within the brain is thought to trigger a number of different pathophysiological processes including neuroinflammation, synaptic dysfunction, network breakdown, and neuronal loss, all of which also occur before the

onset of symptoms. Whilst the exact mechanisms are unclear, it is likely, that individuals with pre-symptomatic AD pathology are more vulnerable to the effects of stressors, including opiates, pain, and inflammatory changes seen in surgery (34), which may result in delirium.

Recent observational data has demonstrated an association between postoperative delirium and subsequent cognitive impairment (35, 36), and also between postoperative delirium and a more significant rate and magnitude of decline in cognitive function with reference to preoperative levels (37). Future work will explore whether delirium itself exerts detrimental pathophysiological effects.

Studies of longer term post-operative cognitive dysfunction often do not assess for delirium in the initial postoperative days. Evered *et al* have demonstrated an association between preoperative CSF A β 42, but not T-tau or P-Tau, concentrations and cognitive dysfunction three months following elective hip arthroplasty (38) however it is not clear whether this association was mediated by delirium. That the effects of surgery, and anaesthesia, could possibly mediate AD pathophysiology changes has been investigated with conflicting results (39-42).

The much less significant difference in CSF T-tau between the delirium and no delirium groups in this study is perhaps unsurprising given the closer temporal association of CSF T-tau, reflecting neurodegeneration, and cognitive decline and the exclusion of patients with a diagnosis of dementia from this study. This is supported by the further weakening of the association once those participants with preoperative MMSE \leq 24 were excluded. CSF A β 42/p tau and t tau/A β 42 ratios were significantly different between delirium and no delirium groups however they could not be included in the multivariable regression as they are too highly correlated with the biomarker concentration variables. Whilst ratios offer information over and above the individual biomarker concentrations it is likely, in this study, the significance is driven by the CSF A β 42 results.

We are aware of only three similar studies, of which only one has been undertaken in an elective population. Xie *et al* showed an association between quartiles of CSF A β /tau ratios and delirium but no association between individual biomarker concentrations. Of note their CSF analyses were

conducted outwith the Alzheimer's Association Quality Control program (28) and they reported relatively high intra-assay CVs (21.8, 18.7 and 6.8%) (10). Of the two studies conducted in hip fracture populations, the findings of Idland et al contradicted Witlox et al by demonstrating an association between CSF A β 42 and tau in a non-demented population (12, 13). Like us Idland et al analysed samples in an expert laboratory however hip fracture and elective orthopaedic populations vary significantly in age, comorbidity and baseline functional ability as well as circumstances precipitating delirium (3, 43) and so whilst these studies are relevant the populations should be considered independently.

CCI scores in this study were largely reliant on diabetes and vascular disease. This ties in with these AD pathophysiological processes but more work is needed to further elicit these associations.

It is not clear why delirium should be more common following knee rather than hip arthroplasty, the greater pain, and by extension analgesic burdens, seen following knee compared to hip surgery may be contributory. In addition, the utilisation of a tourniquet during total knee replacement, as was common practice for this cohort, could increase neuroinflammatory burden via the washout of an ischaemic limb. Further work is needed.

All participants in this study received intrathecal diamorphine. The independent association between additional intraoperative intravenous opioid administration and postoperative delirium is in keeping with broader published evidence associating opioids and delirium in hospitalised patients (44) however studies examining intraoperative intravenous opioids are limited (45). The numbers of participants receiving fentanyl in our study are too small to challenge recent findings suggesting it is a safer opioid however the relatively high numbers receiving alfentanil, and the limited evidence base available assessing delirium risk in elderly elective surgical populations require further study. That the association does not withstand our sensitivity analysis suggests patients with established cognitive impairment are the most vulnerable, which supports existing guidelines advocating an individualised, opioid-sparing anaesthetic approach in the elderly (46). The significant interaction

effect of CCI and intravenous opioid raises the possibility opioids were preferred for those frailer more comorbid participants, or that opioids carried an additive delirium risk for these participants. Further research is needed. It is important to note that this study was not powered to examine for anaesthetic factors as predictors of delirium.

Given that *APOE* $\epsilon 4$ is a strong risk factor for AD through reduction of CSF $A\beta 42$ (47) it is perhaps surprising that the rate of $\epsilon 4$ positivity was similar, and not significantly different, between the two groups (28% vs 25%). Further studies are required to replicate this, and also to assess for the other genetic risk factors for sporadic AD.

This study has a number of strengths, including the relatively large sample size, the prospective ascertainment of delirium via daily assessment by a trained researcher utilising a recognised tool, supplemented by notes review, and high accuracy of CSF measurements, as reflected by the very low CVs, conducted in a centre of excellence. Potential limitations to the study include the possible inclusion of patients with undiagnosed dementia, although our sensitivity analyses using the standard MMSE ≥ 24 cut-off goes a way towards addressing this concern. Patients must be well to undergo elective surgery. Any evidence of intercurrent illness would mandate cancelling of surgery in our unit therefore it is unlikely participants would have been delirious preoperatively however the absence of explicit preoperative screening for delirium is a limitation of this study. Correction was not made for the multiple univariable comparisons, thereby increasing the risk of a type 1 error, although a multivariable regression model was subsequently utilised.

Conclusions

Postoperative delirium is common and is associated with subsequent cognitive impairment, functional decline and death. This study is the first to show an independent association between CSF $A\beta 42$ and delirium and in so doing informs the evolving understanding of delirium pathophysiology and raises the possibility that delirium is indicative of an underlying AD process. Further work is required to understand this potential relationship in more detail, and how it interacts with vascular

risk, using CSF measures of inflammation, and structural and molecular brain imaging. Long term cognitive follow-up is needed to determine if, as predicted, postoperative delirium is a marker for the subsequent development of Alzheimer's disease: if so this has implications for ongoing follow-up and management of these patients and in due course may provide a means of identifying high risk individuals who will benefit from disease modifying therapies as/when they become available.

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