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ORIGINAL ARTICLE

Changes in plasma and cerebrospinal fluid biomarkers in aged patients with early postoperative cognitive dysfunction following total hip-replacement surgery

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Abstract
Purpose We hypothesized that different patterns of biomarkers of brain injury and inflammation exist in aged patients with postoperative cognitive dysfunction (POCD) after total hip-replacement with spinal anesthesia.
Methods Eighty-three patients older than 65 years undergoing elective total hip-replacement surgery were enrolled in this prospective observational study. The CSF levels of Tau, phosphorylated-tau (pTau), amyloidβ1–42 (Aβ1–42), Tau/Aβ1–42, pTau/Aβ1–42, BDNF, IL-6, and IL-1β were measured preoperatively. Perioperative plasma levels of IL-1β, IL-6, brain-derived neurotrophic factor (BDNF), C-reactive protein (CRP), and malonaldehyde (MDA) as well as neurocognitive tests were determined preoperatively and seven days postoperatively.
Results Sixty-one patients completed both the CSF and blood samples collection and the neurocognitive tests. POCD occurred in 24.6 % of patients at seven days after surgery. Patients with POCD had significantly higher IL-1β, Tau/Aβ1–42, pTau/Aβ1–42, and a lower level of Aβ1–42 in CSF when compared with the Non-POCD group ($P < 0.05$). Furthermore, POCD patients displayed significantly higher plasma levels of MDA when compared with Non-POCD patients at seven days after surgery ($P < 0.05$). There was no difference in preoperative CSF levels of Tau, IL-6, and pTau as well as plasma levels of IL-1β, IL-6, BDNF and CRP between POCD and Non-POCD groups ($P > 0.05$).
Conclusion The POCD patients were associated with higher postoperative plasma levels of MDA, and higher IL-1β and lower Aβ1–42 levels in preoperative CSF that might predispose the development of POCD in aged patients following total hip-replacement surgery with spinal anesthesia.

Keywords Postoperative cognitive dysfunction · Biomarker · Spinal anesthesia · Cerebrospinal fluid · Plasma

Introduction

Postoperative cognitive dysfunction (POCD) is a well-recognized complication after major surgery in the elderly, which is associated with a prolonged hospitalization, a reduced quality of life and will increase morbidity and mortality [1–6]. A recent multicenter trial has demonstrated that POCD is present in 25.8 % at one week and 9.9 % at three months postoperatively in patients older than 60 years after major non-cardiac surgery [5]. Although most POCD patients resolve fast, a small proportion of this population may display permanent cognitive impairment postoperatively. However, identified risk factors are largely limited to demographic characteristics in the reports [6]. Thus, investigation of biomarkers is required to improve the accuracy of diagnosis and better understanding of the complex pathophysiology for POCD.

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Patients undergoing hip-replacement surgery constitute a frail population, which seems to be frequently associated with POCD in the elderly [7]. Surgical trauma can increase the levels of inflammatory cytokines in both the peripheral and central nervous system, and thus impairing cognitive function by altering hippocampal function [2]. Indeed, increased expression of pro-inflammatory cytokines results in performance deficits in hippocampus-dependent cognitive memory [2, 6]. On the other hand, POCD may represent a preclinical stage of dementia and share similar pathological mechanisms with neurodegenerative disorders including Alzheimer's disease (AD) [8]. Some biomarkers such as inflammatory mediators, oxidation stress and cerebral damage parameters that reflect the pathophysiology of neurodegenerative diseases may serve as diagnostic biomarkers for POCD. However, little is known about those parameters in POCD patients.

In this context, we hypothesized that the changes of inflammatory mediators, Aβ1–42, Tau, brain-derived neurotrophic factor (BDNF), and parameters of oxidation stress such as malonaldehyde (MDA) would be predictive factors for POCD in aged patients following total hip-replacement surgery with spinal anesthesia.

Patients and methods

The present study was approved by the Ethics Committee of Jinling Hospital. Eligible subjects were ASA I or II patients between 65 to 85 years of age who underwent total hip-replacement surgery. After obtaining written patient consent, all of the subjects had spinal anesthesia. Exclusion criteria included: Mini-Mental State Examination (MMSE) score <23; history of alcoholism, drug dependence, psychiatric or neurological diseases (AD, stroke and psychosis, etc.); unwillingness to comply with the protocol or procedures; terminal status or inability to understand the language (Mandarin Chinese) used.

Anesthesia and post-operative analgesia

All the participants had spinal anesthesia and received the same postoperative pain control protocol of patient controlled analgesia (PCA, a constant infusion rate of 2 mL/h with a lock time of 15 min) with tramadol 12 mg/kg plus ondansetron 24 mg for two days. Electrocardiogram, pulse oximetry and invasive blood pressure were continuously monitored during anesthesia. The subjective pain scale was assessed with a 10-cm linear visual analogue scale (VAS), where 0 represented "no pain" and 10 represented "most severe pain". Pain scores were determined at one, two, and seven days after surgery.

CSF and plasma samples

Lumbar punctures were performed using a 25-G needle. CSF samples were obtained using lumbar puncture in the L_2–L_3 or L_3–L_4 intervertebral space. After a successful puncture, 3 mL of CSF was obtained and collected in polypropylene tubes and brought to the laboratory immediately. Then 1.2–1.4 mL (a man $\leq$75 years with 1.4 mL, a man >75 years or a woman $\leq$75 years with 1.3 mL, a woman >75 years with 1.2 mL) of 0.75 % bupivacaine was diluted with the same volume of injection water and then was administered into the spinal space via the 25-G needle for spinal anesthesia. The CSF was aliquoted into polypropylene tubes and immediately stored at −80 °C until analysis. The CSF levels of total-tau (Tau), phosphorylated-tau (pTau), Aβ1–42, BDNF, IL-6, and IL-1β were measured using a commercially available sandwich enzyme-linked immunosorbent assay (All kits were provided by Jiancheng Biologic Project Company, Nanjing, China) following the manufacturer's instructions.

The blood samples were collected before induction of anesthesia and seven days after the end of surgery. After centrifuging at 3500*g* for 10 min, the plasma samples were stored at −80 °C for use. Plasma levels of IL-1β, IL-6, BDNF, and CRP were measured by using an enzyme-linked immunosorbent assay (All kits were provided by Dizhao Biologic Project Company, Nanjing, China) following the manufacturer's instructions. Malonaldehyde (MDA) concentrations were determined using enzymatic methods (Jiancheng Biologic Project Company, Nanjing, China).

Cognitive function measurement

Cognitive function was assessed pre-operatively and seven days post-operatively in a quiet room with a Chinese version of a protocol. Briefly, the subjects' attention, sustained attention, and visual scanning ability were measured by the Digit Symbol Substitution Test of the Wechsler Adult Intelligence Scale [9], and 'Concentration Endurance Test d2' tests [10], whereas cognitive processing speed was determined by the 'number connection test' [11]. A decline of more than 10 % in neuropsychological test results was regarded as POCD in the study. Thereafter, the patients were divided into the POCD group and the Non-POCD group according to whether POCD occurred at seven days after the operation.

Statistical analysis

Statistical analysis was performed using the SPSS 16.0 software for Windows (SPSS, Chicago, IL, USA). Data are presented as mean ± SD. Intergroup comparisons were

compared by independent-samples t tests. Intragroup comparisons were analyzed by paired-samples t tests. Categorical variables were analyzed using Chi-square or Fisher exact tests. $P < 0.05$ was considered to be statistically significant.

Results

From May 2011 to January 2012, a total of 83 eligible patients participated in the study. Of these, successful spinal anesthesia was achieved in 79 patients and the other 4 patients received general anesthesia. All the patients underwent total hip-replacement surgery successfully and uneventfully. No patients suffered from major complications such as infection or stroke after surgery during hospitalization in the present study. Eighteen patients were discharged before completing the test for cognitive function at seven days post-operatively, thus only 61 patients completed both the samples collection and neurocognitive tests.

Demographic data of patients were shown in Table 1. There was no significant difference in age, body weight, height, gender, education level, ASA classification, length of surgery and estimated blood loss during surgery between POCD and Non-POCD groups. POCD occurred in 15 patients (15/61, 24.6 %) at seven days after surgery (Table 2). There was no difference in VAS scores at seven days after surgery between the two groups (1.3 ± 0.8 vs. 1.3 ± 0.8) ($P > 0.05$).

Plasma levels of IL-1β were lower while BDNF were higher at seven days after surgery when compared with baseline levels in both POCD and Non-POCD groups ($P < 0.05$) (Fig. 1a, c). There was no significant difference in plasma levels of IL-6 and CRP at seven days after surgery between POCD and Non-POCD groups ($P > 0.05$) (Fig. 1b, e).

Patients with POCD had a significantly higher CSF IL-1β (Fig. 2f), Tau/Aβ1–42 (Fig. 2g), pTau/Aβ1–42 (Fig. 2h), and a lower level of Aβ1–42 (Fig. 2f) when compared with the Non-POCD group ($P < 0.05$; Fig. 2). However, there was no difference in CSF levels of Tau, pTau, BDNF, and IL-6 between POCD and Non-POCD groups ($P > 0.05$) (Fig. 2a–d). On the other hand, POCD patients displayed significantly higher plasma levels of MDA when compared with Non-POCD patients at seven days after surgery ($P < 0.05$) (Fig. 1d).

Table 1 Demographic data in POCD and Non-POCD patients

Admission characteristics	POCD ($n = 15$)	Non-POCD ($n = 46$)
Age (years)	75 ± 6	73 ± 7
Height (cm)	164 ± 9	165 ± 9
Body weight (kg)	60 ± 4	57 ± 5
Gender (M/F)	(5/10)	(13/33)
Education (year)	8.3 ± 1.7	9.2 ± 2.0
ASA classification (I/II)	2/13	5/41
Length of surgery (min)	75 ± 9	73 ± 11
Estimated blood loss (mL)	576 ± 87	544 ± 79
MMSE scores	27.1 ± 2.1	27.4 ± 2.1

Values are mean ± SD. There was no statistical difference with respect to the demographics between the two groups

POCD postoperative cognitive dysfunction, *Non-POCD* non-postoperative cognitive dysfunction, *MMSE* Mini-Mental State Examination

Table 2 Number of patients with 10 % decline in the test battery at 7 days postoperatively

Days after surgery	7
Patients investigated	61
>10 % decline in one test	11
>10 % decline in two tests	4
>10 % decline in three tests	0

Eighteen patients discharged before completing the test for cognitive function at 7 days postoperatively. POCD occurred in 15 patients (15/61, 24.6 %) at 7 days after surgery

Discussion

In this preliminary study, we demonstrated that there were significantly higher IL-1β, Tau/Aβ1–42, pTau/Aβ1–42 and lower Aβ1–42 levels in preoperative CSF in patients with POCD. Moreover, we suggested that POCD patients displayed significantly higher postoperative plasma levels of MDA than Non-POCD patients.

The prevalence of POCD reported in the reports varies widely at seven days postoperatively among studies [3, 6, 12, 13]. The conflicting results may be attributable to different diagnosis criteria for POCD [12]. The incidence of POCD (24.6 %) in the present study at seven days after surgery was higher than one comparable study [7], which may be due to different diagnosis criteria of POCD and different population and surgery type.

Conceptually, POCD can be considered as a disorder situated in the spectrum between normal cognition and dementia, and belongs to mild cognitive impairment [1, 8]. However, the underlying mechanisms leading to POCD remain largely unknown but likely involve a combination of patient, surgical and anesthetic factors [1–5]. A large body of evidence suggests that POCD can be a consequence of neuropathological processes in the brain and can be reflected in the CSF [6–8, 14]. Tau protein is a highly soluble microtubule-associated protein, which is believed to reflect the intensity of the neuronal degeneration [15,

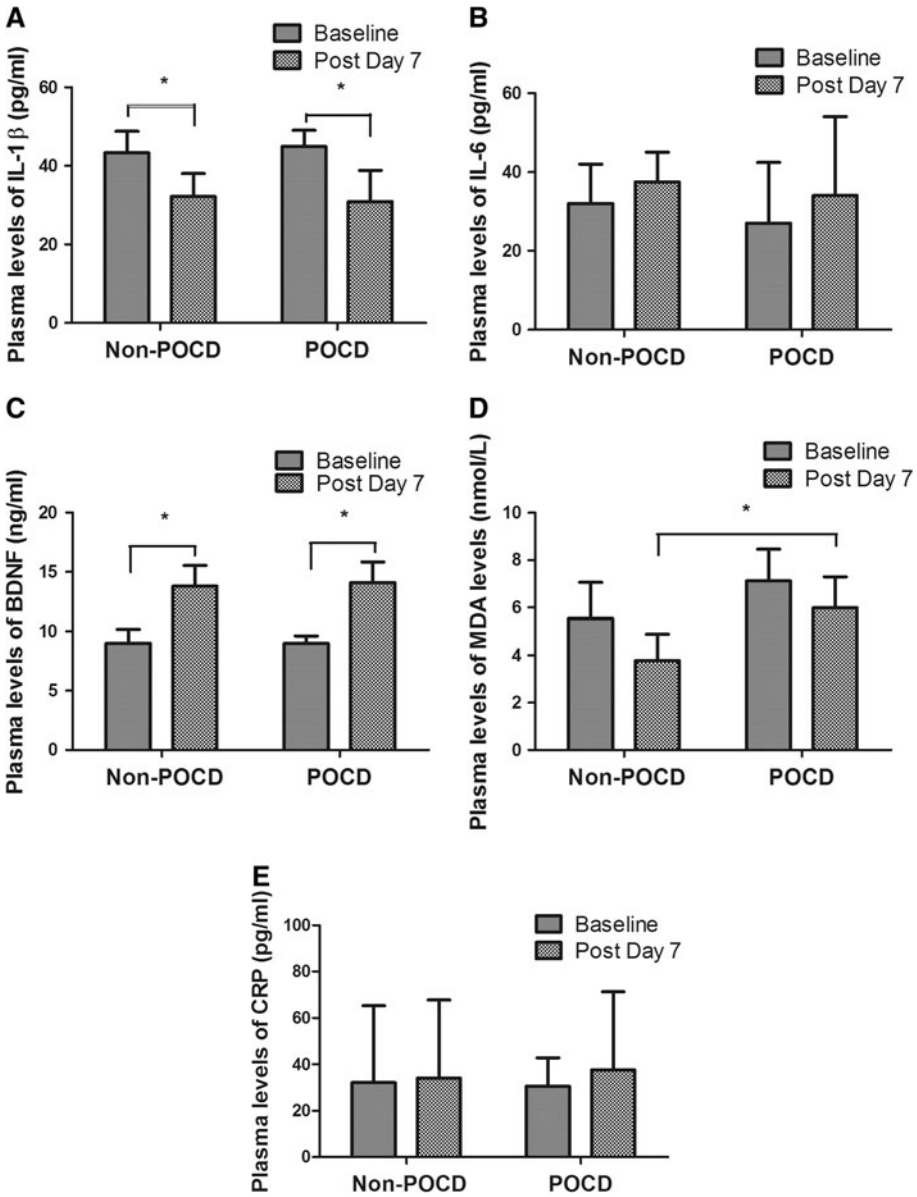

Fig. 1 Plasma levels of IL-1β, IL-6, BDNF, MDA, CRP before and at 7 days after surgery in POCD and Non-POCD Patients. *POCD* post-operative cognitive dysfunction, *Non-POCD* nonpost-operative cognitive dysfunction. *$P < 0.05$, versus baseline

16]. The pTau may indicate the phosphorylation state of the Tau and is involved in the pathogenesis of AD [14–16]. Hence, it is possible that changes in Tau and Aβ1–42 may be related to POCD, which have been suggested as possible diagnostic biomarkers for AD [8]. Furthermore, it has been demonstrated that the predictive value of CSF biomarkers may increase when a combination of Aβ1–42 and Tau (Tau/Aβ1–42) is used [15], with a higher CSF levels in AD patients when compared with normal controls [16]. Notably, Aβ1–42 and pTau have been more specifically implicated in the underlying pathological mechanisms of AD, and the ratio of pTau/Aβ1–42 has been shown to accurately predict the presence or absence of AD [15, 16]. Despite this, there was no difference in preoperative MMSE between POCD and Non-POCD patients in the present study, we found that patients with POCD had significantly higher Tau/Aβ1–42 and pTau/Aβ1–42 as well as lower level of Aβ1–42 in the CSF when compared with Non-POCD patients, a pattern consistent with AD [16]. It is possible that CSF biomarkers reflect neuronal damage even before any cognitive deficits appear, and thus may influence susceptibility to the development of POCD. The results suggested CSF biomarkers that reflect the neuropathological features of AD are also related to POCD in aged patients undergoing total hip-replacement.

The magnitude of the inflammatory response has been implicated as a risk factor for neurocognitive decline after major surgery [17]. Both experimental and clinical studies suggest that increased expression of pro-inflammatory cytokines is associated with cognitive decline after anesthesia and surgery [2, 6]. It has been postulated that a peripheral inflammatory response to a stress may induce

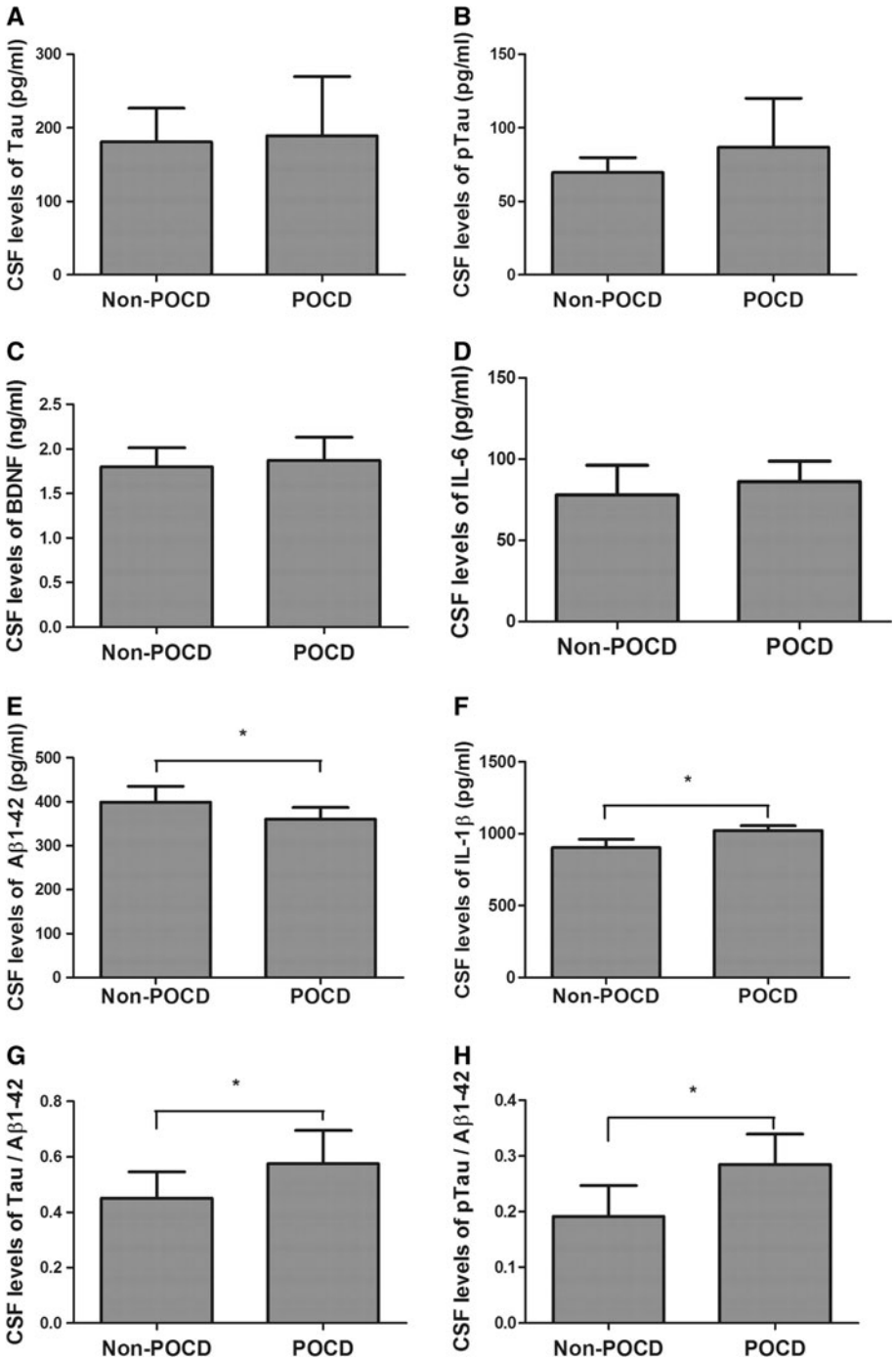

Fig. 2 Preoperative CSF levels of Aβ1–42, Tau, pTau, Tau/Aβ1–42, pTau/Aβ1–42, IL-1β, IL-6, and BDNF between POCD and Non-POCD groups. *POCD* post-operative cognitive dysfunction, *Non-POCD* nonpost-operative cognitive dysfunction.*$P < 0.05$, versus Non-POCD group

neuroinflammation, which can result in cognitive dysfunction via interruption of long-term potentiation in the hippocampus [18]. In the present study, significantly higher preoperative CSF levels of IL-1β were observed in POCD patients than Non-POCD patients, suggesting that increased preoperative CSF IL-1β levels may be an important factor for the development of POCD. However, no difference in plasma IL-1β and IL-6 levels was observed between the two groups. Previously, it has been suggested that inflammatory markers peak within 24 h after surgery and return to baseline levels in 2–4 days [19]. Thus, we might fail to detect the difference in plasma inflammatory mediators at seven days after surgery between the two groups.

On the other hand, a large body of evidence suggests that oxidative stress is related to brain pathology, neurotransmitters release, and thus contributes to cognitive impairment [20]. One recent study suggests that elevated production of lipid peroxidation was associated with poor cognitive performance in AD patients [20]. Consistently,

our studies suggested that MDA was higher in POCD patients than Non-POCD patients, suggesting a role of oxidative stress in POCD. Besides, it has been demonstrated that BDNF plays a key role in neuronal development and synaptic plasticity that underlie circuit formation and cognitive function [21]. In parallel, reduced secretion of BDNF in the central nerve system is one mechanism that contributing to age-related cognitive decline [22]. Therefore, it is possible that BDNF may be evaluated as a diagnostic marker for POCD. However, we did not find any difference in both CSF and plasma levels of BDNF in patients with or without POCD. However, due to the small sample size, whether the occurrence of POCD is associated with BDNF level needs to be further clarified.

It has been suggested that acute postoperative pain is also associated with poorer postoperative cognitive function [23]. This confounding factor can be avoided because all patients received a standard protocol of post operative pain control. However, it must be taken into consideration that this study has a relatively small sample size and short duration of follow-up. Furthermore, as there are no subsequent CSF levels, the relationship between Tau, pTau, Aβ1–42, Tau/Aβ1–42, BDNF and IL-1β and POCD is not established.

Conclusions

Although our results need to be confirmed and extended in future studies, the present study showed that POCD patients were associated with higher postoperative plasma levels of MDA. Furthermore, POCD patients presented higher levels of IL-1β, Tau/Aβ1–42, pTau/Aβ1–42, and lower Aβ1–42 in preoperative CSF following total hip-replacement surgery with spinal anesthesia, such changes may predispose the occurrence of POCD. However, further studies are needed to clarify its role as biomarkers for POCD.

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Conflict of interest The authors have no potential conflicts of interest to disclose.

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