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## Preliminary study of serum biomarkers associated with delirium after major cardiac surgery

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### Abstract

**Objectives:** The objective of this study was to identify novel serum biomarkers specific to postoperative delirium following major cardiac surgery to provide insight into the pathological processes involved in delirium and its sequelae.

**Design:** Nested, case-control study.

**Setting:** Cardiac surgical intensive care unit in a single-site hospital setting.

**Participants:** Twenty-four older adults (>60 years old) undergoing major cardiac surgery with cardiopulmonary bypass.

**Interventions:** None.

**Measurements and Main Results:** The primary outcome was a positive screen for delirium from postoperative day 1 through 3 based on criteria included in the long form of the Confusion Assessment Method. We applied a multiplexed proteomic approach using proximity extension assays to identify and quantify proteins found in serum collected on the day of surgery and postoperative day 1 in delirious and non-delirious patient cohorts. We identified an increase in serum fibroblast growth factor (FGF)-21 levels in the delirious cohort from a pre-surgery baseline of (mean  $\pm$ stdev) 5.0 ( $\pm$ 1.1) log<sub>2</sub> abundance (95% confidence interval (CI) [4.3 to 5.7]) to 6.7 ( $\pm$ 1.6) log<sub>2</sub> abundance (95% CI [5.7 to 7.7],  $p=0.01$ ) post-surgery. We identified a similar increase in FGF-23 from a pre-surgery baseline of 1.7 ( $\pm$ 1.3) log<sub>2</sub> abundance (95% CI [0.8 to 2.5]) to 3.4 ( $\pm$ 2.2) log<sub>2</sub> abundance (95% CI [2.0 to 4.8]),  $p=0.06$ ) post-surgery. We also identified an increase

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#### Author Contributions

TBM analyzed data and wrote the paper; JR and JQ conceptualized the study, aided in study design, and reviewed the paper; KC, KA, and IT collected clinical data and processed blood samples; AM reviewed the paper and statistical analyses; OA conceptualized the study, acquired funding, analyzed data, and wrote the paper.

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#### Declaration of Competing Interest

The authors declare no conflict of interest.

in interleukin-6 serum levels in the delirious cohort from a pre-surgery baseline of 3.8 ( $\pm$ 1.1) log<sub>2</sub> abundance (95% CI [3.1 to 4.5]) to 8.7 ( $\pm$ 1.9) log<sub>2</sub> abundance (95% CI [7.5 to 9.9],  $p$ <0.0001) post-surgery. However, the increase in interleukin-6 serum levels of the non-delirious cohort also met our threshold for statistical significance ( $p$ <0.0001). Finally, we identified an increase in monocyte chemotactic protein-3 serum levels in the delirious cohort from a pre-surgery baseline of 4.1 ( $\pm$ 0.9) log<sub>2</sub> abundance (95% CI [3.6 to 4.7]) to 6.1 ( $\pm$ 2.0) log<sub>2</sub> abundance (95% CI [4.8 to 7.4],  $p$ =0.009) post-surgery.

**Conclusion:** FGF-21, FGF-23, interleukin-6, and monocyte chemotactic protein-3 serum levels were increased postoperatively in patients that developed delirium following major cardiac surgery. This study identifies two members of the FGF family as potential putative systemic biomarkers for postoperative delirium after cardiac surgery, suggesting a possible role for metabolic recovery in the pathophysiological mechanisms underlying neurocognitive dysfunction.

### Keywords

Delirium; FGF; neurodegeneration; cognition; metabolism; cardiac surgery; cardiopulmonary bypass; inflammation

### Introduction

Delirium is an acute brain dysfunction characterized by disturbances in attention, awareness, and cognition not explained by a preexisting neurocognitive disorder.<sup>1</sup> Delirium disproportionately affects older individuals (>65 years) and is associated with increased mortality, prolonged hospitalization, and long-term cognitive deficits. In the United States alone, an estimated \$39.2 billion dollars (95% confidence interval (CI) [\$25.7 billion-\$42.2 billion]) are spent on healthcare costs associated with postoperative delirium.<sup>2</sup> Older patients account for nearly double the number of hospitalization stays compared to younger individuals (45–64 years)<sup>3</sup> and are at increased risk for developing delirium while in the hospital.<sup>4</sup>

Metabolic disturbance, organ failure, sleep disturbance, hyperthermia, and central nervous system insults are a few of the etiological factors that may contribute to developing delirium.<sup>5, 6</sup> Delirium commonly occurs after critical illness, surgery, and invasive diagnostic procedures and is associated with increased mortality, prolonged hospitalization, long-term cognitive deficits, and higher healthcare costs.<sup>7, 8</sup> In terms of mechanism, systemic activation of the inflammatory cascade involving interleukin-6 (IL-6) and C-reactive protein have been associated with delirium in humans.<sup>9, 10</sup> While blood-brain barrier breakdown and microglial activation appear to be involved in neurocognitive dysfunction,<sup>11, 12</sup> the pathophysiological mechanisms underlying delirium in the context of the aging brain and the response to the physiological and biological changes associated with surgery and invasive diagnostic procedures remain unclear. Thus, our ability to modify the public health burden of postoperative delirium is presently limited by the lack of objective biomarkers that can be related to delirium onset or its sequelae. The discovery of systemic biomarkers of delirium may provide a defined biochemical signature associated with delirium progression, resolution, and/or response to treatment (e.g., prognostic biomarkers) or as factors correlated with delirium onset (e.g., 'disease' biomarkers). We hypothesize that development of

delirium may be associated with fluctuations in systemic factors that can be detected in serum and relate to the underlying pathophysiology involved in neurocognitive dysfunction. The identification of prognostic or predictive biomarkers of delirium may aid in identifying patients that are at high-risk for developing postoperative delirium, independent of age, as a means to personalize postoperative care and develop therapeutic interventions to improve patient-centered outcomes.

Here, we performed a nested case-control study of 24 patients to identify proteins found in serum that might provide insights into the underlying pathological processes involved in delirium and potentially serve as prognostic or diagnostic biomarkers.

## Methods

### Study Design

This nested, case-control study involves an unbiased proteomic screen of serum from age- and sex-matched non-delirious control and delirious patients, as an approved substudy of the Minimizing ICU Neurological Dysfunction with Dexmedetomidine-induced Sleep (MINDDS) trial.<sup>13</sup> All recruited patients provided written and informed consent prior to enrollment in the MINDDS study in accordance with institutional and federal guidelines (Institutional Review Board Protocol #: 20168000742). A sample size of  $n=24$  patients ( $n=12$  non-delirious control patients and  $n=12$  delirious patients) was selected to determine biochemical differences in serum protein levels. Study details for the MINDDS trial have been previously described.<sup>13</sup> As the MINDDS trial is ongoing, the researchers remained blinded to treatment with placebo or the adrenergic sedative, dexmedetomidine. Seven of the 12 control patients and 7 of the 12 delirious patients did not receive either placebo or dexmedetomidine. Briefly, inclusion criteria included patients aged  $\geq 60$  years old who were undergoing cardiac surgery with an expected same-day admission to the CSICU for  $>24$  hours for sample collection. Exclusion criteria was applied to patients with greater than two days in the ICU during the month prior to surgery, renal or liver failure, and severe neurocognitive damage. All patients underwent cognitive and physical function assessments before and after surgery. The 3D Cognitive Assessment Method (3D CAM) and Telephone-Montreal Cognitive Assessment (T-MoCA) were performed preoperatively to establish baseline cognitive scores. The Patient-Reported Outcomes Measurement Information System (PROMIS) clinical outcome assessments were performed preoperatively to define baseline physical and mental functional scores (PROMIS v.1.1 Global Health Physical, PROMIS v.1.1 Global Health Mental, PROMIS v1.2 Physical Function 8b, PROMIS v1.0 Applied Cognition-Abilities-Short Form 8a, PROMIS Sleep Disturbance-Short Form 4a, and PROMIS v1.0 Pain Interference-Short Form 8a).<sup>14-18</sup> Cognitive assessments were performed twice daily for three days following surgery using the Confusion Assessment Method (CAM) short- and long-forms. The T-MoCA and PROMIS questionnaires were performed preoperatively and at postoperative days 30, 90, and 180. Details regarding patient-centered clinical outcomes, including delirium onset, delirium severity, mortality, and hospital length of stay were recorded.

## Serum Isolation and Processing

Blood samples were collected before surgery and the morning of postoperative day one in a glass serum separator tube (BD Vacutainer tube, BD Life Sciences, Franklin Lakes, NJ) and allowed to clot for 30 minutes, followed by centrifugation at 3000 rpm at 22°C for 30 minutes. The supernatant was then collected, aliquoted, and stored at -20°C for at least 24 hours, followed by transfer to -80°C until further processing.

## Protein Analysis

Serum samples were analyzed using proximity extension assays (Olink Inflammation Panel, v.3004 and Olink Neuro Exploratory Panel (v.3901)) that utilize targeted antibodies coupled to oligonucleotide sequences for amplification and quantification by real-time polymerase chain reaction (Olink Proteomics Inc., Watertown, MA).<sup>19, 20</sup> Serum samples (~40 µL/patient sample) were added to individual wells of a 96-well plate, sealed, and sent on dry ice to the Olink Proteomics Analysis laboratory (Watertown, MA). Internal controls were included within the biochemical analysis to verify specificity and quality control of the incubation, extension, and detection steps. The average intra-assay and inter-assay % coefficient of variation for the proximity extension assay method has been reported as 8% and 12%, respectively.<sup>19</sup> Biological replicates were compared for significant differences (>2-linear fold increase and p<0.05) between control and delirium cohorts. Graphs of the proteins detected in serum samples from each cohort and individual patient responses from preoperative to postoperative states are provided within the Supplemental Materials (Appendix A and B).

## Statistical Analysis

All statistical analyses were performed using GraphPad Prism (version 8.4.3) (GraphPad Software, San Diego, CA). Our primary hypothesis to determine significant biomarker regulation in the delirium cohort compared to the control group was assessed with an ordinary two-way ANOVA and a Šídák's multiple comparisons test for each protein detected. Each biomarker was first analyzed using a univariate analysis. A multivariable analysis of each protein considering the timepoint and cohort (control versus delirium) was performed. A Kolmogorov-Smirnov normality test was used to determine if the data were normally distributed. Secondary comparisons of clinical features were assessed with a Mann-Whitney U test. Pearson correlation coefficients (*r*) were calculated using multiple variables, e.g., age, BMI, CPB time, FGF-21<sub>pre-op</sub>, FGF-21<sub>post-op</sub>, IL-6<sub>pre-op</sub>, and IL-6<sub>post-op</sub> with significance determined using a two-tailed p-value. Multiple logistic regression models were applied to identify biomarker selectivity, specificity, and predictive power using bivariate analyses. The odds ratio was calculated for delirium based on FGF-21<sub>post-op</sub> with a Wald confidence interval reported. A p<0.05 was considered statistically significant.

## Results

Control and delirium cohorts were defined by matching patients by age- and sex- with no significant differences in body mass index, cardiopulmonary bypass time, or overall cognitive or physical health detected between groups at the preoperative evaluation, thereby excluding underlying dementia or other late-stage neurodegenerative diseases (Fig. 1).

We applied a sensitive, multiplexed proteomics-based approach using proximity extension assays to identify the relative abundance of select proteins found in serum samples. We detected a specific and significant increase in a pleiotropic hormone and metabolic regulator, known as fibroblast growth factor (FGF)-21, in serum from patients that developed delirium following surgery compared to patients that *did not* develop delirium (mean postoperative values = 6.7 ( $\pm$ 1.6) log<sub>2</sub> abundance, 95% CI [5.7 to 7.7] and 4.6 ( $\pm$ 1.0) log<sub>2</sub> abundance, 95% CI [4.0 to 5.3] for delirious and control cohorts, respectively,  $p=0.002$ ) (Fig. 2A). FGF-23 was elevated postoperatively in the delirious cohort (3.4 ( $\pm$ 2.2), 95% CI [2.0 to 4.8]), though not statistically significant, compared to the control group (2.1 ( $\pm$ 1.5), 95% CI [1.1 to 3.0],  $p=0.2$ ). In agreement with previous reports, we also detected a significant increase in the pro-inflammatory factor, IL-6, in the delirium cohort following surgery (8.7 ( $\pm$ 1.9) log<sub>2</sub> abundance, 95% CI [7.5 to 9.9],  $p=0.02$ ) compared to non-delirious control patients (7.2 ( $\pm$ 0.6) log<sub>2</sub> abundance, 95% CI [6.8 to 7.6]). Monocyte chemotactic protein-3 (MCP-3) and chemokine (C-C motif) ligand (CCL) 20 levels at the postoperative state were not significantly different between delirious patients (6.1 ( $\pm$ 2.0) log<sub>2</sub> abundance, 95% CI [4.8 to 7.4] and 6.3 ( $\pm$ 2.0) log<sub>2</sub> abundance, 95% CI [5.0 to 7.6], respectively) and non-delirious patients (5.1 ( $\pm$ 1.2) log<sub>2</sub> abundance, 95% CI [4.4 to 5.9],  $p=0.5$ ; and 5.1 ( $\pm$ 1.0) log<sub>2</sub> abundance, 95% CI [4.5 to 5.8],  $p=0.3$ , respectively). CCL19 decreased with surgery in both cohorts with a significant difference detected between delirious and control patients at the postoperative state (mean postoperative values = 6.9 ( $\pm$ 1.5) log<sub>2</sub> abundance, 95% CI [5.9 to 7.8] and 5.3 ( $\pm$ 1.0) log<sub>2</sub> abundance, 95% CI [4.7 to 6.0] for delirious and control cohorts, respectively,  $p=0.04$ ).

In terms of individual patient responses from preoperative to postoperative states, FGF-21 was increased at least 2-linear fold in 8 out of 12 patients (67%) that screened positive for delirium and 3 out of 12 patients (25%) that screened negative for delirium (mean preoperative values = 5.0 ( $\pm$ 1.1) log<sub>2</sub> abundance, 95% CI [4.3 to 5.7] and 4.3 ( $\pm$ 1.3) log<sub>2</sub> abundance, 95% CI [3.5 to 5] for delirious and control cohorts, respectively) (Fig. 2B). Similarly, FGF-23 was increased at least 2-linear fold following surgery in 8 out 12 delirious patients (67%) and 4 out of 12 patients (33%) that screened negative for delirium (mean preoperative values = 1.7 ( $\pm$ 1.3) log<sub>2</sub> abundance, 95% CI [0.8 to 2.5] and 1.1 ( $\pm$ 0.6) log<sub>2</sub> abundance, 95% CI [0.7 to 1.4] for delirious and control cohorts, respectively) (Fig. 2B). IL-6 was increased from preoperative levels in every patient following surgery regardless of the development of delirium (mean preoperative values = 3.8 ( $\pm$ 1.1) log<sub>2</sub> abundance, 95% CI [3.1 to 4.5] and 3.3 ( $\pm$ 0.9) log<sub>2</sub> abundance, 95% CI [2.7 to 3.8] for delirious and control cohorts, respectively) (Fig. 2B). MCP-3 was increased at least 2-linear fold following surgery in 7 out of 12 delirious patients (58%) and 8 out of 12 non-delirious patients (67%) (mean preoperative values = 4.1 ( $\pm$ 0.9) log<sub>2</sub> abundance, 95% CI [3.6 to 4.7] and 3.8 ( $\pm$ 1.3), 95% CI [3.0 to 4.7] for delirious and control cohorts, respectively). Though overall postoperative CCL19 levels were higher in the delirious cohort, most patients (11 of the 12 delirious patients (92%) and 11 of the 12 non-delirious patients (92%)) showed a reduction in CCL19 serum levels following surgery ranging from 1.03–4.1 and 1.3–4.8 linear fold-change in the delirious and control cohorts, respectively (mean preoperative values = 7.6 ( $\pm$ 1.5) log<sub>2</sub> abundance, 95% CI [6.7 to 8.6] and 6.5 ( $\pm$ 1.2) log<sub>2</sub> abundance, 95% CI [5.7 to 7.2] for delirious and control cohorts, respectively). Showing a similar

pattern to MCP-3, CCL20 increased at least 2-linear fold postoperatively in most patients (8 out of 12 delirious patients (67%) and 7 out of 12 non-delirious patients (58%) from preoperative serum levels (mean preoperative value = 4.2 ( $\pm$ 1.0) log<sub>2</sub> abundance, 95% CI [3.6 to 4.9] and 4.0 ( $\pm$ 1.7) log<sub>2</sub> abundance, 95% CI [2.9 to 5.1] for delirious and control cohorts, respectively).

The positive and negative predictive power of FGF-21 for delirium was 73% and 69%, respectively, with a sensitivity and specificity for delirium of 80% and 75%, respectively, based on multiple logistic regression analyses (odds ratio 3.7, 95% CI [1.3 to 10.2]). Delirium severity scores in the delirium cohort were higher on postoperative day one, highly variable through postoperative day three, and correlated with elevated average FGF-21 levels on postoperative day one compared to non-delirious control patients (Supplemental Fig. 2). Significant Pearson's correlation ( $r$ ) were identified for postoperative FGF-21 and IL-6 serum levels ( $r=0.53$ ,  $p=0.007$ ) with comparable positive correlations for the presence of delirium ( $r=0.6$ ,  $p=0.004$  and  $r=0.49$ ,  $p=0.014$ , respectively) (Supplemental Fig. 3).

## Discussion

In this study, we utilized a multiplexed proteomic approach to screen for >150 proteins in serum isolated from a sub-population of patients ( $n=24$ ) enrolled in the MINDDS trial.<sup>13</sup> We found that after cardiac surgery with cardiopulmonary bypass, IL-6 serum levels increased in all patients and were 2.8-linear fold higher on average in patients with delirium. Interestingly, postoperative serum FGF-21 levels were higher by an average of 4.3-linear fold in patients that developed delirium compared to non-delirious control patients. To-date, studies have focused heavily on the role of pro-inflammatory pathways and sensitization of the blood-brain barrier in the pathobiology of delirium,<sup>9, 21</sup> however, the role of systemic metabolism in the development of postoperative delirium remains unclear.

FGF-21 is a potent endocrine factor secreted by the liver known to influence circadian behavior, insulin sensitivity, and physical activity in animal models.<sup>22, 23</sup> Initially identified for its importance in metabolic regulation and stimulation of glucose and fatty acid uptake, FGF-21 and related mimetics have been studied in animal and human clinical trials as a potential treatment for Type II diabetes mellitus, obesity and metabolic disorders.<sup>24–27</sup> Beneficial neuroprotective properties of FGF-21 in insulin-resistant mouse models have also been described.<sup>28, 29</sup> FGF-21 has also been reported as a strong biomarker of mitochondrial dysfunction in human populations,<sup>30</sup> which is predictive of increased mortality during sepsis<sup>31</sup> and a hallmark of aging.<sup>32</sup>

In terms of downstream signaling, both FGF-21 and FGF-23 share high-affinity binding interactions with the klotho family of receptors ( $\beta$ Klotho and  $\alpha$ Klotho for FGF-21 and FGF-23, respectively), as obligate co-receptors to the FGF receptor (FGFR1-R4).<sup>33, 34</sup> FGF-23 is a bone-derived hormone involved in phosphate reabsorption and Vitamin D metabolism.<sup>35</sup> Elevated circulating FGF-23 levels have been associated with increased incident dementia in a community-based cohort<sup>36</sup> and cognitive impairment in a cohort of hemodialysis patients,<sup>37</sup> though associations between FGF-23 levels and baseline cognitive function or development of cognitive impairment over time in well-functioning older adults

remain disputed.<sup>38</sup> Of note, the increase in FGF-21 (and FGF-23) in patients that developed postoperative delirium in our study occurred at postoperative day 1, with no significant difference detected between cohorts preoperatively suggesting an acute response to surgery, rather than constitutive differences in kidney or liver function at baseline. However, the presence of numerous confounding factors in the development of postoperative delirium and the dynamic changes in circulating protein levels must be considered. Further studies evaluating changes in protein biomarkers associated with postoperative delirium in a larger cohort will require stratification based on the presence of a metabolic disorder, kidney and liver function, and Vitamin D levels, among others.

Given the importance of metabolic stores and energy switching in the brain, we posit that systemic FGF-21 may be an early signal of cerebral metabolic insufficiency and inflammation, two putative processes involved in developing delirium.<sup>5</sup> The increase in serum FGF-21 in postoperative delirious patients may also be presenting as a compensatory mechanism to counteract a systemic or localized metabolic failure, in a similar manner to that found in Type II diabetic patients with elevated basal levels of circulating FGF-21.<sup>39, 40</sup> As delirium is a clinical diagnosis with no current biochemical approach for standardized detection, the detection of elevated FGF-21 in delirious patients following major cardiac surgery is a promising finding in the search for objective delirium biomarkers.

Limitations of this study include the small sample size (n=24) and the dependency of statistical significance on individual samples in evaluating associations between increased IL-6, MCP-3, CCL19 and CCL20 with delirium. We also detected a modest sensitivity and specificity of FGF-21 for delirium (80% and 75%, respectively). While we performed cognitive evaluations up to 180 days following surgery, the progressive reduction in clinical data collected with time (e.g., as patients were lost to follow-up, n=3 in the delirious cohort by postoperative day 180) leads to difficulty in determining any differences in control and delirium cohorts in regard to long-term effects of an episode of postoperative delirium on permanent cognitive dysfunction.

Our findings show an increase in FGF-21 in a small cohort of patients with delirium, which may represent an initial signal of mitochondrial dysfunction and oxidative stress, both prominent features also associated with chronic neurodegenerative diseases.<sup>41, 42</sup> Thus, principled insights into the regulation of FGF-21, and potentially FGF-23, as biomarkers of acute neurocognitive dysfunction may be highly relevant to developing therapeutics to prevent long-term neurocognitive decline that may follow an episode of delirium. Further studies evaluating if FGF-21 may be prognostic for delirium severity and whether its upregulation *precedes* or *follows* development of postoperative delirium are warranted. The long-term goals of this work will investigate whether mitochondrial dysfunction and metabolic stores (two factors that regulate FGF-21 expression) influence the predisposition of elderly patients to delirium and whether therapies that induce mitochondrial preconditioning prior to surgical stress can improve postoperative neurocognitive function and recovery.

## Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

## Acknowledgements

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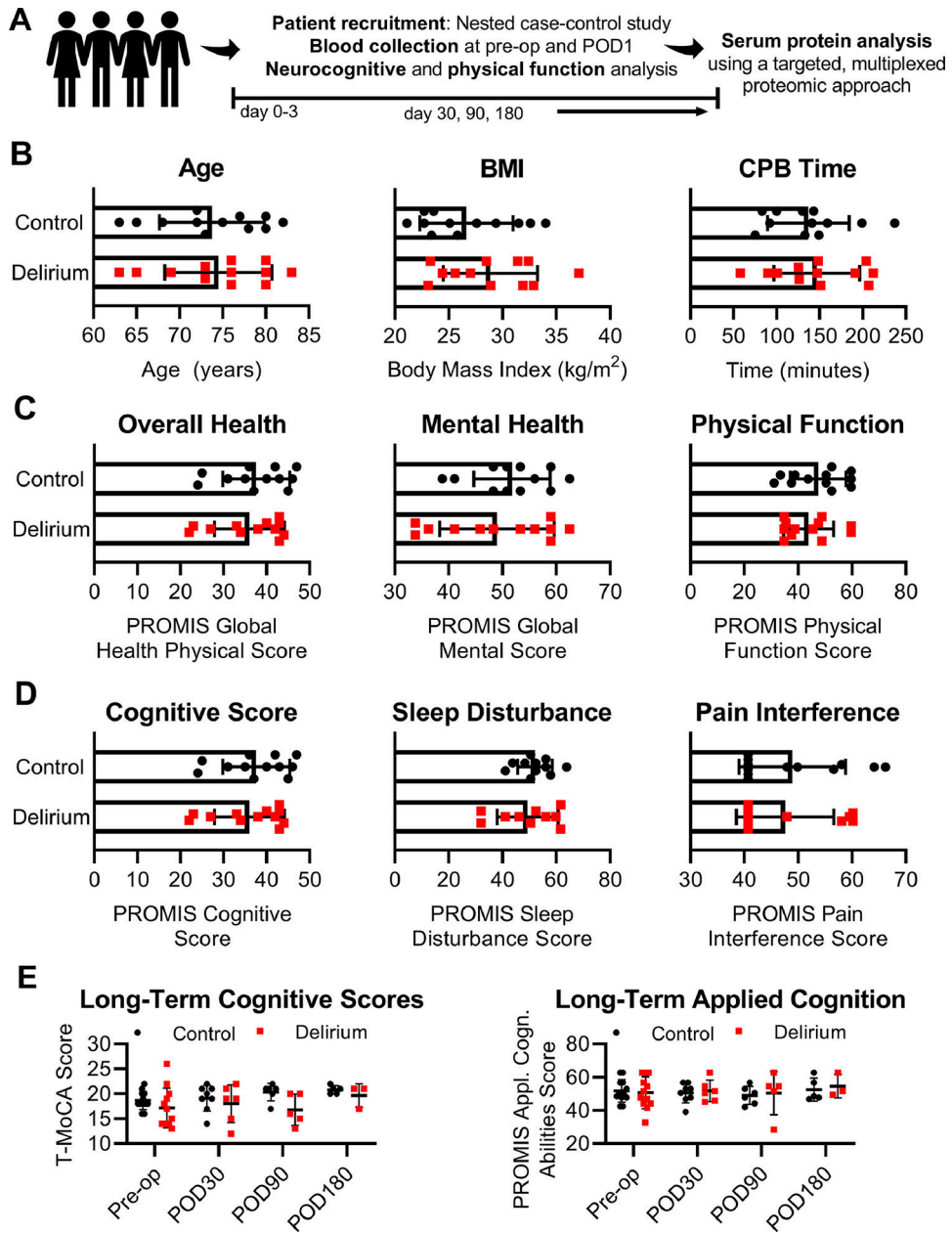
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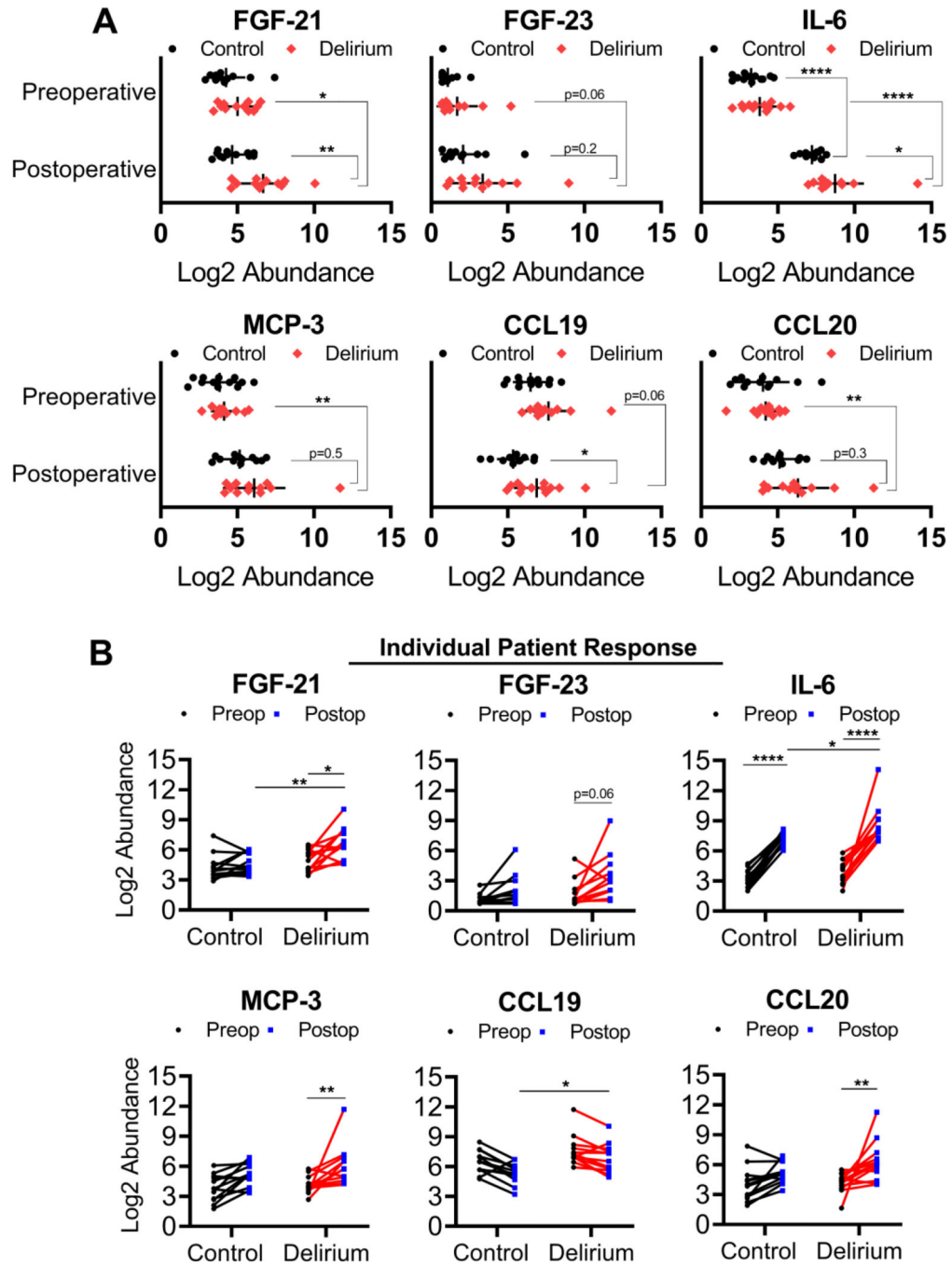
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**Figure 1.** Clinical characteristics and physical and cognitive assessments of patients enrolled in a substudy to evaluate serum biomarkers detected at pre- and postoperative states in control and delirium cohorts. (A) Study design and data collection; (B) Age, body mass index (BMI), and total cardiopulmonary bypass (CPB) time; (C) Patient-Reported Outcomes Measurement Information System (PROMIS) scores of the patients’ overall physical health, and mental and physical function reported at pre-op; (D) PROMIS cognitive score, sleep disturbance, and pain interference scores reported at pre-op; and (E) temporal evaluation of overall cognitive abilities and applied cognition from pre- to postoperative day (POD) 30, 90, and 180. Bars show mean ± standard deviation. Statistical significance was evaluated using a Mann-Whitney U test.



**Figure 2.** Select proteins detected in serum from patients with delirium compared to age- and sex-matched control patients. (A) FGF-21, FGF-23, IL-6, MCP-3, CCL19, and CCL20 expression in control and delirium cohorts at preoperative day 0 and postoperative day (POD) 1; (B) The individual patient response of FGF-21, FGF-23, IL-6, MCP-3, CCL19, and CCL20 from preoperative day 0 to POD1. \*p<0.05, \*\*p<0.01, \*\*\*p<0.001, and

\*\*\* $p < 0.0001$  based on an ordinary two-way ANOVA with Šídák's multiple comparisons test.

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