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Effects of general versus regional anesthesia on circadian melatonin rhythm and its association with postoperative delirium in elderly patients undergoing hip fracture surgery: study protocol for a prospective cohort clinical trial

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Complete List of Authors:	Yuan, Yi; Beijing Jishuitan Hospital Song, Yanan; Peking University Third Hospital, Department of Anesthesiology Wang, Geng; Beijing Jishuitan Hospital and Fourth Medical College of Peking University Department of Radiology, Department of Anesthesiology Jia, Yunyang; Beijing Jishuitan Hospital zhou, Yang; Peking University Third Hospital Mi, Xinning; Peking University Third Hospital Jia, Xixi; Peking University Third Hospital Wang, Xiaoxiao; Peking University Third Hospital, Research Center of Clinical Epidemiology Liu, Chang; Peking University Third Hospital Li, Yue ; Peking University Third Hospital Shi, Chengmei; Peking University Third Hospital Han, Yongzheng; Peking University Third Hospital, Anesthesiology Guo, Xiangyang; Peking University Third Hospital Zhang, Wenchao; Beijing Jishuitan Hospital Li, Zhengqian; Peking University Third Hospital
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Title

Effects of general versus regional anesthesia on circadian melatonin rhythm and its association with postoperative delirium in elderly patients undergoing hip fracture surgery: study protocol for a prospective cohort clinical trial

Author

Yi Yuan[1#], Yanan Song[2#], Geng Wang[1], Yunyang Jia[3], Yang Zhou[2], Xinning Mi[2], Xixi Jia[2], Xiaoxiao Wang[4], Chang Liu[2], Yue Li[2], Chengmei Shi[2], Yongzheng Han[2], Xiangyang Guo[2], Wenchao Zhang[1*], Zhengqian Li [2*]

# Yi Yuan and Yanan Song contributed equally to this study.

* Zhengqian Li and Wenchao Zhang are co-correspondents for this article.

Affiliations

1. Department of Anesthesiology, Beijing Jishuitan Hospital, 31 Xinjiekou East Street, Xicheng District, Beijing 100035, China.

2. Department of Anesthesiology, Peking University Third Hospital, No. 49, North Garden Street, Haidian District, Beijing, 100191 China.

3. Department of Orthopaedics & Traumatology, Beijing Jishuitan Hospital, 31 Xinjiekou East Street, Xicheng District, Beijing 100035, China.

4. Research Center of Clinical Epidemiology, Peking University Third Hospital, No. 49, North Garden Street, Haidian District, Beijing, 100191 China.

Correspondence

Zhengqian Li,

Department of Anesthesiology

Peking University Third Hospital

Beijing, China, Tel: 8610-82267276; E-mail: zhengqianli@bjmu.edu.cn

Or

Wenchao Zhang

Department of Anesthesiolgy

Beijing Jishuitan Hospital,

Beijing, China, Tel:8610-58516688-6907; E-mail: wenchaozhang116@163.com

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Effects of general versus regional anesthesia on circadian melatonin rhythm and its association with postoperative delirium in elderly patients undergoing hip fracture surgery: study protocol for a prospective cohort clinical trial

Abstract

Introduction: Postoperative delirium (POD) is a common neurological complication after hip fracture surgery and is associated with high morbidity and mortality in elderly patients. Although, the specific mechanism of POD remains unclear, circadian rhythm disruptions have recently drawn increased attention. To date, only limited postoperative time points of plasma melatonin level measurements were recorded in previous studies, and such data cannot represent a comprehensive melatonin rhythm. The process of anesthesia (either general anesthesia [GA] or regional anesthesia [RA]) is known to influence the melatonin rhythm. However, how these two anesthesia methods differently affect the postoperative melatonin rhythm is still unknown. Therefore, we hypothesized that RA may attenuate disruption of the melatonin rhythm, which might decrease the incidence of POD in elderly patients undergoing hip surgery.

Methods and analysis: In this prospective cohort clinical trial, 140 patients scheduled for hip fracture surgery will be divided into two groups to receive either GA or RA. The primary aim is to compare the circadian rhythm of melatonin secretion between the two groups and explore its association with the incidence of POD.

Ethics and dissemination: The study is ethically approved by the Medical Science Research Ethics Committees of Beijing Jishuitan Hospital (JLKS201901-04). The results of the study will be published in peer-reviewed international journals.

Trial registration number: Chinese Clinical Trial Registry, ChiCTR1900027393.

Keywords: Postoperative delirium; Circadian rhythm; Melatonin; General anesthesia; Regional anesthesia; Elderly patients.

Strengths and limitations of this study

(1) A major strength of this study is that to our knowledge, this is the first trial to evaluate the effects of different anesthesia methods (RA and GA) on the circadian rhythm of melatonin secretion and explore their association with POD in elderly patients undergoing hip fracture surgery.

(2) Both objective and subjective sleep quality are included in the current study. This can provide a better and more comprehensive understanding of the connection between circadian rhythms and anesthesia.

(3) The limitation of trial is that melatonin is considered to be an optimal peripheral biomarker of circadian timing, more biomarkers such as cytokines and genes (peripheral clock gene) should be included.

(4) Although we will record both subjective and objective sleep quality, such measurements are incomplete, for example polysomnography is the gold standard

for diagnosis of sleep disorders.

(5) Our study will be conducted until discharge from the hospital, which might be insufficient for delirium screening.

INTRODUCTION

Postoperative delirium (POD), a common neurological complication in elderly patients undergoing hip fracture surgery (range, 4%–53%), is usually associated with increased morbidity and mortality rates, long-term cognitive decline, and a heavier healthcare economic burden. [1-6] Many studies have indicated that preoperative brain dysfunction (frail brain) in elderly patients is a predisposing factor for POD [7, 8], while the perioperative change of neuroinflammatory markers (e.g., C-reactive protein and interleukin-6), oxidative stressor markers (cortisol) and the crucial hormone (melatonin) within elderly frail brain are the precipitating factor for POD. [8, 9] However, the specific mechanism of POD remains unclear. [9, 10]

Circadian rhythm disruptions have recently drawn increased attention as a possible mechanism of POD. [10, 11] Circadian rhythms refer to the behavioral and physiological cycles that occur within a period of approximately 24 hours, including the sleep–wake cycles, hormone secretion (e.g., melatonin), and core body temperature. [12] Among these indicators, plasma melatonin is considered to be the best peripheral marker of endogenous circadian rhythms.[13-15] Previous studies have revealed potential correlations between plasma melatonin secretion disruption and POD. [10, 16-20] Nevertheless, only limited postoperative time points (e.g., once a day) of plasma

melatonin level measurements were recorded in those studies, and such data cannot represent a comprehensive melatonin rhythm. Moreover, one study indicated that the urinary excretion of 6-sulfatoxymelatonin (6-SMT), the main metabolite of melatonin, during delirium was associated with clinical subtypes of delirium (higher in hypoactive and lower in hyperactive delirium). [21] However, measuring the melatonin level in a urine sample instead of a plasma sample at only one time point is insufficient to describe the real pattern of the melatonin rhythm in the acute phase of delirium. Therefore, the exact association between the melatonin rhythm and delirium requires further exploration.

The process of anesthesia (either general anesthesia [GA] or regional anesthesia [RA]) is known to influence the circadian rhythms. [22-24] On one hand, GA is associated with unconsciousness similar to a dreamless sleep, which may strongly alter circadian rhythms through the use of drugs such as N-methyl-D-aspartate (NMDA) receptor antagonists or gamma-aminobutyric acid (GABA) receptor agonists [22, 25, 26]. On the other hand, RA is more helpful in the relief of sleep disturbances after surgery than GA [27], which may result from better maintenance of the melatonin rhythm postoperatively. However, the effects of these two anesthesia methods on the postoperative melatonin rhythm have not been compared. Therefore, we hypothesized that RA may attenuate disruption of the melatonin rhythm, which might decrease the incidence of POD in elderly patients undergoing hip surgery. In this report, we present the design of a prospective cohort clinical trial to compare the effects of GA versus RA on the circadian

rhythm of melatonin secretion and the incidence of POD in elderly patients undergoing hip fracture surgery.

METHODS AND ANALYSIS

Trial design

This prospective cohort clinical trial is designed to compare the effects of GA and RA on the melatonin rhythm and incidence of POD. We aim to recruit 140 patients with hip fracture (≥65 years of age) who will be assigned to either the GA group (n = 70) or RA group (n = 70) depending on their individual situation and personal willingness as well as the preference of the anesthesiologists and surgeons. We will conduct the trial at the Beijing Jishuitan Hospital in China. Figure 1 shows the flow chart of this study.

Primary outcome

The primary outcome is to compare the circadian rhythm of melatonin secretion between the GA and RA groups and explore its association with the incidence of POD until discharge from the hospital.

Secondary outcomes

The secondary outcomes are to compare the subtype and severity of POD, sleep quality, and pain until discharge from the hospital, and postoperative cognitive function at 6 months and 1 year after surgery between the GA and RA groups.

Ethics approval

The study protocol has been approved by the Medical Science Research Ethics Committees of Beijing Jishuitan Hospital (JLKS201901-04). Written informed consent

will be obtained from each enrolled patient accompanied by at least one family member or proxy. This study has been registered at the Chinese Clinical Trial Registry (ChiCTR1900027393).

Eligibility criteria

Inclusion criteria

Participants will be included if they meet the following criteria: age of ≥65 years, hospital admission for surgical treatment of hip fracture, and American Society of Anesthesiologists (ASA) physical status classification of I to III.

Exclusion criteria

Patients who meet any of the following criteria will be excluded: Parkinson's disease, dementia (including dementia cause by Parkinson's disease, Alzheimer's disease, or Lewy body dementia), a stroke within the prior 6 months or any other central nervous system disease, alcohol-related disorders, multiple trauma, preoperative delirium, severe deafness or vision problems, communication difficulties, night shift duty, taking medication related to melatonin, a plan to be transferred to the intensive care unit postoperatively, and refusal to participate in the study or unexpected discharge.

Preoperative baseline data collection and evaluation

Trained investigators who are blind to the patient grouping will evaluate the patients and their medical charts to screen out potential participants according to the inclusion and exclusion criteria. Baseline data will be collected, including demographic data (e.g., age, sex, race, education level, height, and weight), surgical diagnosis, comorbidities, medication history, surgical history, smoking and drinking status, and the results of

physical, laboratory, and instrumental examinations. We will also record the ASA physical status classification. Specifically, preoperative cognitive scans will be performed using the Mini-Mental State Examination (MMSE) [28]; preoperative delirium will be diagnosed with the Confusion Assessment Method (CAM) [29, 30]; activities of daily living and instrumental activities of daily living will be assessed with the Katz Index of Independence in Activities of Daily Living [31]; sleep quality will be assessed by the Pittsburgh Sleep Quality Index (PSQI) [32]; and pain intensity will be assessed using the numerical rating scale (NRS) [33].

Preoperative management

All individuals will be placed in a standard private room with a consistent light–dark cycle and room temperature, and the lights will be turned off between 21:00 and 06:00 even at the time of blood sample collection. The participants will be requested to eat the same diet and refrain from alcohol, caffeine, and chocolate. Their activities will be restricted at night from 21:00 to 06:00, during which time they will be free from interference factors including noise, television, and mobile phones during the study period.

Anesthesia and analgesia

All patients will receive an ultrasound-guided fascia iliac block (a single injection of 30 mL of 0.33% ropivacaine) after admission to the operating room. During the operation, the patients' eyes will be carefully covered with eye patches to avoid light effects.

In the GA group, standard GA will be administered to the patients. Anesthesia will be intravenously induced with propofol (or etomidate), an opiate (sufentanil or fentanyl),

and a muscle relaxant (rocuronium or cisatracurium). Sevoflurane will be used for anesthesia maintenance, remifentanil for analgesia, and rocuronium or cisatracurium for muscle relaxation. Mechanical ventilation will be continued to keep the end-tidal carbon dioxide concentration at 35 to 40 mmHg, and the bispectral index will be maintained between 40 and 60.

In the RA group, bupivacaine or ropivacaine will be used for spinal anesthesia or combined spinal and epidural anesthesia at the L2-3 or L3-4 levels.

On the day of surgery, intraoperative parameters will be recorded, including the mean arterial pressure, heart rate, bispectral index, start time, duration and type of anesthesia and surgery, anesthetic drugs and doses, sedative doses, blood loss, fluid balance and transfusion of blood or clotting products, complications, and adverse events.

For postoperative analgesia management, the patients will receive intravenous patient-controlled analgesia (sufentanil 100 μg and ondansetron 8 mg mixed with normal saline to a total volume of 100 mL).

Postoperative management and follow-up

All patients will be followed up twice a day until discharge from the hospital. The investigators blind to grouping will record the total hospitalization cost, the in-hospital length of stay, and the occurrence of postoperative complications during hospitalization. Long-term cognitive function will be assessed for 1 year after surgery.

1. Delirium assessment

Delirium will be diagnosed using the CAM, and the subtype and severity of delirium will be assessed by using the Memorial Delirium Assessment Scale [34]. Surveillance

will involve twice-daily visits (8:00 and 20:00) by a trained geriatrician. The duration of delirium will be measured as the number of days from onset of delirium symptoms to resolution of symptoms. If the researcher is in doubt regarding this assessment, the diagnosis of POD will be referred to an external expert.

2. Sleep assessment

All participants will continuously wear a sleep tracker (Fitbit Charge 3; Fitbit, Inc., San Francisco, CA, USA), which is an available and inexpensive method to quantify sleep patterns [35]. The investigators will provide instructions on wearing the sleep tracker, a mobile device capable of downloading Fitbit Connect software, and completing the corresponding sleep logs (bedtime, wake time, and nap times). The frequency of syncing will be checked by the research staff weekly, and the Fitbit Charge proprietary algorithm will process the sensor data to automate the sleep variables [36]. All patients will wear sleep tracker until discharge from hospital. Based on the data from the sleep tracker, sleep logs, and direct observation by a third party, we will derive the following data: total sleep time (min), sleep onset latency (min), wake time after sleep onset (min), time in rapid eye movement sleep , and time spent in “light sleep” and “deep sleep” (according to Fitbit Inc.). Subjective sleep quality will be assessed with an NRS (0 = worst sleep and 10 = best sleep) [37].

3. The intensity of postoperative pain both at rest and with movement will be evaluated twice daily with the NRS.

4. Cognitive function will be assessed by the same geriatrician at 6 months and 1 year after surgery. The Modified Telephone Interview for Cognitive Status will be used to

test the patient's cognitive function in non-face-to-face interviews [37].

Blinding

An investigator will be assigned to preserve and distribute randomization results. Investigators were not blinded to the study group assignment, but the trained geriatrician responsible for postoperative evaluation including POD diagnosis, as well as PSQI, IADLs, and NRS scores are blinded to the grouping.

Light exposure

Considering that light suppresses melatonin secretion except in life-threatening situations, only illumination from a penlight will be used during these interventions [15, 19]. Blood sample collection and nursing interventions at night will be performed with a penlight to avoid light exposure. Light intensity readings will be obtained with a digital meter to ensure that light exposure does not exceed 10 lux.

Sample Collection

Usually, the melatonin concentration is low throughout the day and then rises to a peak between 02:00 and 04:00 [15]. To establish the circadian rhythm, the plasma concentration of melatonin will be measured at least four times a day [15, 19]. Postoperative blood samples will be dynamically collected every day at 04:00, 10:00, 16:00, and 22:00 until the patient discharged through venipuncture in the heparinized tube (2 ml per sample) [16, 19]. The samples will be stored at 4°C and brought to the laboratory in a nitrogen canister, where they will be stored at −80°C. The concentration of melatonin will be measured with the enzyme immunoassay method (cat no. RE 54021; IBL International GmbH, Hamburg, Germany) with a limit of detection of 1.6 pg/mL [38].

Sample size estimation

Melatonin is secreted in a biphasic pattern in which nighttime secretion increases from 20:00 to 22:00 with a peak at 02:00 to 04:00, and daytime secretion decreases from 06:00 to 08:00 with a nadir at 12:00 [14, 39]. Cronin et al. [15] researched the nighttime melatonin secretion of adult patients for 3 days after gynecologic surgery and found that the lowest level occurred on the first night postoperatively. In another study of elderly patients undergoing major abdominal surgery, Shigeta et al. [40] found that the postoperative amplitude of nighttime melatonin secretion was significantly lower than the preoperative amplitude. These findings indicate that the peak of melatonin secretion is suppressed to the greatest degree in the first postoperative night, possibly leading to a melatonin rhythm shift. Therefore, for the present study, we will choose 04:00 on the first postoperative night as the time point for comparing the melatonin concentration between the groups. Our pilot study showed that the mean ± standard deviation of melatonin was 10.5 ± 4.0 and 13.4 ± 6.9 pg/mL in the GA and RA groups (n=15 in each group), respectively. Using a two-sided α value of 0.05 and 85% power and considering a 10% dropout rate, we plan to include 140 patients in this study, with 70 patients in each group (1:1).

Circadian melatonin rhythm data

Circadian markers of the melatonin rhythm, including the mesor (mean concentration) and amplitude (peak value minus the mean value), acrophase time (peak time), will be calculated by Chronos-Fit program data and cosinor software [41]. The area under the curve (AUC) will be calculated as a measure of the secreted amount of melatonin with

the trapezoidal method. The data will be subsequently smoothed with the Lowess (Cleveland) curve-fitting procedure curve-fitting procedure [42].

Statistical analysis

Case report forms will be used for data collection. Demographic data and information on pain scores, delirium and sleep assessment data, and adverse events during and after surgery will be recorded. Data will be presented as mean and standard deviation, median and interquartile range (25th–75th percentile), or frequency and proportion depending on the variable type and distribution. Categorical variables will be assessed with the χ^2 test or Fisher's exact test. Differences in continuous variables between the groups will be assessed with a parametric *t*-test, the Wilcoxon rank-sum test, or the Kruskal–Wallis test. The Mann–Whitney U test will be used to analyze non-normal variables. Results will be presented with 95% confidence intervals. A *P* value of <0.05 will be considered statistically significant. All statistical analyses will be performed with SPSS software, version 21.0 (IBM Corp., Armonk, NY, USA).

DISCUSSION

This clinical trial is designed to test whether RA can relieve perioperative melatonin rhythm disruption compared with GA and decrease the incidence of POD in elderly patients with hip fracture.

Although some previous studies have shown connections between melatonin secretion disruption and delirium, few have elucidated the real melatonin rhythm pattern [43-45]. For example, a prospective observational study by Yoshitaka et al. showed that the delta plasma melatonin concentration (calculated based on the preoperative and

postoperative values at selected time points) at 1 hour after surgery was significantly lower in patients with than without delirium [46]. Melatonin secretion exhibits a biphasic pattern in which daytime secretion is low (5 pg/mL on average) and nighttime secretion is relatively high (50–100 pg/ml) [14, 39]. However, the sample collection time points of melatonin in the above-mentioned study (before the operation, 1 hour after the operation, and at 08:00 on postoperative days 1 and 2) represented either nighttime secretion on the operation day or daytime secretion on the postoperative days; thus, the measurements do not represent the comprehensive rhythm of melatonin. In contrast, in the current study the blood samples will be taken before anesthesia, postoperative blood samples (melatonin) every day at 04:00, 10:00, 16:00 and 22:00 until discharge. These measurements are adequate to establish a melatonin rhythm curve. They will not only show the individual postoperative melatonin concentrations but also allow us to analyze the irregular pattern of melatonin secretion in patients with delirium.

Previous trials have shown that GA may influence postoperative melatonin secretion [22-24]. First, in patients who received combined intravenous and inhaled anesthesia, the melatonin levels decreased the first night after surgery [24, 47]. Second, GA by inhalation was associated with a significant decrease in the melatonin concentration on the night of surgery, and this decrease was 350% greater than that on the third night after surgery [15]. Third, total intravenous anesthesia induced a phase delay in the 6-SMT rhythm; the peak time was late by about 1 hour, and the amplitude of the melatonin rhythm decreased by 85% on the night of surgery [12]. In contrast, another study demonstrated that RA was helpful to relieve sleep disturbance after surgery [27], which may result from

better maintenance of the melatonin rhythm postoperatively. However, little is known about how the two anesthesia methods differently affect the melatonin rhythm. Karkela et al. [24] conducted a similar clinical study and found that anesthesia and surgery disturbed the circadian melatonin rhythm, but there was no significant difference in the melatonin secretion between the spinal anesthesia and GA groups for minor orthopedic operations [24]. We do not consider that these results are especially robust and generalizable because of several limitations of the trial, such as the small sample size (40 participants), measurement of only the saliva and urine concentrations of melatonin (no plasma or serum concentrations), and insufficient melatonin measurement timing (measurement of only nighttime secretion levels). We hypothesize that RA can maintain an almost normal melatonin rhythm for the following two reasons. First, RA may significantly reduce the types and amounts of general anesthetics used compared with GA, which can strongly alter melatonin secretion through different signal pathways. Second, RA allows better pain control and improved sleep quality compared with GA, which further facilitates a decrease in the severity of melatonin rhythm disruption.

CONCLUSION

In conclusion, to our knowledge, this is the first study to compare the circadian rhythm of melatonin secretion between GA and RA groups and to explore the potential association between circadian rhythm disruptions and the development of delirium. Our findings will promote a greater understanding of delirium pathophysiology and provide clinical evidence for the optimal anesthesia method in elderly patients

undergoing hip fracture surgery.

Declarations

The results of the study will be published in peer-reviewed international journals and will be presented at national and international conferences and symposiums.

Acknowledgments: Not applicable

Supplementary Material

Contributors: YS and YY are joint first authors. ZL and XG obtained funding. XG, ZL and GW designed the study. YS, YY, YJ and WZ collected the data. LY and YC were involved in data cleaning, mortality follow-up, and verification. JL, CY, and DS analyzed the data. YS, YY and ZL drafted the manuscript. WZ contributed to the interpretation of the results and critical revision of the manuscript for important intellectual content and approved the final version of the manuscript. All authors have read and approved the final manuscript. ZL and WZ are the study guarantors.

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Patient consent: Obtained.

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Data sharing: No additional data are available.

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Figuer legend

Figure 1 Flow chart of the trial. ASA, American Society of Anesthesiologists; CAM, Confusion Assessment Method; MMSE, Mini-Mental State Examination; ADL, activities of daily living; PSQI, Pittsburgh Sleep Quality Index; IL, interleukin; CRP, C-reactive protein; Cor, cortisol; MAP, mean arterial pressure; HR, heart rate; BIS, bispectral index; MDAS, Memorial Delirium Assessment Scale; TICS-m, Modified telephone interview for cognitive status.

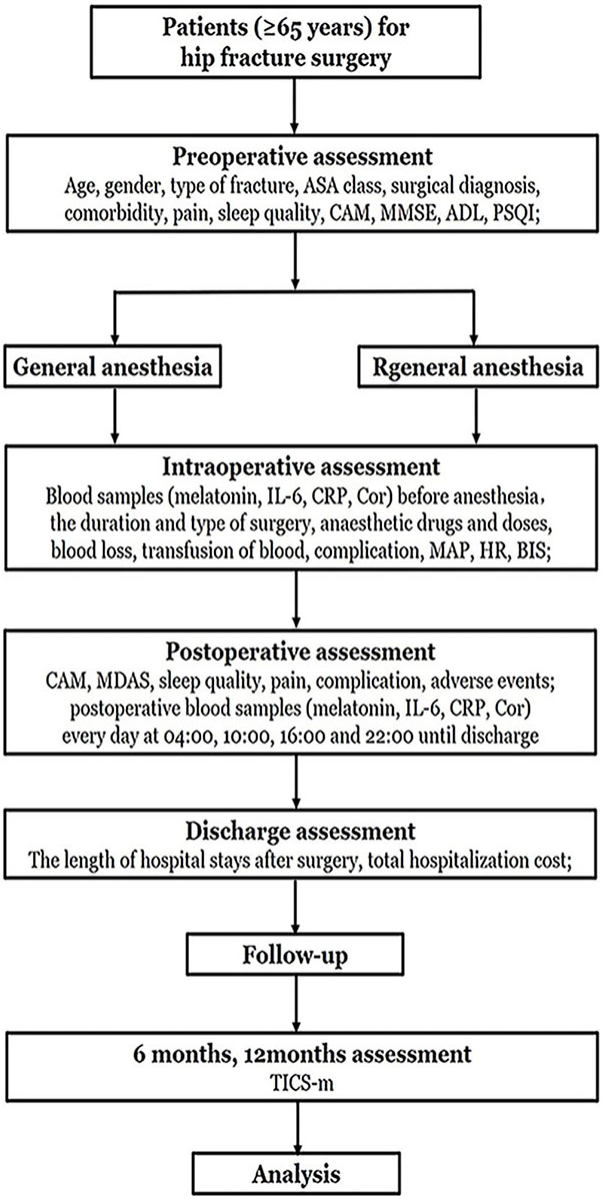

Figure 1

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Effects of general versus regional anesthesia on circadian melatonin rhythm and its association with postoperative delirium in elderly patients undergoing hip fracture surgery: study protocol for a prospective cohort clinical trial

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Title

Effects of general versus regional anesthesia on circadian melatonin rhythm and its association with postoperative delirium in elderly patients undergoing hip fracture surgery: study protocol for a prospective cohort clinical trial

Author

Yi Yuan[1#], Yanan Song[2#], Geng Wang[1], Yunyang Jia[3], Yang Zhou[2], Xinning Mi[2], Xixi Jia[2], Xiaoxiao Wang[4], Chang Liu[2], Yue Li[2], Chengmei Shi[2], Yongzheng Han[2], Xiangyang Guo[2], Wenchao Zhang[1*], Zhengqian Li [2*]

# Yi Yuan and Yanan Song contributed equally to this study.

* Zhengqian Li and Wenchao Zhang are co-correspondents for this article.

Affiliations

1. Department of Anesthesiology, Beijing Jishuitan Hospital, 31 Xinjiekou East Street, Xicheng District, Beijing 100035, China.

2. Department of Anesthesiology, Peking University Third Hospital, No. 49, North Garden Street, Haidian District, Beijing, 100191, China.

3. Department of Orthopaedics & Traumatology, Beijing Jishuitan Hospital, 31 Xinjiekou East Street, Xicheng District, Beijing 100035, China.

4. Research Center of Clinical Epidemiology, Peking University Third Hospital, No. 49, North Garden Street, Haidian District, Beijing, 100191, China.

Correspondence

Zhengqian Li,

Department of Anesthesiology

Peking University Third Hospital

Beijing, China, Tel: 8610-82267276; E-mail: zhengqianli@bjmu.edu.cn

Or

Wenchao Zhang

Department of Anesthesiology

Beijing Jishuitan Hospital,

Beijing, China, Tel:8610-58516688-6907; E-mail: wenchaozhang116@163.com

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Effects of general versus regional anesthesia on circadian melatonin rhythm and its association with postoperative delirium in elderly patients undergoing hip fracture surgery: study protocol for a prospective cohort clinical trial

Abstract

Introduction: Postoperative delirium (POD) is a common neurological complication after hip fracture surgery, and is associated with high morbidity and mortality in elderly patients. Although, the specific mechanism of POD remains unclear, circadian rhythm disruptions have recently drawn increased attention. To date, only limited postoperative time points of plasma melatonin level measurements were recorded in previous studies, and such data cannot represent a comprehensive melatonin rhythm. The process of anesthesia (either general anesthesia [GA] or regional anesthesia [RA]) is known to influence the melatonin rhythm. However, how these two anesthesia methods differently affect the postoperative melatonin rhythm is still unknown. Therefore, we hypothesize that RA may attenuate the disruption of the melatonin rhythm, which might decrease the incidence of POD in elderly patients undergoing hip surgery.

Methods and analysis: In this prospective cohort clinical trial, 138 patients scheduled for hip fracture surgery will be divided into two groups to receive either GA or RA. The primary aim is to compare the circadian rhythm of melatonin secretion between the two groups and explore its association with the incidence of POD.

Ethics and dissemination: The study has been approved by the Medical Science Research Ethics Committees of Beijing Jishuitan Hospital (JLKS201901-04). The results of the study will be published in peer-reviewed international journals.

Trial registration number: The study has been registered at the Chinese Clinical Trial Registry (www.chictr.org.cn) with identifier ChiCTR1900027393. The latest of the trial protocol has been approved on 11 November 2019.

Keywords: Postoperative delirium; Circadian rhythm; Melatonin; General anesthesia; Regional anesthesia; Elderly patients.

Strengths and limitations of this study

(1) A major strength of this study is that to our knowledge, this is the first trial to evaluate the effects of different anesthesia methods (RA and GA) on the circadian rhythm of melatonin secretion and explore their association with POD in elderly patients undergoing hip fracture surgery.

(2) Both objective and subjective sleep quality are included in the current study. This can provide a better and more comprehensive understanding of the connection between circadian rhythms and anesthesia.

(3) The limitation of the trial is that melatonin is the optimal peripheral biomarker of circadian timing, more biomarkers such as cytokines and genes (peripheral clock gene) should be included.

(4) Although we will record both subjective and objective sleep quality, such

measurements are incomplete, for example, polysomnography is the gold standard for diagnosis of sleep disorders.

(5) Our study will be conducted until discharge from the hospital, which might be insufficient for delirium screening.

INTRODUCTION

Postoperative delirium (POD), a common neurological complication in elderly patients undergoing hip fracture surgery (range, 4%–53%), is usually associated with increased morbidity and mortality rates, long-term cognitive decline, and a heavier healthcare economic burden. [1-6] Many studies have indicated that preoperative brain dysfunction (frail brain) in elderly patients is a predisposing factor for POD [7, 8], while the perioperative change of neuroinflammatory markers (e.g., C-reactive protein and interleukin-6), oxidative stressor markers (cortisol) and the crucial hormone (melatonin) within elderly frail brain are the precipitating factors for POD. [8, 9] However, the specific mechanism of POD remains unclear. [9, 10]

Circadian rhythm disruptions have recently drawn increased attention as a possible mechanism of POD. [10, 11] Circadian rhythms refer to the behavioral and physiological cycles that occur within approximately 24 hours, including the sleep-wake cycles, hormone secretion, and core body temperature. [12] Among these indicators, plasma melatonin is considered to be the best peripheral marker of endogenous circadian rhythms.[13-15] Previous studies have revealed potential correlations between plasma melatonin secretion disruption and POD. [10, 16-20] Nevertheless, only limited

postoperative time points (e.g., once a day) of plasma melatonin level measurements were recorded in those studies, and such data cannot represent a comprehensive melatonin rhythm. Moreover, one study indicated that the urinary excretion of 6-sulfatoxymelatonin (6-SMT), the main metabolite of melatonin, during delirium was associated with clinical subtypes of delirium (higher in hypoactive and lower in hyperactive delirium). [21] However, measuring the melatonin level in a urine sample instead of a plasma sample at only one time point is insufficient to describe the real pattern of the melatonin rhythm in the acute phase of delirium. Therefore, the exact association between the melatonin rhythm and delirium requires further exploration.

The process of anesthesia (either general anesthesia [GA] or regional anesthesia [RA]) is known to influence the circadian rhythms. [22-24] On one hand, GA is associated with unconsciousness similar to a dreamless sleep, which may strongly alter circadian rhythms through the use of drugs such as N-methyl-D-aspartate (NMDA) receptor antagonists or gamma-aminobutyric acid (GABA) receptor agonists [22, 25, 26]. On the other hand, RA is more helpful in the relief of sleep disturbances after surgery than GA [27], which may result from better maintenance of the melatonin rhythm postoperatively. However, the effects of these two anesthesia methods on the postoperative melatonin rhythm have not been compared. Therefore, we hypothesize that RA may attenuate the disruption of the melatonin rhythm, which might decrease the incidence of POD in elderly patients undergoing hip surgery. In this report, we present the design of a prospective cohort clinical trial to compare the effects of GA versus RA on the circadian

rhythm of melatonin secretion and the incidence of POD in elderly patients undergoing hip fracture surgery.

METHODS AND ANALYSIS

Trial design

This prospective single-center, 1:1 matched cohort clinical trial is designed to compare the effects of GA and RA on the melatonin rhythm and incidence of POD. We aim to recruit 138 patients with hip fracture (≥65 years of age) who will be assigned to either the GA group (n = 69) or the RA group (n = 69) depending on their situation and personal willingness as well as the preference of the anesthesiologists and surgeons. We will conduct the trial at the Beijing Jishuitan Hospital in China from November 2019 until the target sample size is achieved. Figure 1 shows the flow chart of this study.

Primary outcome

The primary outcome is to compare the circadian rhythm of melatonin secretion between the GA and RA groups and explore its association with the incidence of POD until discharge from the hospital.

Secondary outcomes

The secondary outcomes are to compare the subtype and severity of POD, plasma cortisol levels, C-reactive protein, interleukin-6, sleep quality, and pain until discharge from the hospital, and postoperative cognitive function at 6 months and 1 year after surgery between the GA and RA groups.

Eligibility criteria

Inclusion criteria

Participants will be included if they meet the following criteria: age of ≥65 years, hospital admission for surgical treatment of hip fracture, and American Society of Anesthesiologists (ASA) physical status classification of I to III.

Exclusion criteria

Patients who meet any of the following criteria will be excluded: Parkinson's disease, dementia (including dementia caused by Parkinson's disease, Alzheimer's disease, or Lewy body dementia), a stroke within the prior 6 months, or any other central nervous system disease, alcohol-related disorders, multiple trauma, preoperative delirium, severe deafness or vision problems, communication difficulties, night shift duty, taking medication related to melatonin, a plan to be transferred to the intensive care unit postoperatively, and refusal to participate in the study or unexpected discharge.

Preoperative baseline data collection and evaluation

Trained investigators who are blind to the patient grouping will evaluate the patients and their medical charts to screen out potential participants according to the inclusion and exclusion criteria. Baseline data will be collected, including demographic data (e.g., age, sex, race, education level, height, and weight), surgical diagnosis, comorbidities, medication history, surgical history, smoking and drinking status, and the results of physical, laboratory, and instrumental examinations. We will also record the ASA physical status classification. Specifically, preoperative cognitive scans will be performed using the Mini-Mental State Examination (MMSE) [28]; preoperative delirium will be diagnosed with the Confusion Assessment Method (CAM) [29, 30]; activities of daily

living and instrumental activities of daily living will be assessed with the Katz Index of Independence in Activities of Daily Living (ADL) [31]; sleep quality will be assessed by the Pittsburgh Sleep Quality Index (PSQI) [32]; and pain intensity will be assessed using the numerical rating scale (NRS) [33].

Perioperative management

All individuals will be placed in a standard private room with a consistent light-dark cycle and room temperature during the study period. The lights will be turned off between 20:00 and 06:00 even at the time of blood sample collection, and light exposure (controlled to be < 10 lux) will be measured during the night-time sleep period. The participants will be requested to refrain from alcohol, caffeine, and chocolate. Their activities will be restricted from 20:00 to 06:00, during which time they will be free from interference factors including noise, television, and mobile phones during the study period.

Anesthesia and analgesia

All patients will receive an ultrasound-guided fascia iliac block (a single injection of 30 mL of 0.33% ropivacaine) after admission to the operating room. During the operation, the patients' eyes will be carefully covered with eye patches to avoid light effects. Sedatives (benzodiazepine and dexmedetomidine) or anticholinergics will be avoided during the perioperative phase.

In the RA group, ropivacaine will be used for spinal anesthesia or combined spinal and epidural anesthesia at the L2-3 or L3-4 levels, and the sensory block will be controlled at T8-T10. If regional anesthesia fails, the patients will receive surgery under GA and

will withdraw from this trial to avoid the influence of lumbar puncture. In the GA group, standard GA will be administered to the patients. Anesthesia will be intravenously induced with 0.5–1.5 mg/kg propofol, 0.2 mg/kg etomidate, 0.3 μg/kg sufentanil, and 0.15 mg/kg cisatracurium. Anesthesia will be maintained with sevoflurane, propofol, and remifentanil, and the bispectral (BIS) value will be maintained between 40 and 60. Mechanical ventilation will be continued to keep the end-tidal carbon dioxide concentration at 35 to 40 mmHg in the GA group. A Vigileo/FloTrac device will be used for measuring stroke volume variation and cardiac index to guide fluid therapy. Fluids or vasoactive substances will be administrated to maintain mean arterial pressure (MAP) > 70 mmHg, heart rate < 100 beat per min (bpm), and urine output > 0.5 ml/kg/h. Meanwhile, all patients will be warmed with forced air and intravenous fluids. On the day of surgery, intraoperative parameters will be recorded, including the mean arterial pressure, heart rate, BIS value, start time of anesthesia and surgery, duration and type of anesthesia and surgery, anesthetic drugs and doses, sedative doses, blood loss, fluid balance and transfusion of blood or clotting products, complications, and adverse events.

For postoperative analgesia management, the patients will receive intravenous patient-controlled analgesia (100 μg sufentanil and 8 mg ondansetron mixed with normal saline to a total volume of 100 mL). Intramuscular injection of pethidine (50 mg) or oral use of oxycodone (5 mg)/acetaminophen (325 mg) will be made as remedy analgesia.

Postoperative management and follow-up

All patients will be followed up twice a day (8:00 and 20:00) until discharge from the

hospital. The investigators blind to grouping will record the total hospitalization cost, the in-hospital length of stay, and the occurrence of postoperative complications during hospitalization. Long-term cognitive function will be assessed for 1 year after surgery.

1. Delirium assessment

Delirium will be diagnosed using the CAM, and the subtype and severity of delirium will be assessed by using the Memorial Delirium Assessment Scale [34]. Surveillance will involve twice-daily visits (8:00 and 20:00) by a trained geriatrician. The duration of delirium will be measured as the number of days from onset of delirium symptoms to resolution of symptoms. If the researcher is in doubt regarding this assessment, the diagnosis of POD will be referred to an external expert.

2. Sleep assessment

All participants will continuously wear a sleep tracker (Fitbit Charge 3; Fitbit, Inc., San Francisco, CA, USA), which is an available and inexpensive method to quantify sleep patterns [35]. The investigators will provide instructions on wearing the sleep tracker, a mobile device capable of downloading Fitbit Connect software, and completing the corresponding sleep logs (bedtime, wake time, and nap times). The frequency of syncing will be checked by the research staff weekly, and the Fitbit Charge proprietary algorithm will process the sensor data to automate the sleep variables [36]. All patients will wear sleep trackers until discharge from the hospital. Based on the data from the sleep tracker, sleep logs, and direct observation by a third party, we will derive the following data: total sleep time (min), sleep onset latency (min), wake time after sleep onset (min), time in rapid eye movement sleep,

and time spent in “light sleep” and “deep sleep” (according to Fitbit Inc.). Subjective sleep quality will be assessed with an NRS (0 = worst sleep and 10 = best sleep) [37].

3. The intensity of postoperative pain both at rest and with movement will be evaluated twice daily (8:00 and 20:00) with the NRS.
4. Cognitive function will be assessed by the same geriatrician at 6 months and 1 year after surgery. The Modified Telephone Interview for Cognitive Status will be used to test the patient’s cognitive function in non-face-to-face interviews [37].

Blinding

An investigator will be assigned to preserve and distribute results. Investigators will be not blinded to the study group assignment, but the trained geriatrician responsible for postoperative evaluation including POD diagnosis, as well as PSQI, ADLs, and NRS scores are blinded to the grouping.

Light exposure

Considering that light suppresses melatonin secretion except in life-threatening situations, the only illumination from a penlight will be used during these interventions [15, 19]. Blood sample collection and nursing interventions at night will be performed with a penlight to avoid light exposure. Light intensity readings will be obtained with a digital meter to ensure that light exposure does not exceed 10 lux.

Sample Collection

Usually, the melatonin concentration is low throughout the day and then rises to a peak between 02:00 and 04:00 [15]. To establish the circadian rhythm, the plasma concentration of melatonin will be measured at least four times a day [15, 19]. Postoperative

blood samples will be dynamically collected every day at 04:00, 10:00, 16:00, and 22:00 until the patient is discharged through venipuncture (2 ml per sample) [16, 19]. All blood samples will be collected in the heparinized tubes. The samples will be centrifuged at 3000 rpm for 10 min and will be stored at -80°C until further analysis. We will measure plasma melatonin, cortisol, C-reactive protein (CRP), and interleukin-6 (IL-6). Laboratory technicians will be blinded to the results of all clinical data. The concentration of melatonin will be measured with the enzyme immunoassay method (cat no. RE 54021; IBL International GmbH, Hamburg, Germany) with a limit of detection of 1.6 pg/mL [38]. Plasma cortisol levels will be analyzed using an electrochemiluminescence immunoassay method (Roche Diagnostics GmbH, Germany). To measure inflammation, we will measure plasma C-reactive protein (CRP) and interleukin-6 (IL-6) using immunoturbidimetry (Beckman Coulter, Inc., USA) and electrochemiluminescence immunoassay method (Roche, Germany), respectively.

Sample size estimation

Melatonin is secreted in a biphasic pattern in which nighttime secretion increases from 20:00 to 22:00 with a peak at 02:00 to 04:00, and daytime secretion decreases from 06:00 to 08:00 with a nadir at 12:00 [14, 39]. Cronin et al. [15] researched the nighttime melatonin secretion of adult patients for 3 days after gynecologic surgery and found that the lowest level occurred on the first night postoperatively. In another study of elderly patients undergoing major abdominal surgery, Shigeta et al. [40] found that the postoperative amplitude of nighttime melatonin secretion was significantly lower than the preoperative amplitude. These findings indicate that the peak of melatonin secretion

is suppressed to the greatest degree in the first postoperative night, possibly leading to a melatonin rhythm shift. Therefore, for the present study, we will choose 04:00 on the first postoperative night as the time point for comparing the melatonin concentration between the groups. Our pilot study showed that the mean ± standard deviation of melatonin was 10.48 ± 4.04 and 13.40 ± 6.85 pg/mL in the GA and RA groups (n=15 in each group), respectively. Using a two-sided α value of 0.05 and 85% power and considering a 10% dropout rate, we plan to include 138 patients in this study, with 69 patients in each group (1:1).

Circadian melatonin rhythm data

Circadian markers of the melatonin rhythm, including the mesor (mean concentration) and amplitude (peak value minus the mean value), acrophase time (peak time), will be calculated by Origin (OriginLab Corp, USA) based on cosinor regression $y = a + b*\cos(x*\pi/12-c*\pi/12)$, in which a, b, and c represent mesor, amplitude, and acrophase, respectively [41]. The data will be subsequently smoothed with the cosinor method curve-fitting procedure (GraphPad Prism 8; GraphPad Software, Inc) [41,42].

Adverse events

In the study, the intervention measures of both groups are anesthesia methods currently being used. Therefore, there will be no additional risk to participants. The safety of patients will be monitored, and patients will receive study information including explicit details on whom to contact, and will be told to withdraw from the trial and get the corresponding compensation in case of an adverse event situation.

Data management and storage

At the first meeting, researchers will make appointments for the following dates to improve adherence to the study. All the data will be collected recorded in the Case Report Forms (CRF). After the data is recorded, the data will be stored in a locked cabinet. Data entry will be performed by two investigators with the EpiData3.10 database system. Personal information of participants will be confidentially kept, and all data will be identified by a name acronym and a study identification number in the CRF. The data analysis will be performed by the primary investigator and designated teammates.

Ethics and Declarations

During the study period, we will follow the Declaration of Helsinki and Chinese guidelines of Good Clinical Practice to guarantee the right of the patients. The study protocol has been approved by the Medical Science Research Ethics Committees of Beijing Jishuitan Hospital (JLKS201901-04). This study has been registered at the Chinese Clinical Trial Registry (ChiCTR1900027393). To inform the purpose and risks of the study, each enrolled patient accompanied by at least one family member or proxy will sign a written informed consent. Besides, participants will sign the additional consent provisions for collection and use of participant data and biological specimens in ancillary studies. And all participants and investigators will keep the written informed consent. The primary investigator will regularly report on the progress and changes of the study to the local Ethics Committee. The results of the study will be published in peer-reviewed international journals and will be presented at national and international conferences and symposiums.

Data safety monitoring

The data monitoring committee (DMC) comprised of two specialists outside the trial will be monitoring the progress and safety of the trial. And the DMC can give suggestions on safety issues and even pause the trial.

Statistical analysis

Demographic data and information on pain scores, delirium, and sleep assessment data, and adverse events during and after surgery will be recorded. Data will be presented as mean and standard deviation, median and interquartile range (25th–75th percentile), or frequency and proportion depending on the variable type and distribution. Categorical variables will be assessed with the χ^2 test or Fisher's exact test. Differences in continuous variables between the groups will be assessed with a parametric *t*-test, the Wilcoxon rank-sum test, or the Kruskal–Wallis test. The Mann–Whitney U test will be used to analyze non-normal variables. Results will be presented with 95% confidence intervals. The association between melatonin parameters and sleep quality as well as POD will be also validated by Pearson or Spearman analysis, and linear regression analysis. Multiple imputation methods, i.e., mean completer and regression, will be used to handle missing data. A *P* value of <0.05 will be considered statistically significant. All statistical analyses will be performed with SPSS software, version 21.0 (IBM Corp., Armonk, NY, USA).

DISCUSSION

This clinical trial is designed to test whether RA can relieve perioperative melatonin rhythm disruption compared with GA and decrease the incidence of POD in elderly

patients with hip fracture.

Although some previous studies have shown connections between melatonin secretion disruption and delirium, few have elucidated the real melatonin rhythm pattern [43-45]. For example, a prospective observational study by Yoshitaka et al. showed that the delta plasma melatonin concentration (calculated based on the preoperative and postoperative values at selected time points) at 1 hour after surgery was significantly lower in patients with than without delirium [46]. Melatonin secretion exhibits a biphasic pattern in which daytime secretion is low (5 pg/mL on average) and nighttime secretion is relatively high (50–100 pg/ml) [14, 39]. However, the sample collection time points of melatonin in the above-mentioned study (before the operation, 1 hour after the operation, and at 08:00 on postoperative days 1 and 2) represented either nighttime secretion on the operation day or daytime secretion on the postoperative days; thus, the measurements do not represent the comprehensive rhythm of melatonin. In contrast, in the current study the blood samples will be taken before anesthesia, postoperative blood samples (melatonin) every day at 04:00, 10:00, 16:00 and 22:00 until discharge. These measurements are adequate to establish a melatonin rhythm curve. They will not only show the individual postoperative melatonin concentrations but also allow us to analyze the irregular pattern of melatonin secretion in patients with delirium.

Previous trials have shown that GA may influence postoperative melatonin secretion [22-24]. First, in patients who received combined intravenous and inhaled anesthesia, the melatonin levels decreased the first night after surgery [24, 47]. Second, GA by inhalation was associated with a significant decrease in the melatonin concentration on the night

of surgery, and this decrease was 350% greater than that on the third night after surgery [15]. Third, total intravenous anesthesia induced a phase delay in the 6-SMT rhythm; the peak time was late by about 1 hour, and the amplitude of the melatonin rhythm decreased by 85% on the night of surgery [12]. In contrast, another study demonstrated that RA was helpful to relieve sleep disturbance after surgery [27], which may result from better maintenance of the melatonin rhythm postoperatively. However, little is known about how the two anesthesia methods differently affect the melatonin rhythm. Karkela et al. [24] conducted a similar clinical study and found that anesthesia and surgery disturbed the circadian melatonin rhythm, but there was no significant difference in the melatonin secretion between the spinal anesthesia and GA groups for minor orthopedic operations [24]. We do not consider that these results are especially robust and generalizable because of several limitations of the trial, such as the small sample size (40 participants), measurement of only the saliva and urine concentrations of melatonin (no plasma or serum concentrations), and insufficient melatonin measurement timing (measurement of only nighttime secretion levels). We hypothesize that RA can maintain an almost normal melatonin rhythm for the following two reasons. First, RA may significantly reduce the types and amounts of general anesthetics used compared with GA, which can strongly alter melatonin secretion through different signal pathways. Second, RA allows better pain control and improved sleep quality compared with GA, which further facilitates a decrease in the severity of melatonin rhythm disruption.

CONCLUSION

In conclusion, to our knowledge, this is the first study to compare the circadian rhythm of melatonin secretion between GA and RA groups and to explore the potential association between circadian rhythm disruptions and the development of delirium. Our findings will promote a greater understanding of delirium pathophysiology and provide clinical evidence for the optimal anesthesia method in elderly patients undergoing hip fracture surgery.

Contributorship statement: ZQL and XYG obtained funding. ZQL, WCZ, GW, and XYG contributed to the study design. YY, YNS, YYJ, and YZ performed the trial. XNM ,XXJ and YZH contributed to data collection. CL, YL, CMS and XXW analyzed the data. YNS and YY drafted the manuscript. ZQL and WCZ carefully reviewed the manuscript. All authors have read and approved the final manuscript.

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Figure legend

Figure 1 Flow chart of the trial. ASA, American Society of Anesthesiologists; CAM, Confusion Assessment Method; MMSE, Mini-Mental State Examination; ADL, activities of daily living; PSQI, Pittsburgh Sleep Quality Index; IL, interleukin; CRP, C-reactive protein; Cor, cortisol; MAP, mean arterial pressure; HR, heart rate; BIS, bispectral index; MDAS, Memorial Delirium Assessment Scale; TICS-m, Modified telephone interview for cognitive status.

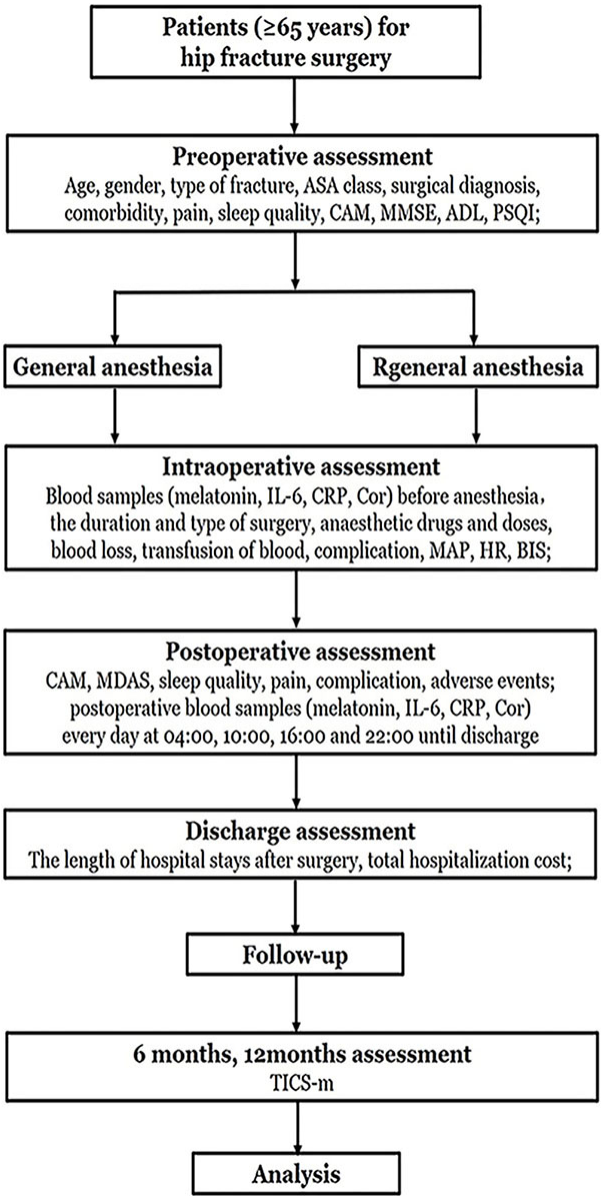

Figure 1

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SPIRIT
STANDARD PROTOCOL ITEMS: RECOMMENDATIONS FOR INTERVENTIONAL TRIALS

SPIRIT 2013 Checklist: Recommended items to address in a clinical trial protocol and related documents*

Section/item	Item No	Description	Addressed on page number
Administrative information			
Title	1	Descriptive title identifying the study design, population, interventions, and, if applicable, trial acronym	Page 1, line 2-4
Trial registration	2a	Trial identifier and registry name. If not yet registered, name of intended registry	Page 15, line 319
	2b	All items from the World Health Organization Trial Registration Data Set	Page 4, line 71-73
Protocol version	3	Date and version identifier	Page 4, line 72-73
Funding	4	Sources and types of financial, material, and other support	Page 19, line 410
Roles and responsibilities	5a	Names, affiliations, and roles of protocol contributors	Page 19, line 402
	5b	Name and contact information for the trial sponsor	Page 19, line 409
	5c	Role of study sponsor and funders, if any, in study design; collection, management, analysis, and interpretation of data; writing of the report; and the decision to submit the report for publication, including whether they will have ultimate authority over any of these activities	Page 19, line 402
	5d	Composition, roles, and responsibilities of the coordinating centre, steering committee, endpoint adjudication committee, data management team, and other individuals or groups overseeing the trial, if applicable (see Item 21a for data monitoring committee)	Page 16, line 329

Background and rationale	6a	Description of research question and justification for undertaking the trial, including summary of relevant studies (published and unpublished) examining benefits and harms for each intervention	Page 5, line 93-132
	6b	Explanation for choice of comparators	Page 5, line 119-132
Objectives	7	Specific objectives or hypotheses	Page 6, line 127-129
Trial design	8	Description of trial design including type of trial (eg, parallel group, crossover, factorial, single group), allocation ratio, and framework (eg, superiority, equivalence, noninferiority, exploratory)	Page 7, line 135
Methods: Participants, interventions, and outcomes			
Study setting	9	Description of study settings (eg, community clinic, academic hospital) and list of countries where data will be collected. Reference to where list of study sites can be obtained	Page 7, line 140
Eligibility criteria	10	Inclusion and exclusion criteria for participants. If applicable, eligibility criteria for study centres and individuals who will perform the interventions (eg, surgeons, psychotherapists)	Page 8, line 153-163
Interventions	11a	Interventions for each group with sufficient detail to allow replication, including how and when they will be administered	Page 7, line 136-139
	11b	Criteria for discontinuing or modifying allocated interventions for a given trial participant (eg, drug dose change in response to harms, participant request, or improving/worsening disease)	Page 9, line 195-196
	11c	Strategies to improve adherence to intervention protocols, and any procedures for monitoring adherence (eg, drug tablet return, laboratory tests)	Page 15, line 307
	11d	Relevant concomitant care and interventions that are permitted or prohibited during the trial	Page 9, line 191-192
Outcomes	12	Primary, secondary, and other outcomes, including the specific measurement variable (eg, systolic blood pressure), analysis metric (eg, change from baseline, final value, time to event), method of aggregation (eg, median, proportion), and time point for each outcome. Explanation of the clinical relevance of chosen efficacy and harm outcomes is strongly recommended	Page 7, line 142-150

Participant timeline	13	Time schedule of enrolment, interventions (including any run-ins and washouts), assessments, and visits for participants. A schematic diagram is highly recommended (see Figure)	Page 8, line 164-186, 216- 246
Sample size	14	Estimated number of participants needed to achieve study objectives and how it was determined, including clinical and statistical assumptions supporting any sample size calculations	Page 13, line 275-291
Recruitment	15	Strategies for achieving adequate participant enrolment to reach target sample size	Page 13, line 275-291
Methods: Assignment of interventions (for controlled trials)			
Allocation:			
Sequence generation	16a	Method of generating the allocation sequence (eg, computer-generated random numbers), and list of any factors for stratification. To reduce predictability of a random sequence, details of any planned restriction (eg, blocking) should be provided in a separate document that is unavailable to those who enrol participants or assign interventions	Page 7, line 137-139
Allocation concealment mechanism	16b	Mechanism of implementing the allocation sequence (eg, central telephone; sequentially numbered, opaque, sealed envelopes), describing any steps to conceal the sequence until interventions are assigned	Page 7, line 136-139
Implementation	16c	Who will generate the allocation sequence, who will enrol participants, and who will assign participants to interventions	Page 12, line 247-251
Blinding (masking)	17a	Who will be blinded after assignment to interventions (eg, trial participants, care providers, outcome assessors, data analysts), and how	Page 12, line 247-251
	17b	If blinded, circumstances under which unblinding is permissible, and procedure for revealing a participant's allocated intervention during the trial	Page 12, line 247-251
Methods: Data collection, management, and analysis			
Data collection methods	18a	Plans for assessment and collection of outcome, baseline, and other trial data, including any related processes to promote data quality (eg, duplicate measurements, training of assessors) and a description of study instruments (eg, questionnaires, laboratory tests) along with their reliability and validity, if known. Reference to where data collection forms can be found, if not in the protocol	Page 8, line 172-177

	18b	Plans to promote participant retention and complete follow-up, including list of any outcome data to be collected for participants who discontinue or deviate from intervention protocols	Page 10, line 218-247
Data management	19	Plans for data entry, coding, security, and storage, including any related processes to promote data quality (eg, double data entry; range checks for data values). Reference to where details of data management procedures can be found, if not in the protocol	Page 15, line 310
Statistical methods	20a	Statistical methods for analysing primary and secondary outcomes. Reference to where other details of the statistical analysis plan can be found, if not in the protocol	Page 16, line 332-346
	20b	Methods for any additional analyses (eg, subgroup and adjusted analyses)	Page 16, line 332-346
	20c	Definition of analysis population relating to protocol non-adherence (eg, as randomised analysis), and any statistical methods to handle missing data (eg, multiple imputation)	Page 16, line 343-344
Methods: Monitoring			
Data monitoring	21a	Composition of data monitoring committee (DMC); summary of its role and reporting structure; statement of whether it is independent from the sponsor and competing interests; and reference to where further details about its charter can be found, if not in the protocol. Alternatively, an explanation of why a DMC is not needed	Page 16, line 330 - 332
	21b	Description of any interim analyses and stopping guidelines, including who will have access to these interim results and make the final decision to terminate the trial	Page 16, line 329 - 331
Harms	22	Plans for collecting, assessing, reporting, and managing solicited and spontaneously reported adverse events and other unintended effects of trial interventions or trial conduct	Page 14, line 300-304
Auditing	23	Frequency and procedures for auditing trial conduct, if any, and whether the process will be independent from investigators and the sponsor	Page 15, line 324-325
Ethics and dissemination			
Research ethics approval	24	Plans for seeking research ethics committee/institutional review board (REC/IRB) approval	Page 15, line 318

Protocol amendments	25	Plans for communicating important protocol modifications (eg, changes to eligibility criteria, outcomes, analyses) to relevant parties (eg, investigators, REC/IRBs, trial participants, trial registries, journals, regulators)	Page 15, line 325
Consent or assent	26a	Who will obtain informed consent or assent from potential trial participants or authorised surrogates, and how (see Item 32)	Page 15, line 323-324
	26b	Additional consent provisions for collection and use of participant data and biological specimens in ancillary studies, if applicable	Page 15, line 321-323
Confidentiality	27	How personal information about potential and enrolled participants will be collected, shared, and maintained in order to protect confidentiality before, during, and after the trial	Page 15, line 310-312
Declaration of interests	28	Financial and other competing interests for principal investigators for the overall trial and each study site	Page 19, line 413
Access to data	29	Statement of who will have access to the final trial dataset, and disclosure of contractual agreements that limit such access for investigators	Page 15, line 312
Ancillary and post-trial care	30	Provisions, if any, for ancillary and post-trial care, and for compensation to those who suffer harm from trial participation	Page 14, line 303-304
Dissemination policy	31a	Plans for investigators and sponsor to communicate trial results to participants, healthcare professionals, the public, and other relevant groups (eg, via publication, reporting in results databases, or other data sharing arrangements), including any publication restrictions	Page 15, line 325-327
	31b	Authorship eligibility guidelines and any intended use of professional writers	Page 16, line 329
	31c	Plans, if any, for granting public access to the full protocol, participant-level dataset, and statistical code	Page 16, line 329
Appendices			
Informed consent materials	32	Model consent form and other related documentation given to participants and authorised surrogates	Page 15, line 323-324
Biological specimens	33	Plans for collection, laboratory evaluation, and storage of biological specimens for genetic or molecular analysis in the current trial and for future use in ancillary studies, if applicable	Page 13, line 263-274

*It is strongly recommended that this checklist be read in conjunction with the SPIRIT 2013 Explanation & Elaboration for important clarification on the items. Amendments to the protocol should be tracked and dated.