CSF PGRN May be Associated With Postoperative Delirium After Knee Replacement in Elderly Patients: The PNDABLE Study

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Research article

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Abstract

Background

Postoperative delirium (POD) represents a serious complication following anesthesia and surgical procedures for patients undergoing surgical intervention (1). Little is known about the mechanisms underlying similarities in the core features between postoperative delirium (POD) and progranulin (PGRN)-related cognitive disorders. We herein investigated the relationship between preoperative CSF PGRN concentration and POD occurrence in the Han Chinese patients undergoing unilateral total knee arthroplasty.

Methods

We conducted an observational, prospective, and 1:1 matched (on age older than 65, the unilateral total knee arthroplasty, American Society of Anesthesiologist’ (ASA) physical status, duration of surgery, and intraoperative bleeding) case-control study. POD cases and non-POD controls were selected from the overall cohort using Confusion Assessment Method (CAM). Delirium severity was measured by the Memorial Delirium Assessment Scale (MDAS). CSF PGRN and core biomarkers were measured by ELISA using the microplate reader. The associations of CSF PGRN levels with POD risk and CSF core biomarkers were assessed.

Results

POD incidence was 9.7% (53/545). There were significant differences in preoperative CSF PGRN concentration between POD patients and controls (P<0.001), and CSF PGRN levels increased with age, as demonstrated by a significantly positive correlation (r=0.796, P<0.001). CSF PGRN levels to increase with CAM scores and MDAS scores, as demonstrated by significantly positive correlations (r=0.693, P<0.001; r=0.590, P<0.001). There were positive associations of CSF PGRN with T-tau (β = -0.501, P < 0.001) and P-tau (β = -0.470, P < 0.001) and negative associations of CSF PGRN with Aβ1–42 (β = -0.576, P < 0.001), Aβ1–40 (β = -0.488, P < 0.001), Aβ42/p-tau (β = -0.422, P < 0.001), and Aβ42 / T- tau (β = -0.395, P < 0.001) in POD patients. The ROC curve analysis of PGRN showed that PGRN concentrations had high diagnostic value for POD.

Conclusions

CSF PGRN may be associated with the POD. Aβ pathology is associated with a decrease in CSF PGRN in the absence of tau deposition and neurodegeneration, whereas tau pathology and neurodegeneration is associated with an increase in CSF PGRN.

Clinical Trial Registration

www.clinicaltrials.gov, identifier ChiCTR2000033439.

Background

Postoperative delirium (POD) represents a serious complication following anesthesia and surgical procedures for patients undergoing surgical intervention (1). POD is characterized by temporary or permanent cognitive decline, memory impairment, deterioration in language comprehension and social adaptation ability, and POD particularly affects elderly people (> 65 years) (2). POD can lead to increased mortality, prolonged hospitalization, other complications such as Alzheimer’s disease, and higher treatment costs (3). Despite the prevalence and clinical
importance of POD, its pathophysiology is poorly understood and no reliable biomarkers have been reported in previous studies.

PGRN, a multifunctional secretory protein, is a neurotrophic growth factor. It is encoded by a single gene on chromosome 17q21. Composed of 593 amino acids, it is rich in cysteine. And it has a molecular weight of 68.5KDa. The precursor proteins are hydrolyzed by extracellular proteases such as elastin into smaller peptide fragments called GRNs or epithelial proteins (4). These segments range in size from 6 KDa to 25KDa. PGRN exists in a large number of tissues and organs of the human body, mainly in epithelial tissues (5). On the body surface, PGRN is mainly involved in post-injury repair and inflammatory response (6). PGRN produces vascular endothelial factors in skin cancer cells (7). In the central nervous system, PGRN gene mainly exists in specific neurons, including microglial cells, cerebellar Purkinje cells and hippocampal pyramidal neurons, which have the functions of neurotrophy, prolongation of axons, promotion of neuron survival and the proliferation of neural stem cells (8). Studies have shown that PGRN expression is significantly increased during neuroinflammation (9), and PGRN growth in microglia cells may play an important role in brain injury, neuroinflammation and neurodegeneration (10). The increase in PGRN expression in neurodegenerative diseases may be a self-protective mechanism to prevent cell damage. Some studies have found that PGRN protein is closely related to changes in cognitive function (11). POD and AD both belong to neurodegenerative diseases. Therefore, to the best of our knowledge, no previous study has studied the association between CSF PGRN and POD.

Therefore, we speculated that patients with higher preoperative CSF PGRN levels were prone to POD. The main objective of this study was to investigate the associations of preoperative CSF PGRN concentration with POD occurrence and CSF core biomarkers for POD, including \( \text{A}^{\beta}_{1-42}, \text{A}^{\beta}_{1-40}, \text{p-tau} \) and \( \text{T-tau} \).

**Methods**

**PNDABLE study**

The Perioperative Neurocognitive Disorder and Biomarker Lifestyle Study (PNDABLE) is intended to explore the pathogenesis, risk factors and biomarkers of perioperative neurocognitive disorders in the northern Chinese Han population. PNDABLE is aimed to identify lifestyle factors that may affect the risk of PND in the non-demented northern Chinese Han population in order to provide a basis for disease prevention and early diagnosis. This study has important scientific and practical values for establishing the standardized model of early diagnosis and prevention for PND in China. Informed consent was obtained from all the included patients before we extracted preoperative cerebrospinal fluid and blood of the patients. This study has been registered in the Chinese Clinical Trial Registry (clinical registration number ChiCTR2000033439) and approved by the Ethics Committee of Qingdao Municipal Hospital.

**Participants**

The Han Chinese patients undergoing unilateral total knee arthroplasty (no gender limitations, aged 65 ~ 90, weight 50~80 kg, ASA ~ I) combined with epidural anesthesia were enrolled in the PNDABLE study at Qingdao Municipal Hospital (East Hospital) from June 2020 to November 2020. The exclusion criteria include: (1) Preoperative CM-MMSE score < 24 points; (2) A history of neurological and mental diseases such as Alzheimer's disease, Parkinson's disease, and cerebrovascular accident, etc.; (3) Drug or psychotropic substance abuse, as well as long-term use of steroid drugs and hormone drugs; (4) preoperative hepatic encephalopathy; (5) Recent major surgery; (6) Severe visual and hearing impairments; (7) Abnormal coagulation function before surgery.
A total of 600 cognitively normal participants from PNDABLE had available information on covariates. We excluded 25 participants who had no information about CM-MMSE, 5 participants without available CSF PGRN data, 17 participants who had no CSF biomarker data or had data outside four standard deviations (SD) of the mean, and 8 participants whose surgeries were suspended. Finally, 545 participants were included in this analysis and they were divided into two groups according to whether POD occurred or not: POD group and non-POD group. POD cases and non-POD controls were frequency-matched (1:1) on five variables using incidence density sampling. Specifically, one non-delirium control was randomly selected for each POD case from the source population according to the five matched variables, including age, diagnosis, American Society of Anesthesiologist’ (ASA) physical status, duration of surgery, and intraoperative blood loss. These variables were listed in the European Society of Anesthesiology evidence-based and consensus-based guideline on POD and were considered to be risk factors for POD after hip fracture surgery (Aldecoa et al., 2017). A patient recruitment flowchart is shown in Fig. 1.

The participants did not receive preoperative medications, and they were instructed not to drink for 6 h and not to eat for 8 h before surgery. After entering the operating room, we routinely monitored ECG, SpO\textsubscript{2} and NBP, opened vein access and extracted 3 ml of whole venous blood. All patients underwent combined spinal-epidural block, with the space between lumbar 3–4 spinous processes (L3-4) as the puncture site. After successful puncture, 2 ml of cerebrospinal fluid was extracted from the subarachnoid space, followed by injection of 2-2.5 ml ropivacaine (0.66%) for about 30 s. After anesthesia, the sensory level was controlled below the T8 level. During the surgery, oxygen was inhaled via mask at 5L/min to maintain blood pressure within ± 80% of the baseline value. If intraoperative NBP < 90 mmHg (1mmHg = 0.133 kPa) or it decreased by more than 20% of the baseline value, ephedrine 5 mg was injected intravenously. If HR < 50 beats/min, atropine 0.5 mg was injected intravenously. Intravenous patient-controlled analgesia (butorphanol 0.1 mg/ml + tropisetron 50 g/ml, diluted with normal saline to a total volume of 100 ml) was used in acute postoperative pain management. After the operation, the patient was sent to the anesthesia resuscitation room (PACU). If no abnormalities were found during a 30-minute observation period, then the patient could return to the ward with low-flow oxygen and continuous monitoring of vital signs.

We interviewed all the patients the day before surgery and collected their baseline data, including age, gender, body mass index (BMI), ASA physical status, years of education, as well as CM-MMSE, CAM and Memorial Delirium Assessment Scale (MDAS) scores. Other information including comorbidities, past medical history, fracture classification, types of anesthesia and surgery, and time from injury to operation were also collected according to the patients’ medical records. All the history collection, physical evaluation and cognitive assessment related to dementia were conducted by neurologists.

**CSF core biomarker and CSF PGRN measurements**

CSF samples were processed immediately within 2 h after standard lumbar puncture. Each sample was centrifuged at 2000 × g for 10 min, and CSF samples were separated and stored in an enzyme-free EP (Eppendorf) tube (AXYGEN; PCR-02-C) at − 80 °C for further use in the subsequent steps of this study. The samples were subjected to a maximum of two freeze-thaw cycles.

CSF PGRN and core biomarkers were measured by ELISA using the microplate reader (X) (Thermo Scientific Multiskan MK3). CSF PGRN measurements were done with ELISA kits (Human PGRN SimpleStep ELISA kit; BioVendor, no. RMEE103R) and CSF core biomarker measurements were done with other ELISA kits (INNOTEST; FUJIREBIO). All ELISA measurements were performed by experienced technicians in strict accordance with the manufacturer's instructions. They were blinded to the clinical information. The samples and standards were measured in duplicate, and the means of duplicates were used for the statistical analyses. All the antibodies and
plates were from a single lot to exclude variability between batches. Moreover, the within-batch CV was < 5% and the inter-batch CV was < 15%.

**Neuropsychological tests**

The Chinese-Modified Mini-Mental State Examination (CM-MMSE) was completed by neurologists 1d before surgery to assess the preoperative cognitive status and record relevant medical history. Patients whose MMSE scores < 23 points were excluded. Participants received interview preoperatively and in PACU, on the first, second, third and seventh (or before discharge) postoperative days. The assessment of delirium was performed in PACU, on the first, second, third and seventh days (or before discharge) after surgery between 9:00 am and 11:00 am by neurologists. We used the visual analog scale (VAS) score of 0–10 (lower scores indicating lower levels of pain (12)) to assess pain at the same time. POD was defined by the Confusion Assessment Method (CAM) (13), and POD severity was measured using the Memorial Delirium Assessment Scale (MDAS) (14). The Chinese versions of CAM and MDAS have been proven to have good reliability and validity in the Chinese elderly population [15, 16]. Therefore, CAM-positive and MDA-positive patients postoperatively in PACU and on the first, second, third and seventh days (or before discharge) were recorded.

**Statistical analysis**

The scheme comprises 4 biomarkers: aggregated $A\beta_1$-$42$, $A\beta_1$-$40$, aggregated P-tau and neurodegeneration (T-tau). And each biomarker is binarized based on whether they are normal or abnormal.

CSF PGRN didn't follow a normal distribution as assessed by Kolmogorov-Smirnov test ($P < 0.001$) and visual inspection of the Q-Q plot (Fig. 1S). Therefore, they were log-transformed to obtain a normal distribution. All the statistical analyses described in this study are performed on the log10-transformed values. We performed the analysis after excluding outliers (defined as 4 SD below or above the group mean) in order to exclude the influence of extreme values. Two independent-samples’ t tests were used for the comparisons between POD and NPOD groups. We used the Correlation analysis to explore whether CSF PGRN is related to CAM score and MDAS score. Given the different trends of PGRN at different ages in the biomarker framework, we applied a one-way ANCOVA followed by Bonferroni post hoc analyses.

We also studied the associations between CSF PGRN and the CSF core biomarkers for POD, using a multiple linear regression adjusted for age, gender, years of education, and APOE $\varepsilon$4 carrier status. The analyses were performed in the total sample and then in subgroups stratified by age, gender, years of education and APOE $\varepsilon$4 carrier status.

ROC curve analysis was used to evaluate the clinical diagnostic value of PGRN in POD. Statistical significance was set at $P < 0.05$. SPSS statistical software, version 21.0 (SPSS, Inc. Chicago, IL, USA), and GraphPad Prism software, version 6.01 (GraphPad Software, Inc., La Jolla, CA, USA), were used for data analysis.

**Results**

**Participant characteristics**

A total of 600 Han Chinese patients over the age of 65 who underwent unilateral total knee arthroplasty were included in the PNDABLE study from January 2018 to January 2020. The reasons for dropping out are shown in Fig. 1. And 545 patients ($n = 545$) remained for analyses. We found the incidence of POD was 9.7% ($n = 53$ of the 545 patients) via our postoperative assessments. Another 53 non-POD patients were also enrolled in this study (Fig. 1).
In this study, we found patients in the POD group had higher CAM and MDAS scores than the NPOD group. The preoperative MMSE score showed no significant difference between the POD group [28(26–29)] and the NPOD group [28(27-29.5), P = 0.330]. Postoperatively, the VAS score did not differ between patients with delirium 2(1−3) and without delirium [2(1–3), P = 0.080]. The demographic and clinical data of the participants are summarized in Table 1.
Table 1
Comparison of general condition and surgical condition, CFS biomarkers of unilateral total knee arthroplasty patients.

<table>
<thead>
<tr>
<th>Variable</th>
<th>POD (N = 53)</th>
<th>Non-POD (N = 53)</th>
<th>P-values</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (year) (mean ± SD)</td>
<td>73.87 ± 6.91</td>
<td>70.43 ± 5.65</td>
<td>0.006</td>
</tr>
<tr>
<td>Gender (female/male)</td>
<td>22/31</td>
<td>20/33</td>
<td>0.421</td>
</tr>
<tr>
<td>Body mass index (kg.m(^{-2})) (mean ± SD)</td>
<td>24.8 ± 3.6</td>
<td>25.7 ± 3.4</td>
<td>0.187</td>
</tr>
<tr>
<td>Education level (year) (median and 25–75 percentile)</td>
<td>9(6-13.5)</td>
<td>12(9-14)</td>
<td>0.326</td>
</tr>
<tr>
<td>ASA physical status (I/II)</td>
<td>27/26</td>
<td>28/25</td>
<td>0.846</td>
</tr>
<tr>
<td>APOE ε4 carriers (%)</td>
<td>7(13)</td>
<td>9(17)</td>
<td>0.587</td>
</tr>
<tr>
<td>Preoperative serum cholesterol (mean ± SD)</td>
<td>4.89 ± 0.97</td>
<td>4.62 ± 0.97</td>
<td>0.398</td>
</tr>
<tr>
<td>Preoperative serum high density lipoprotein (mean ± SD)</td>
<td>1.16 ± 0.23</td>
<td>1.15 ± 0.23</td>
<td>0.798</td>
</tr>
<tr>
<td>Preoperative serum low density lipoprotein (mean ± SD)</td>
<td>2.93 ± 0.65</td>
<td>2.72 ± 0.58</td>
<td>0.200</td>
</tr>
<tr>
<td>Preoperative CFS A(\beta)(_{1-42}) (pg•ml(^{-1})) (mean ± SD)</td>
<td>233.98 ± 135.49</td>
<td>300.57 ± 99.39</td>
<td>0.013</td>
</tr>
<tr>
<td>Preoperative CFS A(\beta)(_{1-40}) (pg•ml(^{-1})) (mean ± SD)</td>
<td>3437.85 ± 2028.87</td>
<td>2645.54 ± 924.07</td>
<td>0.011</td>
</tr>
<tr>
<td>Preoperative CFS T-tau (pg•ml(^{-1})) (mean ± SD)</td>
<td>314.49 ± 206.41</td>
<td>119.33 ± 55.67</td>
<td>0.003</td>
</tr>
<tr>
<td>Preoperative CFS P-tau (pg•ml(^{-1})) (mean ± SD)</td>
<td>130.47 ± 51.15</td>
<td>69.03 ± 29.01</td>
<td>0.001</td>
</tr>
<tr>
<td>Preoperative CFS A(\beta)(_{1-42}) / T-tau (median and 25–75 percentile)</td>
<td>0.64(0.22–2.62)</td>
<td>2.99(1.66–4.13)</td>
<td>0.001</td>
</tr>
<tr>
<td>Preoperative CFS A(\beta)(_{1-42}) / P-tau (median and 25–75 percentile)</td>
<td>1.44(0.63–4.53)</td>
<td>4.79(3.04–6.88)</td>
<td>0.001</td>
</tr>
<tr>
<td>Preoperative CFS A(\beta)(_{1-40}) / T-tau (median and 25–75 percentile)</td>
<td>11.49(3.57–29.79)</td>
<td>25.06(17.18–35.27)</td>
<td>0.002</td>
</tr>
<tr>
<td>Preoperative CFS A(\beta)(_{1-40}) / P-tau (median and 25–75 percentile)</td>
<td>18.56(10.56–54.89)</td>
<td>36.25(24.62–68.35)</td>
<td>0.003</td>
</tr>
<tr>
<td>Preoperative CFS PGRN (pg•ml(^{-1})) (mean ± SD)</td>
<td>2717.23 ± 873.37</td>
<td>3749.06 ± 1004.45</td>
<td>0.001</td>
</tr>
<tr>
<td>Preoperative MMSE scores (median and 25–75 percentile)</td>
<td>28(26–29)</td>
<td>28(27-29.5)</td>
<td>0.330</td>
</tr>
<tr>
<td>Time from injury to operation (h) (mean ± SD)</td>
<td>48.77 ± 9.54</td>
<td>52.13 ± 10.45</td>
<td>0.087</td>
</tr>
<tr>
<td>Duration of anesthesia (min) (mean ± SD)</td>
<td>133.97 ± 26.5</td>
<td>141.25 ± 30.1</td>
<td>0.963</td>
</tr>
<tr>
<td>Duration of surgery (min) (mean ± SD)</td>
<td>125.72 ± 25.13</td>
<td>129.47 ± 26.32</td>
<td>0.455</td>
</tr>
</tbody>
</table>

The categorical variables were expressed as counts. Normal data are given as mean ± SD, whereas non-normal data are expressed as median and 25–75 percentile. Abbreviations: POD, postoperative delirium; MMSE, mini-mental state examination; ASA, American Society of Anesthesiologists; MDAS, memorial delirium assessment scale; VAS, Visual Analogue Scale/Score; SD, standard deviation; CSF, cerebrospinal fluid; A\(\beta\)\(_{1-42}\), amyloid-\(\beta\)\(_{1–42}\); A\(\beta\)\(_{1-40}\), amyloid-\(\beta\)\(_{1–40}\); T-tau, total tau; P-tau, phosphorylated tau; PGRN, Progranulin.
<table>
<thead>
<tr>
<th>Variable</th>
<th>POD(N = 53)</th>
<th>Non-POD(N = 53)</th>
<th>P-values</th>
</tr>
</thead>
<tbody>
<tr>
<td>Transfusion amount (ml) ( mean ± SD)</td>
<td>582.44 ± 148.65</td>
<td>603.91 ± 152.77</td>
<td>0.465</td>
</tr>
<tr>
<td>Postoperative the highest CAM score ( mean ± SD)</td>
<td>31.81 ± 6.18</td>
<td>14.4 ± 2.66</td>
<td>0.001</td>
</tr>
<tr>
<td>Postoperative the highest MDAS score( mean ± SD)</td>
<td>22.75 ± 5.02</td>
<td>5.62 ± 2.43</td>
<td>0.001</td>
</tr>
<tr>
<td>Postoperative the highest VAS score (median and 25–75 percentile)</td>
<td>2(1–3)</td>
<td>2(1–3)</td>
<td>0.080</td>
</tr>
</tbody>
</table>

The categorical variables were expressed as counts. Normal data are given as mean ± SD, whereas non-normal data are expressed as median and 25–75 percentile. Abbreviations: POD, postoperative delirium; MMSE, mini-mental state examination; ASA, American Society of Anesthesiologists; MDAS, memorial delirium assessment scale; VAS, Visual Analogue Scale/Score; SD, standard deviation; CSF, cerebrospinal fluid; Aβ1-42, amyloid-β1–42; Aβ1–40, amyloid-β1–40; T-tau, total tau; P-tau, phosphorylated tau; PGRN, Progranulin

CSF PGRN Concentration

In this study, there were significant differences in preoperative CSF PGRN concentration between POD and NPOD groups (P < 0.001, Table 1). Besides, univariate logistic analysis (adjusted for age, gender, years of education, and APOEε4 carrier status) showed that PGRN was an independent risk factor for POD in elderly patients undergoing unilateral total knee arthroplasty (OR = 1.001, 95% CI 1.001–1.002, P < 0.001, Table 2).

<table>
<thead>
<tr>
<th>Variable</th>
<th>Unadjusted</th>
<th>Adjusted</th>
</tr>
</thead>
<tbody>
<tr>
<td>Preoperative CFS PGRN</td>
<td>0.999(0.998–0.999)</td>
<td>0.998(0.997–0.999)</td>
</tr>
<tr>
<td>Preoperative CFS Aβ1-40</td>
<td>1.000(1.000-1.001)</td>
<td>1.001(1.000-1.001)</td>
</tr>
<tr>
<td>Preoperative CFS Aβ1-42</td>
<td>0.995( 0.992–0.999)</td>
<td>0.995( 0.991–0.999)</td>
</tr>
<tr>
<td>Preoperative CFS T-tau</td>
<td>1.012(1.007–1.018)</td>
<td>1.012(1.009–1.022)</td>
</tr>
<tr>
<td>Preoperative CFS P-tau</td>
<td>1.033(1.020–1.047)</td>
<td>1.043(1.027–1.060)</td>
</tr>
<tr>
<td>Preoperative CFS Aβ1-42 / T-tau</td>
<td>0.660(0.523–0.832)</td>
<td>0.633(0.490–0.817)</td>
</tr>
<tr>
<td>Preoperative CFSAβ1-42 /P-tau</td>
<td>0.776(0.672–0.895)</td>
<td>0.765(0.654–0.896)</td>
</tr>
<tr>
<td>Preoperative CFS Aβ1-40 / T-tau</td>
<td>0.998(0.984–1.012)</td>
<td>1.001(0.985–1.016)</td>
</tr>
<tr>
<td>Preoperative CFS Aβ1-40 /P-tau</td>
<td>0.996(0.989–1.004)</td>
<td>0.998(0.990–1.007)</td>
</tr>
</tbody>
</table>

Since age is the main risk factor for POD, we explored whether CSF PGRN levels were related to aging. We found PGRN levels increased with age, as demonstrated by a significantly positive correlation (r = 0.796, P < 0.001). The results indicated that CSF PGRN did differ significantly between different age subgroups (65–70 years: 2189 ±
119 pg/ml, n = 26, P = 0.001; 65–70 years: 5206 ± 408 pg/ml, n = 18, P = 0.001; >80 years: 7563 ± 502 pg/ml, n = 9; P = 0.037) (Fig. 2).

We found PGRN levels increased with CAM and MDAS scores, as demonstrated by significantly positive correlations (r = 0.781, P < 0.001; r = 0.524, P < 0.001) (Fig. 3).

**Differences in CSF PGRN level between different subgroups stratified by biomarkers**

The associations between CSF PGRN and CSF core biomarkers for POD were tested in linear regression models adjusted for age, gender, years of education and APOE ε4 carrier status. In the whole sample of subjects (n = 659), increased CSF PGRN was associated with lower levels of Aβ1−42 (β = -0.644, P < 0.001), Aβ1−40 (β = 0.275, P = 0.017), Aβ42/p-tau (β = -0.035, P < 0.001) and Aβ42/T-tau (β = -0.073, P < 0.001), as well as higher levels of T-tau (β = 0.557, P < 0.001) and P-tau (β = 0.502, P < 0.001) (Fig. 4).

There were positive associations of CSF PGRN with T-tau (β = -0.501, P < 0.001) and P-tau (β = -0.470, P < 0.001) and negative associations of CSF PGRN with Aβ1−42 (β = -0.576, P < 0.001), Aβ1−40 (β = -0.488, P < 0.001), Aβ42/p-tau (β = -0.422, P < 0.001) and Aβ42/T-tau (β = -0.395, P < 0.001) in POD patients (n = 53) (Fig. 5).

In the NPOD group (n = 53), we found a positive association between CSF PGRN and T-tau (β = 0.495, P < 0.001) and negative associations of CSF PGRN with Aβ1−42 (β = -0.364, P < 0.001), Aβ42/p-tau (β = -0.019, P < 0.001) and Aβ42/T-tau (β = -0.050, P < 0.001), whereas no significant association was found of CSF sTREM2 with p-tau (β = 0.181, P = 0.069) or Aβ1−40 (β = 0.001, P = 0.998) (Fig. 6).

We then calculated the ratios between CSF amyloid and tau biomarkers, and found no associations of CSF PGRN with CSF Aβ1−40/T-tau or Aβ1−40/p-tau (Table 2). Outliers were excluded in our analyses, but we obtained similar results when those were included. These findings indicate that higher CSF PGRN correlates with lower levels of Aβ and higher levels of tau.

**Receiver operating characteristic (ROC) curve analysis of PGRN in CSF**

The ROC curve analysis of PGRN showed that PGRN concentration had high diagnostic value for POD, with all the AUC greater than 0.5 and close to 1.0. (Table 3, Fig. 7).

**Table 3**

The ROC curve analysis of PGRN showed that the concentrations of CSF s PGRN had high diagnostic value for POD

<table>
<thead>
<tr>
<th>CSF’s index</th>
<th>AUC</th>
<th>95%CI(L)</th>
<th>95%CI(U)</th>
<th>Youden’s index</th>
<th>Sensitivity</th>
<th>Specificity</th>
</tr>
</thead>
<tbody>
<tr>
<td>PGRN ((pg•ml⁻¹)</td>
<td>0.795</td>
<td>0.706</td>
<td>0.867</td>
<td>0.528</td>
<td>58.5</td>
<td>94.3</td>
</tr>
</tbody>
</table>

**Discussion**

The incidence of POD in our study was 9.7%, which was consistent with the previous results of 3.6–41% [17]. For example, previous studies have shown that the incidence of POD after the total knee and hip replacement under spine anesthesia is 20% [18]. There is still a great deal of controversy about the pathogenesis of POD. At present, there are several assumptions including cholinergic theory, inflammatory reaction and stress-response theory. There are many risk factors for POD, such as advanced age, preexisting cognitive decline, blood loss and blood transfusion,
anesthetic medications, as well as postoperative pain, etc. [19]. Therefore, our study adopted CAM and MDAS to improve the accuracy of our assessments of POD.

In the present study, we combined biomarker-based classification with age to assess changes in CSF PGRN (a marker for microglial activity) in POD patients. The application of this classification system enabled us to explore the associations between microglial inflammatory response and the pathophysiology of POD (including Aβ pathology, tau pathology, and neurodegeneration). Our study showed that CSF PGRN levels did change dynamically with aging. Aβ pathology (defined as low CSF Aβ1−42 and Aβ1−40) was associated with an increase in CSF PGRN, while tau pathology or neurodegeneration was associated with elevated CSF PGRN. This seems to confirm the potential role of microglial inflammatory response in the pathogenesis of POD. Our results support the hypothesis that Aβ deposition occurs independently of the inflammatory process, but the type and extent of the inflammatory response to Aβ deposition in the brain may trigger or affect subsequent neurodegeneration. Another piece of evidence is that immunotherapy against amyloid can reduce downstream neurodegeneration, a process that may be mediated by changes in microglial activation [20].

In this study, we explored the associations between CSF PGRN and CSF biomarkers for POD to further clarify the pathogenesis of POD and provide theoretical basis for early warning and intervention of POD. Our results showed that in the entire data set and in POD, CSF PGRN was positively correlated with T-tau, P-tau, Aβ42/T-tau and Aβ42/ p-tau, as well as negatively correlated with Aβ1−42 or Aβ1−40, further suggesting that POD was related to reactive microglia proliferation. The underlying mechanism of CSF PGRN remains to be investigated throughout the disease. In NPOD subjects, the correlations of PGRN with Aβ40 and T-tau disappeared, while the correlation of PGRN with p-tau remained. These findings suggest that CSF PGRN may indeed be associated with neuronal injury. It is also suggested that increased CSF PGRN in NPOD patients may be a protective response to mild neuronal injury.

Progranulin (PGRN) is a multifunctional growth factor expressed in a variety of tissues and involved in many physiological and pathological processes [21]. It is widely expressed in various cells of the body. Some studies have found that PGRN is highly expressed in neurons and microglia cells [22]. Little was previously known about the role of PGRN in the nervous system, but since the discovery of PGRN genetic polymorphisms, the number of studies on the role of PGRN in the brain has increased rapidly. Studies have found that PGRN can activate microglia cells and stimulate them to engulf the toxic Aβ around them, which exerts neuroprotective effects [23]. Other studies have found that the content of PGRN in microglia cells which are around Aβ deposits increases in mice [24]. Neurofibrillary tangles are one of the main pathological features of AD, which are closely related to two major proteins – Tau protein and CDK5 [25]. Tau protein is found throughout the nervous system, and its hyperphosphorylation is one of the early cytoskeletal changes during the formation of NFT [26]. Generally speaking, Tau protein is modified by 2–3 phosphate groups. The phosphorylation and dephosphorylation of tau protein maintain a dynamic balance, maintaining the stability of cytoskeleton [27]. In the pathological state of AD, Tau protein has 9–10 phosphate groups, leading to its hyperphosphorylation and the formation of NFT [28]. Hyperphosphorylated tau protein loses its original functions and cannot promote microtubule focusing and maintain cytoskeleton stability [29]. Moreover, hyperphosphorylated Tau protein competes with normal Tau protein to bind microtubules, resulting in an increase in hyperphosphorylated Tau protein and a decrease in normal Tau protein. CDK5, also known as Tau kinase, mainly regulates tau phosphorylation. CDK5 has been shown to be closely associated with neurodegenerative diseases [30]. In the pathological process of AD, the increased expression of CDK5 not only directly aggravates Tau hyperphosphorylation, but also plays a role in regulating phosphatases or kinases of Tau protein. Studies have found that when PGRN is upregulated, it activates central cyclin-dependent kinase (CDK), which leads to reduced clearance
of toxic Aβ and oxidative stress [31]. Neurofibrillary tangles and neuronal loss caused by Tau hyperphosphorylation lead to cognitive dysfunction [32].

The ROC curve analysis showed that PGRN concentrations had the greatest diagnostic value. Therefore, high PGRN concentrations can predict the occurrence and development of POD before surgery. Increased CSF PGRN and its effects have been observed in the brains of AD patients and AD model mice. Increased expression of PGRN in microglia cells around amyloid plaques is a self-protective mechanism to prevent cell damage, which offers prospects for the application of CSF PGRN as a biomarker for patients with cognitive dysfunction. Therefore, it is the future direction of our research to replicate our findings in animal experiments and explore the relevant mechanisms.

Our investigation had two limitations. Firstly, this is a cross-sectional study that limits any conclusions about disease progression. Therefore, results should be replicated in subjects with longitudinal data to analyze whether CSF PGRN levels are associated with disease progression. Second, cerebrospinal fluid collection is an invasive procedure. Monitoring the concentration of PGRN in the plasma of patients will make clinical examination more convenient. This project will monitor the progression of the disease by measuring the changes of PGRN concentration in the peripheral blood through large-scale clinical studies.

**Conclusion**

In conclusion, this study is based on an independent cohort. The results indicate that the occurrence and development of cognitive dysfunction in elderly patients after unilateral total knee arthroplasty may be related to the increased expression of PGRN in CSF, and the concentration of PGRN in CSF increases with age. Aβ pathology is associated with a decrease in CSF PGRN in the absence of tau deposition and neurodegeneration, whereas tau pathology and neurodegeneration are associated with an increase in CSF PGRN. Future studies should use CSF biomarkers to further explore the biological mechanisms underlying POD.

**Abbreviations**

PGRN, progranulin ; AD, Alzheimer's disease; POD, postoperative delirium; NPOD, no postoperative delirium ; CSF, cerebrospinalfluid; ROC, receiver operating characteristic curve ; CDK, cyclin-dependent kinase ; NFT, nerve fiber twineing ; Aβ, β-amyloid ; CAM, Confusion Assessment Method ; MDAS, Modified Dental Anxiety Scale ; ELISA, enzyme linked immunosorbent assay ; MMSE, Mini-mental State Examination ; PACU, postanesthesiacarunit ; ASA, American Society of Anesthesiologist

**Declarations**

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**Authors' contributions**

YLB and YQL conceived and designed the current study and drafted the manuscript. HT, XYD and FHL performed the experiments at the physical laboratory of Qingdao University, China. RD, XL, XJS and BW analyzed data. XL, RD and BW performed the experiments, wrote and revised the manuscript. All authors have contributed to the manuscript.
revising and editing critically for important intellectual content and given final approval of the version and agreed to be accountable for all aspects of the work presented here. All authors read and approved the final manuscript.

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**Availability of data and materials**

The datasets used and/or analyzed during the current study are available from the corresponding author on reasonable request.

**Ethics approval and consent to participate**

The present study followed the recommendations of the National Institute of Health guidelines for the care and use of laboratory animals and obtained approval from the Clinical Trial Ethics Committee of Qingdao Municipal Hospital, Qingdao, China.

**Consent for publication**

Not applicable.

**Competing interests**

The authors declare that they have no competing interests with respect to the research, authorship, and/or publication of this article.

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Figures
Figure 1

Flow diagram showed selection of eligible patients and the enrollment process.
Figure 2

CSF PGRN levels are associated with different ages. P-values were assessed by student's t test.
The POD Patients

Figure 5

Associations of CSF PGRN and CSF core biomarkers. Scatter plots represent the associations of CSF PGRN with CSF core biomarkers: Aβ1–42, Aβ1–40, T-tau, p-tau, Aβ42/p-tau and Aβ42/T-tau in NPOD groups. The normalized regression coefficients (β) and P values computed by multiple linear regression after adjustment for age, gender, educational level, and APOE ε4 carrier status are shown.
Figure 6

Associations of CSF PGRN and CSF core biomarkers. Scatter plots represent the associations of CSF PGRN with CSF core biomarkers: Aβ1–42, Aβ1–40, T-tau, p-tau, Aβ42/p-tau and Aβ42 / T-tau in POD patient groups. The normalized regression coefficients (β) and P values computed by multiple linear regression after adjustment for age, gender, educational level, and APOE ε4 carrier status are shown.

Supplementary Files

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