Low preoperative plasma cholinesterase activity as a risk marker of postoperative delirium in elderly patients

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Abstract

Background: delirium is a frequent neuropsychiatric syndrome affecting medical and surgical elderly patients. Cholinergic dysfunction has been implicated in delirium pathophysiology and plasmatic acetylcholinesterase (AChE) and butyrylcholinesterase (BuChE) activities are suppressed in patients with delirium. In this cohort study, we investigated whether these changes emerge during delirium or whether they are present before its onset.
Methods: plasma activities of AChE and BuChE were measured pre- and postoperatively in consecutive patients ≥60 years old undergoing elective total hip replacement surgery. In addition to a comprehensive clinical and demographic baseline evaluation, venous blood samples were collected from each subject in the morning of hospital admission's day and in the morning of the first postoperative day. Delirium was screened daily with confusion assessment method (confirmed with diagnostic and statistical manual of mental disorders (DSM-IV)-TR).
Results: preoperatively, plasma esterase activity was significantly lower in patients who developed delirium compared with the remaining subjects. Following surgery BuChE activity was lower in the delirium group but this difference disappeared after controlling for preoperative values. Plasma cholinesterase activity correlated positively with calcium and haemoglobin and negatively with total bilirubin and international normalised ratio.
Conclusion: plasma cholinesterase activity can be a useful candidate biomarker to identify subjects at greater risk of developing postoperative delirium.

Keywords: *delirium, acetylcholinesterase, butyrylcholinesterase, acetylcholine, elderly*

Introduction

Delirium is a neuropsychiatric syndrome clinically characterised by a sudden onset and transient impairment of consciousness and attention, with consecutive global cognitive and behavioural disturbance [1]. It is a common postoperative complication particularly in elderly patients undergoing major surgical procedures as a result of the combined action of several risk factors (e.g. medical illness, advanced age and medications) [2].

Failure in cholinergic neurotransmission has long been recognised to be involved in delirium pathophysiology in light of the evidence that cognitive impairment and psychosis are induced by anticholinergic agents (e.g. tricyclic antidepressants, anti-histamines) [3]. Indeed, several studies identified that increased burden of serum anticholinergic activity (e.g. measurements of muscarinic anticholinergic activity in serum to detect global muscarinic anticholinergic properties of various medications) is associated with both delirium [4, 5] and lower cognitive performance in the elderly [6]. Additionally, plasma activity of acetylcholinesterase (AChE) and butyrylcholinesterase (BuChE) is suppressed during a delirium episode [7]. The summated action of these enzymes determines the inactivation of circulating acetylcholine (ACh) and influences the oxidative metabolism of several drugs, including: aspirin, cocaine, heroin, procain and muscle relaxants [8]. Notably, peripheral ACh has been recognised as a key element in the homeostatic control of the innate immune response [9]. Following tissue damage or infection ACh is released from the vagus nerve leading to a dose-dependent inhibition of

proinflammatory cytokine production, including tumour necrosis factor-a, interleukin (IL)-1, IL-6 and IL-18, by immune cells. Thus, the so-called 'cholinergic anti-inflammatory pathway' can be potentially relevant in modulating the putative neuroinflammatory pathway of delirium in response to an acute systemic inflammation (reviewed in [10]). Plasma cholinesterases, therefore, can have a unique role in delirium pathophysiology as they represent a point of convergence between the immune and drug metabolising systems.

Overall changes in plasma cholinergic activity so far have been documented during incident delirium suggesting that they play a role in the aetiology of the syndrome [7]. However, little is known as to whether these changes emerge during a delirium episode as a consequence of the underlying pathophysiological processes (disease marker) or whether they are present before its onset (risk marker of disease). In order to address this, plasma cholinesterase activity was measured before and after a controlled surgical trauma known to be associated to high rates of delirium. By excluding patients with preoperative delirium or dementia, potential confounding risk factors were eliminated. We report that lower levels of plasma cholinesterase activity during postoperative delirium are accounted for pre-existing differences already present before surgery.

Materials and methods

Subjects

All patients over 60 years of age undergoing elective total hip replacement in the Orthopaedic Department of Coimbra University Hospitals from October 2008 to June 2009 were eligible to enter this cohort study. Patients were excluded at the preoperative medical assessment (the day before surgery) if they presented with a diagnosis of dementia according to diagnostic and statistical manual of mental disorders (DSM-IV)-TR criteria; delirium according to the confusion assessment method (CAM) criteria; or hearing or visual deficits prevent them to undergo neuropsychological testing. Ethical approval was given by the local Ethical Committee and written informed consent was obtained for each patient.

Preoperative assessment

All recruited subjects in the study had a baseline evaluation that included a medical history (highlighting the presence of either chronic or acute illness, smoking habits, alcohol consumption and previous psychiatric or neurologic diseases) and a pharmacological history (medication list based on patients' chronic medications and as-needed medications received on the day before surgery). Anticholinergic potency of each medication was rated using the Anticholinergic Drug Scale (ADS) [11]. All subjects were assessed with Charlson Comorbidity Index, Barthel Index, Mini-Mental State Examination and Geriatric Depression Scale (15 items) (Table 1). Data obtained from routine preoperative assessment included whole blood count and routine biochemistry.

Postoperative assessment

All subjects were assessed for delirium with the CAM (performed by a trained psychiatrist) on three occasions: the first assessment occurred in the evening of the surgery day and was repeated on second and third postoperative days.

Table 1. Demographic and clinical description of the analysed sample

	Total sample ($n = 101$)	Delirium ($n = 37$)	Non-delirium ($n = 64$)	P value
Gender (male %)	50 (49.5)	15 (40.54)	35 (54.69)	0.216[a]
Age[b]	73.04 ± 6.29 (60–89)	73.65 ± 5.87 (64–89)	72.69 ± 6.53 (60–87)	0.462[c]
Educational level				
No years of school	20 (19.8%)	8 (21.6%)	12 (18.7%)	0.848[a]
1–4 years	69 (68.3%)	24 (64.9%)	45 (70.3%)	
>5 years	12 (9.9%)	5 (13.5%)	7 (10.9%)	
Smoking				
No smoking	82 (81.2%)	30 (81.1%)	52 (81.3%)	1.000[a]
Past or active smoking	19 (18.8%)	7 (18.9%)	12 (18.7%)	
Alcohol				
No active drinking	46 (45.5%)	24 (64.86%)	22 (34.37)	**0.004**[a]
Active drinking	55 (54.5%)	13 (35.13%)	42 (65.62%)	
Charlson comorbidity index[b]	0.54 ± 0.75 (0–4)	0.68 ± 0.91 (0–4)	0.47 ± 0.64 (0–3)	0.358[d]
MMSE[b]	26.67 ± 2.79 (19–30)	26.43 ± 2.79 (19–30)	26.80 ± 2.83 (19–30)	0.385[d]
Barthel index[b]	90.99 ± 12.59 (45–100)	88.51 ± 14.33 (50–100)	92.42 ± 11.34 (45–100)	0.148[d]
GDS[b]	4.47 ± 3.161 (0–12)	4.62 ± 3.04 (0–12)	4.38 ± 3.25 (0–12)	0.554[d]
Number of preoperative drugs[b]	3.37 ± 2.43 (0–9)	3.97 ± 2.79 (0–9)	3.02 ± 2.14 (0–9)	0.115[d]
Preoperative ADS[b]	0.68 ± 1.06 (0–5)	0.84 ± 1.32 (0–5)	0.59 ± 0.69 (0–3)	0.791[d]

The values are expressed as number and percentages. The bold value is below 0.05 (statistically significant).
[a]χ^2 test.
[b]Mean±standard deviation (range).
[c]t-Student test.
[d]Mann–Whitney test.

Positive cases of delirium (according to CAM criteria) were confirmed with DSM-IV-TR criteria.

Measurements of serum cholinesterase catalytic activities

Venous blood samples were collected from each subject in the morning of hospital admission day and in the morning of the first postoperative day. Plasma was immediately separated by centrifugation and stored at −80°C until analysis. Plasma AChE and BuChE activities were assayed by measuring the production of thiocholine from the hydrolysis of the respective specific substrates acetylthiocholine iodide and S-butyrylthiocholine iodide. The reaction product, thiocholine, reacts with 5,5'-dithio-bis-2-nitrobenzoic acid (DTNB), producing the yellow anion 5-thio-2-nitrobenzoate. The production of 5-thio-2-nitrobenzoate was monitored (Varian Spectrophotometer, Cary 100) at 412 nm over time (every minute for 8 min). Enzyme activity is expressed as micromoles of DTNB transformed per millilitre of plasma per minute. We defined 'cholinergic status' as the summated activity of AChE and BuChE assays.

Statistical analysis

Data were analysed using the *Statistical Package of Social Sciences* (SPSS, 17). Pearson χ^2 test was used for analysis of the relationship between categorical variables; *t*-Student and Mann–Whitney test to compare means between continuous variables with normal and non-normal distribution, respectively. The significance of the difference between the mean plasma activity of each esterase pre- and postoperatively was determined with *t*-Student test for paired samples within-groups and *t*-Student test for independent samples between groups. Relation of AChE and BuChE with continuous variables from preoperative parameters was examined with Pearson (normal distributions) or Spearman (non-normal distributions) correlation coefficients. Factorial analysis of variance and one-way analysis of covariance was conducted to calculate the adjusted means of postoperative values in delirium/non-delirium groups, controlling for the preoperative differences.

Results

One hundred and sixteen patients were eligible to enter the study, two of whom were excluded because they presented with delirium. From the remaining 114 patients, blood samples were not available for 13 subjects due to problems with venous puncture or clotting of the blood sample. Thirty-seven patients of the final sample of 101 subjects (36.6%) met diagnostic criteria for delirium during the study period. Patients who developed delirium were less likely to have regular alcohol consumption (Table 1).

Postoperative AChE and BuChE activities were significantly reduced after surgery with a drop of 24 and 32%, respectively, from preoperative values, representing a 29% decrease in the cholinergic status (Figures 1a–c). This reduction was similarly observed in subjects who developed delirium (22% for AChE and 32% for BuChE) and in the remaining subjects (25% for AChE and 32% for BuChE) (Figures 1d–f).

Patients who developed delirium presented preoperatively with lower activity of AChE and BuChE than those who did not present with postoperative delirium [($F(1,99) = 6.656$, $P = 0.011$ for AChE; $F(1,99) = 4.486$, $P = 0.037$ for BuChE), (Figures 1d and e)]. The differences in plasma cholinergic activity were not influenced by preoperative differences in alcohol consumption pattern ($F(1,97) = 0.565$, $P = 0.565$ for AChE and $F(1,99) = 0.416$, $P = 0.520$ for BuChE). Overall, the total cholinergic status (AChE + BuChE) was decreased by 10% before surgery in the delirium group, representing a difference of moderate magnitude ($\eta_P^2 = 0.05$) (Figure 1f).

Postoperative values were lower for BuChE and cholinergic status in the delirium group (Figures 1e and f). However, adjusted means of patients with and without delirium were not significantly different, after controlling for preoperative BuChE ($F(1, 98) = 1.370$, $P = 0.245$) and preoperative cholinergic status ($F(1, 98) = 0.557$, $P = 0.457$).

Preoperative calcium, haemoglobin, bilirubin and international normalised ratio (INR) significantly correlated with plasma AChE and BuChE activity levels (Table 2), while the remaining demographic and laboratory baseline parameters did not show any association with plasma esterase activity (data not shown).

Conclusions and discussion

The findings of this prospective study show that elective hip-replacement surgery induced a reduction in the plasma activity of AChE and BuChE of 24 and 32%, respectively. Furthermore, we have identified lower preoperative activity levels of plasma cholinesterases in subjects who developed delirium postoperatively.

A decline in plasma cholinergic activity was reported previously in a small sample of individuals submitted to a major surgical trauma [12] although differences were not found following minor surgical procedures [13]. Lower plasma cholinesterase activity has been associated with elevated proinflammatory markers in the circulation during acute illness [14, 15] and following inoculation to peripheral lipopolysaccharide suggesting a significant immune−cholinergic interaction [16, 17]. Thus, the decrement observed in our study is likely related to the pathophysiological response to acute surgical trauma, even though the underlying mechanisms remain unclear.

Similarly to White *et al.* [7] findings, levels of plasma BuChE activity and cholinergic status were decreased, when

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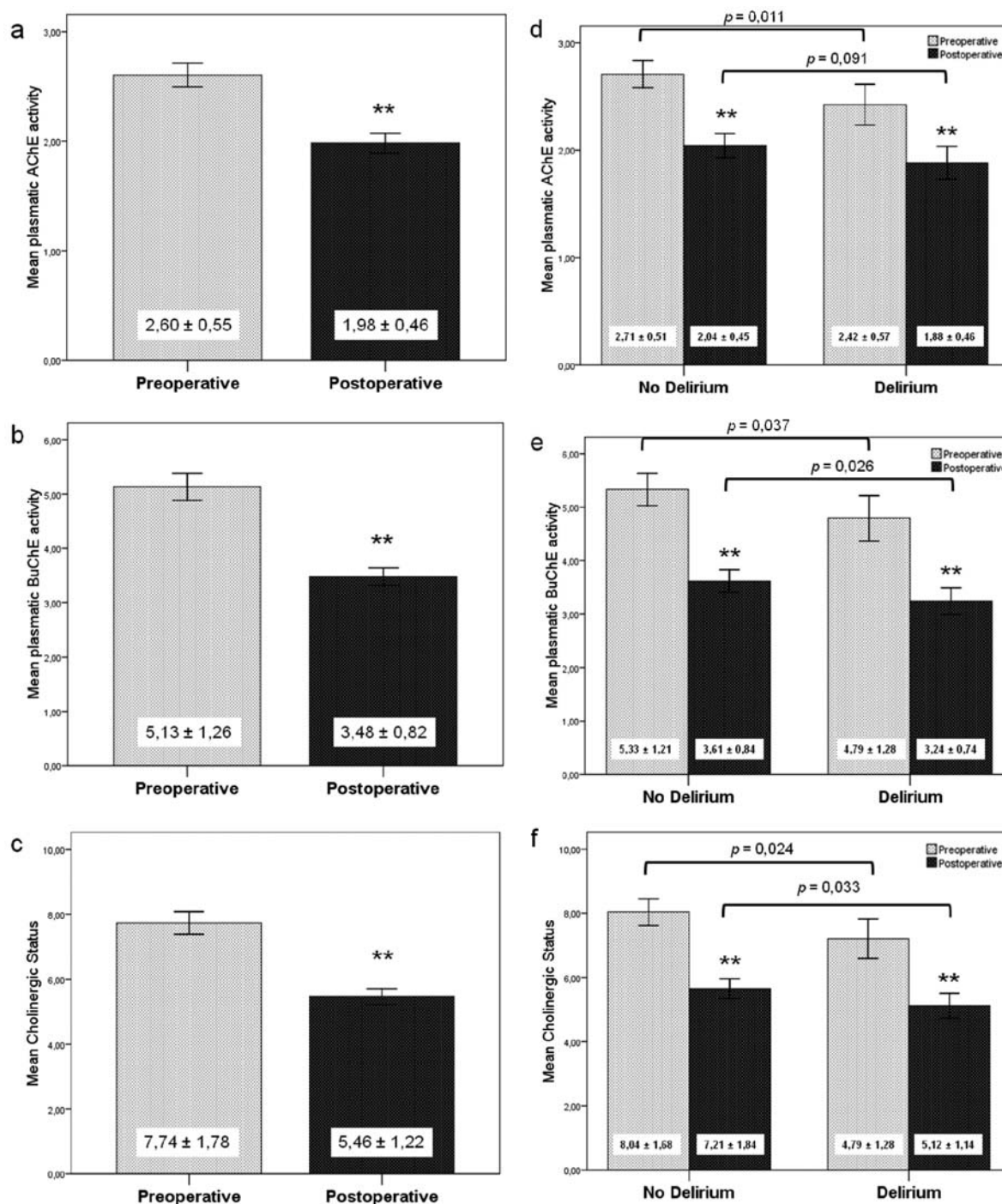
Figure 1. Measures of plasmatic AChE and BuChE activities (μmol/ml/min). Postoperatively, measures of AChE and BuChE as well as the mean cholinergic status were significantly lower than those obtained preoperatively (a–c). Significant decrease in cholinergic markers postoperatively was seen in both subjects who developed and remained devoid of delirium (d–f). Cholinergic status was significantly lower postoperatively in the delirium subjects. However, this difference was accounted for lower levels of plasmatic AChE and BuChE before surgery. **, $P < 0.001$.

compared with non-delirious subjects, during the clinical occurrence of postoperative delirium. Additionally, our study demonstrates that this reduction was already present before surgery. Thus, subjects who developed postoperative delirium presented with lower preoperative plasma activity of AChE and BuChE by over 10% than patients who did not develop delirium. After controlling for preexisting differences, postoperative levels of AChE, BuChE and cholinergic status were similar in the two groups. This suggests that cholinergic homeostasis was equally affected by surgery independently of the pathophysiological changes underlying delirium. Therefore, preoperative rather than

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Table 2. Correlations between preoperative serum biochemical parameters and plasma esterase activity

	Mean ± SD (range)	AChE	BuChE
Calcium (mmol/l)	9.67 ± 0.56 (6.8–10.5)	0.366 <0.001[a]	0.389 <0.001[a]
Haemoglobin (g/dl)	13.54 ± 1.54 (8.3–17.3)	0.317 <0.001[b]	0.350 <0.001[b]
Total Bilirubin (U/l)	0.79 ± 0.38 (0.3–2.9)	−0.239 0.019[a]	−0.246 0.016[a]
INR	1.05 ± 0.15 (0.89–2.28)	−0.227 0.022[a]	−0.300 0.002[a]

[a]Spearman (coefficient and two-tailed significance).
[b]Pearson (coefficient and two-tailed significance).

peri-operative factors appear to play an important role in mediating the association between lower plasma esterase activity and postoperative delirium.

In the current study, the lower preoperative activity levels of plasma cholinesterases in the group of patients who develop delirium were not accounted for differences in age, gender, exposure to anticholinergic drugs or burden of comorbidities. However, preoperative plasma cholinesterase activity was positively correlated with levels of calcium and haemoglobin and correlated negatively with total bilirubin and INR. Reduced plasma esterase activity has been associated with frailty, an increasingly recognised syndrome associated with decreased physiological reserve and increased risk of poor functional outcomes [18]. This is consistent with our results showing that patients with lower plasma cholinesterase activity tended to have lower levels of calcium and haemoglobin and, reversely, higher levels of total bilirubin and INR. Thus, despite the demographic and preoperative clinical homogeneity of our sample, one possible mechanism linking reduced plasma esterase activity to increased risk of delirium is a diminished homeostatic reserve. Indeed, frailty has also been proposed to be a potential useful method of predicting delirium but research in the field has been hampered by lack of a consensual definition and operational criteria [19]. Another possibility is that genetic causes underlie the differences of preoperative plasma esterase activity according to the inheritance of distinct alleles, which has been well documented for BuChE [8].

In conclusion, we found that plasma cholinesterase activity can be a useful candidate biomarker to identify subjects at greater risk of developing postoperative delirium. Future studies should evaluate whether these results can be replicated in more heterogeneous samples and in different clinical settings. Also, further research is needed to identify the factors that influence the baseline levels of plasma esterase activity. Ultimately, these findings can have a major impact on the quality of health care offered to patients if translated into the routine laboratory workout as a readily available way to recognise subjects prone to develop delirium allowing the implementation of prompt preventive measures.

Key points

- Hip-replacement surgery induces a reduction in the plasmatic activity of AChE and BuChE.
- BuChE activity and cholinergic status are decreased, when compared with non-delirious subjects, during delirium.
- One possible mechanism linking reduced plasma esterase activity to increased risk of delirium is a reduced homeostatic reserve.

Acknowledgements

Conflict of interest

None declared.

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