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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

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Department of Anesthesiology

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4 47 **Effects of general versus regional anesthesia on circadian melatonin**
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6 48 **rhythm and its association with postoperative delirium in elderly patients**
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9 49 **undergoing hip fracture surgery: study protocol for a prospective cohort**
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11 50 **clinical trial**
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15 51 **Abstract**

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19 52 **Introduction:** Postoperative delirium (POD) is a common neurological
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21
22 53 complication after hip fracture surgery and is associated with high morbidity and
23
24 54 mortality in elderly patients. Although, the specific mechanism of POD remains unclear,
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26
27 55 circadian rhythm disruptions have recently drawn increased attention. To date, only
28
29
30 56 limited postoperative time points of plasma melatonin level measurements were
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32 57 recorded in previous studies, and such data cannot represent a comprehensive
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34
35 58 melatonin rhythm. The process of anesthesia (either general anesthesia [GA] or regional
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38 59 anesthesia [RA]) is known to influence the melatonin rhythm. However, how these two
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41 60 anesthesia methods differently affect the postoperative melatonin rhythm is still
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43 61 unknown. Therefore, we hypothesized that RA may attenuate disruption of the
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45 62 melatonin rhythm, which might decrease the incidence of POD in elderly patients
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47
48 63 undergoing hip surgery.

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51 64 **Methods and analysis:** In this prospective cohort clinical trial, 140 patients
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54 65 scheduled for hip fracture surgery will be divided into two groups to receive either GA
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56
57 66 or RA. The primary aim is to compare the circadian rhythm of melatonin secretion
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59
60 67 between the two groups and explore its association with the incidence of POD.

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4 68 **Ethics and dissemination:** The study is ethically approved by the Medical
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6 69 Science Research Ethics Committees of Beijing Jishuitan Hospital (JLKS201901-04).
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8
9 70 The results of the study will be published in peer-reviewed international journals.
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11

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13 71 **Trial registration number:** Chinese Clinical Trial Registry,
14
15 72 ChiCTR1900027393.
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19 73 **Keywords:** Postoperative delirium; Circadian rhythm; Melatonin; General
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21 74 anesthesia; Regional anesthesia; Elderly patients.
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26 76 **Strengths and limitations of this study**

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28
29 77 (1) A major strength of this study is that to our knowledge, this is the first trial to
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31 78 evaluate the effects of different anesthesia methods (RA and GA) on the circadian
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33 79 rhythm of melatonin secretion and explore their association with POD in elderly
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35 80 patients undergoing hip fracture surgery.
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39 81 (2) Both objective and subjective sleep quality are included in the current study. This
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41 82 can provide a better and more comprehensive understanding of the connection
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43 83 between circadian rhythms and anesthesia.
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47 84 (3) The limitation of trial is that melatonin is considered to be an optimal peripheral
48
49 85 biomarker of circadian timing, more biomarkers such as cytokines and genes
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51 86 (peripheral clock gene) should be included.
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55 87 (4) Although we will record both subjective and objective sleep quality, such
56
57 88 measurements are incomplete, for example polysomnography is the gold standard
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4 89 for diagnosis of sleep disorders.
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6 90 (5) Our study will be conducted until discharge from the hospital, which might be
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8
9 91 insufficient for delirium screening.
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11 12 13 92 **INTRODUCTION** 14

15
16 93 Postoperative delirium (POD), a common neurological complication in elderly patients
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18 94 undergoing hip fracture surgery (range, 4%–53%), is usually associated with increased
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20 95 morbidity and mortality rates, long-term cognitive decline, and a heavier healthcare
21
22 96 economic burden.¹⁻⁶ Many studies have indicated that preoperative brain dysfunction
23
24 97 (frail brain) in elderly patients is a predisposing factor for POD^{7, 8}, while the
25
26 98 perioperative change of neuroinflammatory markers (e.g., C-reactive protein and
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28 99 interleukin-6), oxidative stressor markers (cortisol) and the crucial hormone
29
30 100 (melatonin) within elderly frail brain are the precipitating factor for POD.^{8,9} However,
31
32 101 the specific mechanism of POD remains unclear.^{9,10}
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34 102 Circadian rhythm disruptions have recently drawn increased attention as a possible
35
36 103 mechanism of POD.^{10, 11} Circadian rhythms refer to the behavioral and physiological
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38 104 cycles that occur within a period of approximately 24 hours, including the sleep–wake
39
40 105 cycles, hormone secretion (e.g., melatonin), and core body temperature.¹² Among these
41
42 106 indicators, plasma melatonin is considered to be the best peripheral marker of
43
44 107 endogenous circadian rhythms.¹³⁻¹⁵ Previous studies have revealed potential
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46 108 correlations between plasma melatonin secretion disruption and POD.^{10, 16-20}
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48 109 Nevertheless, only limited postoperative time points (e.g., once a day) of plasma
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4 110 melatonin level measurements were recorded in those studies, and such data cannot
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6 111 represent a comprehensive melatonin rhythm. Moreover, one study indicated that the
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9 112 urinary excretion of 6-sulfatoxymelatonin (6-SMT), the main metabolite of melatonin,
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11 113 during delirium was associated with clinical subtypes of delirium (higher in hypoactive
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14 114 and lower in hyperactive delirium).²¹ However, measuring the melatonin level in a urine
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17 115 sample instead of a plasma sample at only one time point is insufficient to describe the
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19 116 real pattern of the melatonin rhythm in the acute phase of delirium. Therefore, the exact
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22 117 association between the melatonin rhythm and delirium requires further exploration.

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25
26 118 The process of anesthesia (either general anesthesia [GA] or regional anesthesia [RA])
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28 119 is known to influence the circadian rhythms.²²⁻²⁴ On one hand, GA is associated with
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31 120 unconsciousness similar to a dreamless sleep, which may strongly alter circadian
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34 121 rhythms through the use of drugs such as N-methyl-D-aspartate (NMDA) receptor
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36 122 antagonists or gamma-aminobutyric acid (GABA) receptor agonists^{22, 25, 26}. On the other
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39 123 hand, RA is more helpful in the relief of sleep disturbances after surgery than GA²⁷,
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41 124 which may result from better maintenance of the melatonin rhythm postoperatively.
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44 125 However, the effects of these two anesthesia methods on the postoperative melatonin
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46 126 rhythm have not been compared. Therefore, we hypothesized that RA may attenuate
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49 127 disruption of the melatonin rhythm, which might decrease the incidence of POD in
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52 128 elderly patients undergoing hip surgery. In this report, we present the design of a
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55 129 prospective cohort clinical trial to compare the effects of GA versus RA on the circadian
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4 130 rhythm of melatonin secretion and the incidence of POD in elderly patients undergoing
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6 131 hip fracture surgery.
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10 132 **METHODS AND ANALYSIS**

11 12 13 133 **Trial design**

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15 134 This prospective cohort clinical trial is designed to compare the effects of GA and RA on
16
17
18 135 the melatonin rhythm and incidence of POD. We aim to recruit 140 patients with hip
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20 136 fracture (≥ 65 years of age) who will be assigned to either the GA group (n = 70) or RA
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22
23 137 group (n = 70) depending on their individual situation and personal willingness as well
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25
26 138 as the preference of the anesthesiologists and surgeons. We will conduct the trial at the
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28 139 Beijing Jishuitan Hospital in China. Figure 1 shows the flow chart of this study.
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31 140 **Primary outcome**

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33 141 The primary outcome is to compare the circadian rhythm of melatonin secretion
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36 142 between the GA and RA groups and explore its association with the incidence of POD
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39 143 until discharge from the hospital.
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41 144 **Secondary outcomes**

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44 145 The secondary outcomes are to compare the subtype and severity of POD, sleep quality,
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47 146 and pain until discharge from the hospital, and postoperative cognitive function at 6
48
49 147 months and 1 year after surgery between the GA and RA groups.
50

51 148 **Ethics approval**

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53
54 149 The study protocol has been approved by the Medical Science Research Ethics
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56
57 150 Committees of Beijing Jishuitan Hospital (JLKS201901-04). Written informed consent
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4 151 will be obtained from each enrolled patient accompanied by at least one family member
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6 152 or proxy. This study has been registered at the Chinese Clinical Trial Registry
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9 153 (ChiCTR1900027393).

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12 154 **Eligibility criteria**

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14 155 ***Inclusion criteria***

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17 156 Participants will be included if they meet the following criteria: age of ≥ 65 years,
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19 157 hospital admission for surgical treatment of hip fracture, and American Society of
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21
22 158 Anesthesiologists (ASA) physical status classification of I to III.

23
24 159 ***Exclusion criteria***

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27 160 Patients who meet any of the following criteria will be excluded: Parkinson's disease,
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29 161 dementia (including dementia cause by Parkinson's disease, Alzheimer's disease, or
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32 162 Lewy body dementia), a stroke within the prior 6 months or any other central nervous
33
34
35 163 system disease, alcohol-related disorders, multiple trauma, preoperative delirium,
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37 164 severe deafness or vision problems, communication difficulties, night shift duty, taking
38
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40 165 medication related to melatonin, a plan to be transferred to the intensive care unit
41
42
43 166 postoperatively, and refusal to participate in the study or unexpected discharge.

44
45 167 **Preoperative baseline data collection and evaluation**

46
47
48 168 Trained investigators who are blind to the patient grouping will evaluate the patients
49
50 169 and their medical charts to screen out potential participants according to the inclusion
51
52
53 170 and exclusion criteria. Baseline data will be collected, including demographic data (e.g.,
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56 171 age, sex, race, education level, height, and weight), surgical diagnosis, comorbidities,
57
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59 172 medication history, surgical history, smoking and drinking status, and the results of
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4 173 physical, laboratory, and instrumental examinations. We will also record the ASA
5
6 174 physical status classification. Specifically, preoperative cognitive scans will be
7
8
9 175 performed using the Mini-Mental State Examination (MMSE) ²⁸; preoperative delirium
10
11 176 will be diagnosed with the Confusion Assessment Method (CAM) ^{29,30}; activities of daily
12
13 177 living and instrumental activities of daily living will be assessed with the Katz Index of
14
15 178 Independence in Activities of Daily Living ³¹; sleep quality will be assessed by the
16
17 179 Pittsburgh Sleep Quality Index (PSQI) ³²; and pain intensity will be assessed using the
18
19 180 numerical rating scale (NRS) ³³.

181 **Preoperative management**

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

189 **Anesthesia and analgesia**

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

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

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4 195 and a muscle relaxant (rocuronium or cisatracurium). Sevoflurane will be used for
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6 196 anesthesia maintenance, remifentanyl for analgesia, and rocuronium or cisatracurium
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9 197 for muscle relaxation. Mechanical ventilation will be continued to keep the end-tidal
10
11
12 198 carbon dioxide concentration at 35 to 40 mmHg, and the bispectral index will be
13
14 199 maintained between 40 and 60.

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16
17 200 In the RA group, bupivacaine or ropivacaine will be used for spinal anesthesia or
18
19 201 combined spinal and epidural anesthesia at the L2-3 or L3-4 levels.

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21
22 202 On the day of surgery, intraoperative parameters will be recorded, including the mean
23
24 203 arterial pressure, heart rate, bispectral index, start time, duration and type of anesthesia
25
26 204 and surgery, anesthetic drugs and doses, sedative doses, blood loss, fluid balance and
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28 205 transfusion of blood or clotting products, complications, and adverse events.

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31 206 For postoperative analgesia management, the patients will receive intravenous patient-
32
33 207 controlled analgesia (sufentanil 100 µg and ondansetron 8 mg mixed with normal saline
34
35 208 to a total volume of 100 mL).

36 37 38 39 40 209 **Postoperative management and follow-up**

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43 210 All patients will be followed up twice a day until discharge from the hospital. The
44
45 211 investigators blind to grouping will record the total hospitalization cost, the in-hospital
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47 212 length of stay, and the occurrence of postoperative complications during hospitalization.
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50 213 Long-term cognitive function will be assessed for 1 year after surgery.

51 52 53 214 1. Delirium assessment

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56 215 Delirium will be diagnosed using the CAM, and the subtype and severity of delirium
57
58 216 will be assessed by using the Memorial Delirium Assessment Scale³⁴. Surveillance

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4 217 will involve twice-daily visits (8:00 and 20:00) by a trained geriatrician. The
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6 218 duration of delirium will be measured as the number of days from onset of delirium
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9 219 symptoms to resolution of symptoms. If the researcher is in doubt regarding this
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12 220 assessment, the diagnosis of POD will be referred to an external expert.

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14 221 2. Sleep assessment

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17 222 All participants will continuously wear a sleep tracker (Fitbit Charge 3; Fitbit, Inc.,
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19 223 San Francisco, CA, USA), which is an available and inexpensive method to quantify
20
21
22 224 sleep patterns ³⁵. The investigators will provide instructions on wearing the sleep
23
24
25 225 tracker, a mobile device capable of downloading Fitbit Connect software, and
26
27 226 completing the corresponding sleep logs (bedtime, wake time, and nap times). The
28
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30 227 frequency of syncing will be checked by the research staff weekly, and the Fitbit
31
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33 228 Charge proprietary algorithm will process the sensor data to automate the sleep
34
35 229 variables ³⁶. All patients will wear sleep tracker until discharge from hospital. Based
36
37
38 230 on the data from the sleep tracker, sleep logs, and direct observation by a third party,
39
40 231 we will derive the following data: total sleep time (min), sleep onset latency (min),
41
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43 232 wake time after sleep onset (min), time in rapid eye movement sleep , and time spent
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45 233 in “light sleep” and “deep sleep” (according to Fitbit Inc.). Subjective sleep quality
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48 234 will be assessed with an NRS (0 = worst sleep and 10 = best sleep) ³⁷.

49
50 235 3. The intensity of postoperative pain both at rest and with movement will be evaluated
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52
53 236 twice daily with the NRS.

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56 237 4. Cognitive function will be assessed by the same geriatrician at 6 months and 1 year
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59 238 after surgery. The Modified Telephone Interview for Cognitive Status will be used to
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4 239 test the patient's cognitive function in non-face-to-face interviews ³⁷.

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7 240 **Blinding**

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9 241 An investigator will be assigned to preserve and distribute randomization results.

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11 242 Investigators were not blinded to the study group assignment, but the trained

12
13 243 geriatrician responsible for postoperative evaluation including POD diagnosis, as well

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15 244 as PSQI, IADLs, and NRS scores are blinded to the grouping.

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19 245 **Light exposure**

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21 246 Considering that light suppresses melatonin secretion except in life-threatening

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23 247 situations, only illumination from a penlight will be used during these interventions ¹⁵,

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25 248 ¹⁹. Blood sample collection and nursing interventions at night will be performed with a

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27 249 penlight to avoid light exposure. Light intensity readings will be obtained with a digital

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29 250 meter to ensure that light exposure does not exceed 10 lux.

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33 251 **Sample Collection**

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35 252 Usually, the melatonin concentration is low throughout the day and then rises to a peak

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37 253 between 02:00 and 04:00 ¹⁵. To establish the circadian rhythm, the plasma

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39 254 concentration of melatonin will be measured at least four times a day ^{15, 19}. Postoperative

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41 255 blood samples will be dynamically collected every day at 04:00, 10:00, 16:00, and 22:00

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43 256 until the patient discharged through venipuncture in the heparinized tube (2 ml per

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45 257 sample) ^{16, 19}. The samples will be stored at 4°C and brought to the laboratory in a

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47 258 nitrogen canister, where they will be stored at -80°C. The concentration of melatonin

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49 259 will be measured with the enzyme immunoassay method (cat no. RE 54021; IBL

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58 260 International GmbH, Hamburg, Germany) with a limit of detection of 1.6 pg/mL ³⁸.

261 **Sample size estimation**

262 Melatonin is secreted in a biphasic pattern in which nighttime secretion increases from
263 20:00 to 22:00 with a peak at 02:00 to 04:00, and daytime secretion decreases from
264 06:00 to 08:00 with a nadir at 12:00^{14, 39}. Cronin et al.¹⁵ researched the nighttime
265 melatonin secretion of adult patients for 3 days after gynecologic surgery and found that
266 the lowest level occurred on the first night postoperatively. In another study of elderly
267 patients undergoing major abdominal surgery, Shigeta et al.⁴⁰ found that the
268 postoperative amplitude of nighttime melatonin secretion was significantly lower than
269 the preoperative amplitude. These findings indicate that the peak of melatonin secretion
270 is suppressed to the greatest degree in the first postoperative night, possibly leading to
271 a melatonin rhythm shift. Therefore, for the present study, we will choose 04:00 on the
272 first postoperative night as the time point for comparing the melatonin concentration
273 between the groups. Our pilot study showed that the mean \pm standard deviation of
274 melatonin was 10.5 ± 4.0 and 13.4 ± 6.9 pg/mL in the GA and RA groups (n=15 in each
275 group), respectively. Using a two-sided α value of 0.05 and 85% power and considering
276 a 10% dropout rate, we plan to include 140 patients in this study, with 70 patients in
277 each group (1:1).

278 **Circadian melatonin rhythm data**

279 Circadian markers of the melatonin rhythm, including the mesor (mean concentration)
280 and amplitude (peak value minus the mean value), acrophase time (peak time), will be
281 calculated by Chronos-Fit program data and cosinor software⁴¹. The area under the
282 curve (AUC) will be calculated as a measure of the secreted amount of melatonin with

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4 283 the trapezoidal method. The data will be subsequently smoothed with the Lowess
5
6 284 (Cleveland) curve-fitting procedure curve-fitting procedure ⁴².

285 **Statistical analysis**

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

297 **DISCUSSION**

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

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

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4 305 postoperative values at selected time points) at 1 hour after surgery was significantly
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6 306 lower in patients with than without delirium ⁴⁶. Melatonin secretion exhibits a biphasic
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9 307 pattern in which daytime secretion is low (5 pg/mL on average) and nighttime secretion
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11 308 is relatively high (50–100 pg/ml) ^{14, 39}. However, the sample collection time points of
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14 309 melatonin in the above-mentioned study (before the operation, 1 hour after the
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17 310 operation, and at 08:00 on postoperative days 1 and 2) represented either nighttime
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19 311 secretion on the operation day or daytime secretion on the postoperative days; thus, the
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22 312 measurements do not represent the comprehensive rhythm of melatonin. In contrast,
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25 313 in the current study the blood samples will be taken before anesthesia, postoperative
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27 314 blood samples (melatonin) every day at 04:00, 10:00, 16:00 and 22:00 until discharge.
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30 315 These measurements are adequate to establish a melatonin rhythm curve. They will not
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32 316 only show the individual postoperative melatonin concentrations but also allow us to
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35 317 analyze the irregular pattern of melatonin secretion in patients with delirium.
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37
38 318 Previous trials have shown that GA may influence postoperative melatonin secretion ²²⁻
39
40 319 ²⁴. First, in patients who received combined intravenous and inhaled anesthesia, the
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42
43 320 melatonin levels decreased the first night after surgery ^{24, 47}. Second, GA by inhalation
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46 321 was associated with a significant decrease in the melatonin concentration on the night
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48 322 of surgery, and this decrease was 350% greater than that on the third night after surgery
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51 323 ¹⁵. Third, total intravenous anesthesia induced a phase delay in the 6-SMT rhythm; the
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53 324 peak time was late by about 1 hour, and the amplitude of the melatonin rhythm
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56 325 decreased by 85% on the night of surgery ¹². In contrast, another study demonstrated
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58 326 that RA was helpful to relieve sleep disturbance after surgery ²⁷, which may result from
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4 327 better maintenance of the melatonin rhythm postoperatively. However, little is known
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6 328 about how the two anesthesia methods differently affect the melatonin rhythm. Karkela
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9 329 et al. ²⁴ conducted a similar clinical study and found that anesthesia and surgery
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11 330 disturbed the circadian melatonin rhythm, but there was no significant difference in the
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14 331 melatonin secretion between the spinal anesthesia and GA groups for minor orthopedic
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17 332 operations ²⁴. We do not consider that these results are especially robust and
18
19 333 generalizable because of several limitations of the trial, such as the small sample size
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22 334 (40 participants), measurement of only the saliva and urine concentrations of
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25 335 melatonin (no plasma or serum concentrations), and insufficient melatonin
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27 336 measurement timing (measurement of only nighttime secretion levels). We hypothesize
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29
30 337 that RA can maintain an almost normal melatonin rhythm for the following two reasons.
31
32 338 First, RA may significantly reduce the types and amounts of general anesthetics used
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35 339 compared with GA, which can strongly alter melatonin secretion through different
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38 340 signal pathways. Second, RA allows better pain control and improved sleep quality
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41 341 compared with GA, which further facilitates a decrease in the severity of melatonin
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43 342 rhythm disruption.

343 **CONCLUSION**

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

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4 349 undergoing hip fracture surgery.
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7 350 **Declarations**

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9 351 The results of the study will be published in peer-reviewed international journals and will be
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11 352 presented at national and international conferences and symposiums.
12

13
14 353 **Acknowledgments:** Not applicable
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17 354 **Supplementary Material**

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19
20 355 **Contributors:** YS and YY are joint first authors. ZL and XG obtained funding. XG, ZL and GW
21
22 356 designed the study. YS, YY, YJ and WZ collected the data. LY and YC were involved in data
23
24 357 cleaning, mortality follow-up, and verification. JL, CY, and DS analyzed the data. YS, YY and ZL
25
26 358 drafted the manuscript. WZ contributed to the interpretation of the results and critical revision
27
28 359 of the manuscript for important intellectual content and approved the final version of the
29
30 360 manuscript. All authors have read and approved the final manuscript. ZL and WZ are the study
31
32 361 guarantors.
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43
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50 366 **Competing interests:** The authors declare that they have no competing interests.
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53 367 **Patient consent:** Obtained.
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4 368 **Ethics approval:** The study was approved by the research ethics board of
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6 369 Beijing Jishuitan Hospital (JLKS201901-04). All participants and their caregivers will provide
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9 370 written consent to participate in the study.
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13 371 **Data sharing:** No additional data are available.
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14 503 **Figuer legend**

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17 504 **Figure 1 Flow chart of the trial.** ASA, American Society of Anesthesiologists; CAM,

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19 505 Confusion Assessment Method; MMSE, Mini-Mental State Examination; ADL,

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21 506 activities of daily living; PSQI, Pittsburgh Sleep Quality Index; IL, interleukin; CRP, C-

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23 507 reactive protein; Cor, cortisol; MAP, mean arterial pressure; HR, heart rate; BIS,

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25 508 bispectral index; MDAS, Memorial Delirium Assessment Scale; TICS-m, Modified

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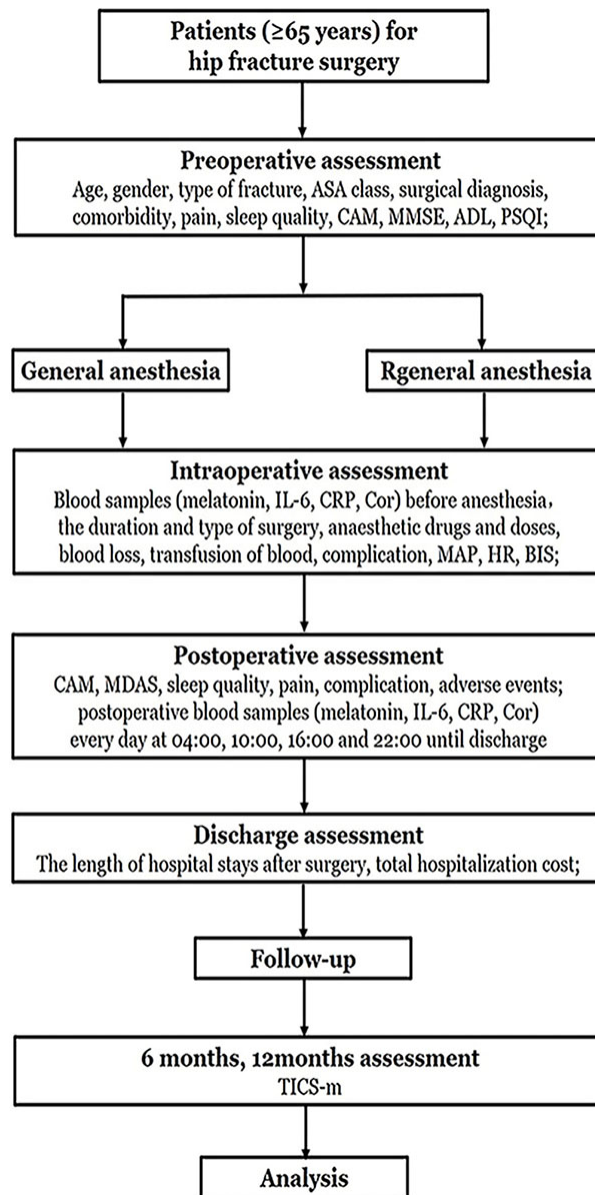


Figure 1

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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

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Yi Yuan and Yanan Song contributed equally to this study.

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32 **Word count**

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4 47 **Effects of general versus regional anesthesia on circadian melatonin**
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9 49 **undergoing hip fracture surgery: study protocol for a prospective cohort**
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11 50 **clinical trial**
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15 51 **Abstract**
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19 52 **Introduction:** Postoperative delirium (POD) is a common neurological
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22 53 complication after hip fracture surgery, and is associated with high morbidity and
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24 54 mortality in elderly patients. Although, the specific mechanism of POD remains unclear,
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27 55 circadian rhythm disruptions have recently drawn increased attention. To date, only
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30 56 limited postoperative time points of plasma melatonin level measurements were
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32 57 recorded in previous studies, and such data cannot represent a comprehensive
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35 58 melatonin rhythm. The process of anesthesia (either general anesthesia [GA] or regional
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37 59 anesthesia [RA]) is known to influence the melatonin rhythm. However, how these two
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40 60 anesthesia methods differently affect the postoperative melatonin rhythm is still
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42 61 unknown. Therefore, we hypothesize that RA may attenuate the disruption of the
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44 62 melatonin rhythm, which might decrease the incidence of POD in elderly patients
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47 63 undergoing hip surgery.
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51 64 **Methods and analysis:** In this prospective cohort clinical trial, 138 patients
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53 65 scheduled for hip fracture surgery will be divided into two groups to receive either GA
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55 66 or RA. The primary aim is to compare the circadian rhythm of melatonin secretion
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58 67 between the two groups and explore its association with the incidence of POD.
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4 68 **Ethics and dissemination:** The study has been approved by the Medical
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6 69 Science Research Ethics Committees of Beijing Jishuitan Hospital (JLKS201901-04).
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9 70 The results of the study will be published in peer-reviewed international journals.
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13 71 **Trial registration number:** The study has been registered at the Chinese
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15 72 Clinical Trial Registry (www.chictr.org.cn) with identifier ChiCTR1900027393. The
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18 73 latest of the trial protocol has been approved on 11 November 2019.
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22 74 **Keywords:** Postoperative delirium; Circadian rhythm; Melatonin; General
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24 75 anesthesia; Regional anesthesia; Elderly patients.
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28 29 30 77 **Strengths and limitations of this study**

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32 78 (1) A major strength of this study is that to our knowledge, this is the first trial to
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34 79 evaluate the effects of different anesthesia methods (RA and GA) on the circadian
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37 80 rhythm of melatonin secretion and explore their association with POD in elderly
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40 81 patients undergoing hip fracture surgery.

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42 82 (2) Both objective and subjective sleep quality are included in the current study. This
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44 83 can provide a better and more comprehensive understanding of the connection
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47 84 between circadian rhythms and anesthesia.

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50 85 (3) The limitation of the trial is that melatonin is the optimal peripheral biomarker of
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52 86 circadian timing, more biomarkers such as cytokines and genes (peripheral clock
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55 87 gene) should be included.

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58 88 (4) Although we will record both subjective and objective sleep quality, such
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4 89 measurements are incomplete, for example, polysomnography is the gold standard
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6 90 for diagnosis of sleep disorders.
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9 91 (5) Our study will be conducted until discharge from the hospital, which might be
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11 92 insufficient for delirium screening.
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15 93 **INTRODUCTION**

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18 94 Postoperative delirium (POD), a common neurological complication in elderly patients
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20 95 undergoing hip fracture surgery (range, 4%–53%), is usually associated with increased
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22 96 morbidity and mortality rates, long-term cognitive decline, and a heavier healthcare
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24 97 economic burden.¹⁻⁶ Many studies have indicated that preoperative brain dysfunction
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26 98 (frail brain) in elderly patients is a predisposing factor for POD^{7, 8}, while the
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28 99 perioperative change of neuroinflammatory markers (e.g., C-reactive protein and
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30 100 interleukin-6), oxidative stressor markers (cortisol) and the crucial hormone
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32 101 (melatonin) within elderly frail brain are the precipitating factors for POD.^{8,9} However,
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34 102 the specific mechanism of POD remains unclear.^{9,10}
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42 103 Circadian rhythm disruptions have recently drawn increased attention as a possible
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44 104 mechanism of POD.^{10, 11} Circadian rhythms refer to the behavioral and physiological
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46 105 cycles that occur within approximately 24 hours, including the sleep-wake cycles,
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48 106 hormone secretion, and core body temperature.¹² Among these indicators, plasma
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50 107 melatonin is considered to be the best peripheral marker of endogenous circadian
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52 108 rhythms.¹³⁻¹⁵ Previous studies have revealed potential correlations between plasma
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54 109 melatonin secretion disruption and POD.^{10, 16-20} Nevertheless, only limited
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4 110 postoperative time points (e.g., once a day) of plasma melatonin level measurements
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6 111 were recorded in those studies, and such data cannot represent a comprehensive
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9 112 melatonin rhythm. Moreover, one study indicated that the urinary excretion of 6-
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11 113 sulfatoxymelatonin (6-SMT), the main metabolite of melatonin, during delirium was
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14 114 associated with clinical subtypes of delirium (higher in hypoactive and lower in
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17 115 hyperactive delirium).²¹ However, measuring the melatonin level in a urine sample
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19 116 instead of a plasma sample at only one time point is insufficient to describe the real
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21
22 117 pattern of the melatonin rhythm in the acute phase of delirium. Therefore, the exact
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25 118 association between the melatonin rhythm and delirium requires further exploration.

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27
28 119 The process of anesthesia (either general anesthesia [GA] or regional anesthesia [RA])
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30
31 120 is known to influence the circadian rhythms.²²⁻²⁴ On one hand, GA is associated with
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34 121 unconsciousness similar to a dreamless sleep, which may strongly alter circadian
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36
37 122 rhythms through the use of drugs such as N-methyl-D-aspartate (NMDA) receptor
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39 123 antagonists or gamma-aminobutyric acid (GABA) receptor agonists^{22, 25, 26}. On the other
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41
42 124 hand, RA is more helpful in the relief of sleep disturbances after surgery than GA²⁷,
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44
45 125 which may result from better maintenance of the melatonin rhythm postoperatively.
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48 126 However, the effects of these two anesthesia methods on the postoperative melatonin
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51 127 rhythm have not been compared. Therefore, we hypothesize that RA may attenuate the
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54 128 disruption of the melatonin rhythm, which might decrease the incidence of POD in
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57 129 elderly patients undergoing hip surgery. In this report, we present the design of a
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60 130 prospective cohort clinical trial to compare the effects of GA versus RA on the circadian

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4 131 rhythm of melatonin secretion and the incidence of POD in elderly patients undergoing
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6 132 hip fracture surgery.
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10 133 **METHODS AND ANALYSIS**

11 12 13 134 **Trial design**

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15 135 This prospective single-center, 1:1 matched cohort clinical trial is designed to compare
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17
18 136 the effects of GA and RA on the melatonin rhythm and incidence of POD. We aim to
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20
21 137 recruit 138 patients with hip fracture (≥ 65 years of age) who will be assigned to either
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23
24 138 the GA group (n = 69) or the RA group (n = 69) depending on their situation and
25
26 139 personal willingness as well as the preference of the anesthesiologists and surgeons. We
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28
29 140 will conduct the trial at the Beijing Jishuitan Hospital in China from November 2019
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31 141 until the target sample size is achieved. Figure 1 shows the flow chart of this study.
32

33 142 **Primary outcome**

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35
36 143 The primary outcome is to compare the circadian rhythm of melatonin secretion
37
38
39 144 between the GA and RA groups and explore its association with the incidence of POD
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41
42 145 until discharge from the hospital.
43

44 146 **Secondary outcomes**

45
46
47 147 The secondary outcomes are to compare the subtype and severity of POD, plasma
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49
50 148 cortisol levels, C-reactive protein, interleukin-6, sleep quality, and pain until discharge
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52
53 149 from the hospital, and postoperative cognitive function at 6 months and 1 year after
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55 150 surgery between the GA and RA groups.
56

57 151 **Eligibility criteria**

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4 152 ***Inclusion criteria***

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6 153 Participants will be included if they meet the following criteria: age of ≥ 65 years,
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8
9 154 hospital admission for surgical treatment of hip fracture, and American Society of
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11
12 155 Anesthesiologists (ASA) physical status classification of I to III.

13
14 156 ***Exclusion criteria***

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16
17 157 Patients who meet any of the following criteria will be excluded: Parkinson's disease,
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19 158 dementia (including dementia caused by Parkinson's disease, Alzheimer's disease, or
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22 159 Lewy body dementia), a stroke within the prior 6 months, or any other central nervous
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25 160 system disease, alcohol-related disorders, multiple trauma, preoperative delirium,
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28 161 severe deafness or vision problems, communication difficulties, night shift duty, taking
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31 162 medication related to melatonin, a plan to be transferred to the intensive care unit
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33 163 postoperatively, and refusal to participate in the study or unexpected discharge.

34
35 164 **Preoperative baseline data collection and evaluation**

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37
38 165 Trained investigators who are blind to the patient grouping will evaluate the patients
39
40
41 166 and their medical charts to screen out potential participants according to the inclusion
42
43
44 167 and exclusion criteria. Baseline data will be collected, including demographic data (e.g.,
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46
47 168 age, sex, race, education level, height, and weight), surgical diagnosis, comorbidities,
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51 169 medication history, surgical history, smoking and drinking status, and the results of
52
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54 170 physical, laboratory, and instrumental examinations. We will also record the ASA
55
56
57 171 physical status classification. Specifically, preoperative cognitive scans will be
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60 172 performed using the Mini-Mental State Examination (MMSE)²⁸; preoperative delirium
173 will be diagnosed with the Confusion Assessment Method (CAM)^{29,30}; activities of daily

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4 174 living and instrumental activities of daily living will be assessed with the Katz Index of
5
6 175 Independence in Activities of Daily Living (ADL)³¹; sleep quality will be assessed by the
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9 176 Pittsburgh Sleep Quality Index (PSQI)³²; and pain intensity will be assessed using the
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11
12 177 numerical rating scale (NRS)³³.

178 **Perioperative management**

179 All individuals will be placed in a standard private room with a consistent light-dark
18
19 180 cycle and room temperature during the study period. The lights will be turned off
20
21
22 181 between 20:00 and 06:00 even at the time of blood sample collection, and light
23
24
25 182 exposure (controlled to be < 10 lux) will be measured during the night-time sleep period.
26
27 183 The participants will be requested to refrain from alcohol, caffeine, and chocolate. Their
28
29
30 184 activities will be restricted from 20:00 to 06:00, during which time they will be free
31
32
33 185 from interference factors including noise, television, and mobile phones during the
34
35
36 186 study period.

187 **Anesthesia and analgesia**

188 All patients will receive an ultrasound-guided fascia iliac block (a single injection of 30
41
42
43 189 mL of 0.33% ropivacaine) after admission to the operating room. During the operation,
44
45
46 190 the patients' eyes will be carefully covered with eye patches to avoid light effects.
47
48 191 Sedatives (benzodiazepine and dexmedetomidine) or anticholinergics will be avoided
49
50
51 192 during the perioperative phase.

52
53 193 In the RA group, ropivacaine will be used for spinal anesthesia or combined spinal and
54
55
56 194 epidural anesthesia at the L2-3 or L3-4 levels, and the sensory block will be controlled
57
58
59 195 at T8-T10. If regional anesthesia fails, the patients will receive surgery under GA and
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4 196 will withdraw from this trial to avoid the influence of lumbar puncture. In the GA group,
5
6 197 standard GA will be administered to the patients. Anesthesia will be intravenously
7
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9 198 induced with 0.5–1.5 mg/kg propofol, 0.2 mg/kg etomidate, 0.3 µg/kg sufentanil, and
10
11
12 199 0.15 mg/kg cisatracurium. Anesthesia will be maintained with sevoflurane, propofol,
13
14 200 and remifentanil, and the bispectral (BIS) value will be maintained between 40 and 60.
15
16
17 201 Mechanical ventilation will be continued to keep the end-tidal carbon dioxide
18
19 202 concentration at 35 to 40 mmHg in the GA group. A Vigileo/FloTrac device will be used
20
21
22 203 for measuring stroke volume variation and cardiac index to guide fluid therapy. Fluids
23
24 204 or vasoactive substances will be administered to maintain mean arterial pressure (MAP)
25
26
27 205 > 70 mmHg, heart rate < 100 beat per min (bpm), and urine output > 0.5 ml/kg/h.
28
29
30 206 Meanwhile, all patients will be warmed with forced air and intravenous fluids. On the
31
32 207 day of surgery, intraoperative parameters will be recorded, including the mean arterial
33
34
35 208 pressure, heart rate, BIS value, start time of anesthesia and surgery, duration and type
36
37
38 209 of anesthesia and surgery, anesthetic drugs and doses, sedative doses, blood loss, fluid
39
40 210 balance and transfusion of blood or clotting products, complications, and adverse
41
42
43 211 events.

44
45 212 For postoperative analgesia management, the patients will receive intravenous patient-
46
47
48 213 controlled analgesia (100 µg sufentanil and 8 mg ondansetron mixed with normal saline
49
50
51 214 to a total volume of 100 mL). Intramuscular injection of pethidine (50 mg) or oral use
52
53
54 215 of oxycodone (5 mg)/acetaminophen (325 mg) will be made as remedy analgesia.

216 **Postoperative management and follow-up**

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

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4 218 hospital. The investigators blind to grouping will record the total hospitalization cost,
5
6 219 the in-hospital length of stay, and the occurrence of postoperative complications during
7
8
9 220 hospitalization. Long-term cognitive function will be assessed for 1 year after surgery.
10

11 221 1. Delirium assessment
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14 222 Delirium will be diagnosed using the CAM, and the subtype and severity of delirium
15
16 223 will be assessed by using the Memorial Delirium Assessment Scale ³⁴. Surveillance
17
18 224 will involve twice-daily visits (8:00 and 20:00) by a trained geriatrician. The
19
20 225 duration of delirium will be measured as the number of days from onset of delirium
21
22 226 symptoms to resolution of symptoms. If the researcher is in doubt regarding this
23
24 227 assessment, the diagnosis of POD will be referred to an external expert.
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30 228 2. Sleep assessment
31

32 229 All participants will continuously wear a sleep tracker (Fitbit Charge 3; Fitbit, Inc.,
33
34 230 San Francisco, CA, USA), which is an available and inexpensive method to quantify
35
36 231 sleep patterns ³⁵. The investigators will provide instructions on wearing the sleep
37
38 232 tracker, a mobile device capable of downloading Fitbit Connect software, and
39
40 233 completing the corresponding sleep logs (bedtime, wake time, and nap times). The
41
42 234 frequency of syncing will be checked by the research staff weekly, and the Fitbit
43
44 235 Charge proprietary algorithm will process the sensor data to automate the sleep
45
46 236 variables ³⁶. All patients will wear sleep trackers until discharge from the hospital.
47
48 237 Based on the data from the sleep tracker, sleep logs, and direct observation by a
49
50 238 third party, we will derive the following data: total sleep time (min), sleep onset
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52 239 latency (min), wake time after sleep onset (min), time in rapid eye movement sleep,
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4 240 and time spent in “light sleep” and “deep sleep” (according to Fitbit Inc.). Subjective
5
6 241 sleep quality will be assessed with an NRS (0 = worst sleep and 10 = best sleep) ³⁷.
7
8
9 242 3. The intensity of postoperative pain both at rest and with movement will be evaluated
10
11 243 twice daily (8:00 and 20:00) with the NRS.
12
13
14 244 4. Cognitive function will be assessed by the same geriatrician at 6 months and 1 year
15
16 245 after surgery. The Modified Telephone Interview for Cognitive Status will be used to
17
18 246 test the patient’s cognitive function in non-face-to-face interviews ³⁷.
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22 247 **Blinding**

23
24 248 An investigator will be assigned to preserve and distribute results. Investigators will be
25
26 249 not blinded to the study group assignment, but the trained geriatrician responsible for
27
28 250 postoperative evaluation including POD diagnosis, as well as PSQI, ADLs, and NRS
29
30 251 scores are blinded to the grouping.
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34 252 **Light exposure**

35
36 253 Considering that light suppresses melatonin secretion except in life-threatening
37
38 254 situations, the only illumination from a penlight will be used during these interventions
39
40 255 ^{15, 19}. Blood sample collection and nursing interventions at night will be performed with
41
42 256 a penlight to avoid light exposure. Light intensity readings will be obtained with a digital
43
44 257 meter to ensure that light exposure does not exceed 10 lux.
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50 258 **Sample Collection**

51
52 259 Usually, the melatonin concentration is low throughout the day and then rises to a peak
53
54 260 between 02:00 and 04:00 ¹⁵. To establish the circadian rhythm, the plasma
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56 261 concentration of melatonin will be measured at least four times a day ^{15, 19}. Postoperative
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4 262 blood samples will be dynamically collected every day at 04:00, 10:00, 16:00, and 22:00
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6 263 until the patient is discharged through venipuncture (2 ml per sample) ^{16, 19}. All blood
7
8 264 samples will be collected in the heparinized tubes. The samples will be centrifuged at
9
10 265 3000 rpm for 10 min and will be stored at -80°C until further analysis. We will measure
11
12 266 plasma melatonin, cortisol, C-reactive protein (CRP), and interleukin-6 (IL-6).
13
14 267 Laboratory technicians will be blinded to the results of all clinical data. The
15
16 268 concentration of melatonin will be measured with the enzyme immunoassay method
17
18 269 (cat no. RE 54021; IBL International GmbH, Hamburg, Germany) with a limit of
19
20 270 detection of 1.6 pg/mL ³⁸. Plasma cortisol levels will be analyzed using an
21
22 271 electrochemiluminescence immunoassay method (Roche Diagnostics GmbH,
23
24 272 Germany). To measure inflammation, we will measure plasma C-reactive protein (CRP)
25
26 273 and interleukin-6 (IL-6) using immunoturbidimetry (Beckman Coulter, Inc., USA) and
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28 274 electrochemiluminescence immunoassay method (Roche, Germany), respectively.
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37 **Sample size estimation**

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40 276 Melatonin is secreted in a biphasic pattern in which nighttime secretion increases from
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42 277 20:00 to 22:00 with a peak at 02:00 to 04:00, and daytime secretion decreases from
43
44 278 06:00 to 08:00 with a nadir at 12:00 ^{14, 39}. Cronin et al. ¹⁵ researched the nighttime
45
46 279 melatonin secretion of adult patients for 3 days after gynecologic surgery and found that
47
48 280 the lowest level occurred on the first night postoperatively. In another study of elderly
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50 281 patients undergoing major abdominal surgery, Shigeta et al. ⁴⁰ found that the
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52 282 postoperative amplitude of nighttime melatonin secretion was significantly lower than
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54 283 the preoperative amplitude. These findings indicate that the peak of melatonin secretion
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4 284 is suppressed to the greatest degree in the first postoperative night, possibly leading to
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6 285 a melatonin rhythm shift. Therefore, for the present study, we will choose 04:00 on the
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9 286 first postoperative night as the time point for comparing the melatonin concentration
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11 287 between the groups. Our pilot study showed that the mean \pm standard deviation of
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13
14 288 melatonin was 10.48 ± 4.04 and 13.40 ± 6.85 pg/mL in the GA and RA groups (n=15 in
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16
17 289 each group), respectively. Using a two-sided α value of 0.05 and 85% power and
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19
20 290 considering a 10% dropout rate, we plan to include 138 patients in this study, with 69
21
22 291 patients in each group (1:1).

292 **Circadian melatonin rhythm data**

293 Circadian markers of the melatonin rhythm, including the mesor (mean concentration)
294 and amplitude (peak value minus the mean value), acrophase time (peak time), will be
295 calculated by Origin (OriginLab Corp, USA) based on cosinor regression
296 $y = a + b \cdot \cos(x \cdot \pi / 12 - c \cdot \pi / 12)$, in which a, b, and c represent mesor, amplitude, and
297 acrophase, respectively ⁴¹. The data will be subsequently smoothed with the cosinor
298 method curve-fitting procedure (GraphPad Prism 8; GraphPad Software, Inc) ^{41,42}.

299 **Adverse events**

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

305 **Data management and storage**

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4 306 At the first meeting, researchers will make appointments for the following dates to
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6 307 improve adherence to the study. All the data will be collected recorded in the Case
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8
9 308 Report Forms (CRF). After the data is recorded, the data will be stored in a locked
10
11 309 cabinet. Data entry will be performed by two investigators with the EpiData3.10
12
13
14 310 database system. Personal information of participants will be confidentially kept, and
15
16
17 311 all data will be identified by a name acronym and a study identification number in the
18
19 312 CRF. The data analysis will be performed by the primary investigator and designated
20
21
22 313 teammates.

23 24 25 314 **Ethics and Declarations**

26
27 315 During the study period, we will follow the Declaration of Helsinki and Chinese
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30 316 guidelines of Good Clinical Practice to guarantee the right of the patients. The study
31
32 317 protocol has been approved by the Medical Science Research Ethics Committees of
33
34
35 318 Beijing Jishuitan Hospital (JLKS201901-04). This study has been registered at the
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38 319 Chinese Clinical Trial Registry (ChiCTR1900027393). To inform the purpose and risks
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41 320 of the study, each enrolled patient accompanied by at least one family member or proxy
42
43 321 will sign a written informed consent. Besides, participants will sign the additional
44
45 322 consent provisions for collection and use of participant data and biological specimens
46
47
48 323 in ancillary studies. And all participants and investigators will keep the written
49
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51 324 informed consent. The primary investigator will regularly report on the progress and
52
53 325 changes of the study to the local Ethics Committee. The results of the study will be
54
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56 326 published in peer-reviewed international journals and will be presented at national and
57
58
59 327 international conferences and symposiums.
60

328 **Data safety monitoring**

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

332 **Statistical analysis**

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

347 **DISCUSSION**

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

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4 350 patients with hip fracture.
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6 351 Although some previous studies have shown connections between melatonin secretion
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9 352 disruption and delirium, few have elucidated the real melatonin rhythm pattern ⁴³⁻⁴⁵.
10
11 353 For example, a prospective observational study by Yoshitaka et al. showed that the delta
12
13 354 plasma melatonin concentration (calculated based on the preoperative and
14
15 355 postoperative values at selected time points) at 1 hour after surgery was significantly
16
17 356 lower in patients with than without delirium ⁴⁶. Melatonin secretion exhibits a biphasic
18
19 357 pattern in which daytime secretion is low (5 pg/mL on average) and nighttime secretion
20
21 358 is relatively high (50–100 pg/ml) ^{14, 39}. However, the sample collection time points of
22
23 359 melatonin in the above-mentioned study (before the operation, 1 hour after the
24
25 360 operation, and at 08:00 on postoperative days 1 and 2) represented either nighttime
26
27 361 secretion on the operation day or daytime secretion on the postoperative days; thus, the
28
29 362 measurements do not represent the comprehensive rhythm of melatonin. In contrast,
30
31 363 in the current study the blood samples will be taken before anesthesia, postoperative
32
33 364 blood samples (melatonin) every day at 04:00, 10:00, 16:00 and 22:00 until discharge.
34
35 365 These measurements are adequate to establish a melatonin rhythm curve. They will not
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37 366 only show the individual postoperative melatonin concentrations but also allow us to
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39 367 analyze the irregular pattern of melatonin secretion in patients with delirium.
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50 368 Previous trials have shown that GA may influence postoperative melatonin secretion ²²⁻
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52 369 ²⁴. First, in patients who received combined intravenous and inhaled anesthesia, the
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54 370 melatonin levels decreased the first night after surgery ^{24, 47}. Second, GA by inhalation
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56 371 was associated with a significant decrease in the melatonin concentration on the night
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4 372 of surgery, and this decrease was 350% greater than that on the third night after surgery
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6 373 ¹⁵. Third, total intravenous anesthesia induced a phase delay in the 6-SMT rhythm; the
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9 374 peak time was late by about 1 hour, and the amplitude of the melatonin rhythm
10
11 375 decreased by 85% on the night of surgery ¹². In contrast, another study demonstrated
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13
14 376 that RA was helpful to relieve sleep disturbance after surgery ²⁷, which may result from
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16
17 377 better maintenance of the melatonin rhythm postoperatively. However, little is known
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19 378 about how the two anesthesia methods differently affect the melatonin rhythm. Karkela
20
21
22 379 et al. ²⁴ conducted a similar clinical study and found that anesthesia and surgery
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24
25 380 disturbed the circadian melatonin rhythm, but there was no significant difference in the
26
27
28 381 melatonin secretion between the spinal anesthesia and GA groups for minor orthopedic
29
30
31 382 operations ²⁴. We do not consider that these results are especially robust and
32
33
34 383 generalizable because of several limitations of the trial, such as the small sample size
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36
37 384 (40 participants), measurement of only the saliva and urine concentrations of
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40 385 melatonin (no plasma or serum concentrations), and insufficient melatonin
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43 386 measurement timing (measurement of only nighttime secretion levels). We hypothesize
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45
46 387 that RA can maintain an almost normal melatonin rhythm for the following two reasons.
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48
49 388 First, RA may significantly reduce the types and amounts of general anesthetics used
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51
52 389 compared with GA, which can strongly alter melatonin secretion through different
53
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55 390 signal pathways. Second, RA allows better pain control and improved sleep quality
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58 391 compared with GA, which further facilitates a decrease in the severity of melatonin
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60 392 rhythm disruption.

393 **CONCLUSION**

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4 394 In conclusion, to our knowledge, this is the first study to compare the circadian rhythm
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6 395 of melatonin secretion between GA and RA groups and to explore the potential
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9 396 association between circadian rhythm disruptions and the development of delirium.
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11 397 Our findings will promote a greater understanding of delirium pathophysiology and
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14 398 provide clinical evidence for the optimal anesthesia method in elderly patients
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17 399 undergoing hip fracture surgery.

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21
22 401 **Contributorship statement:** ZQL and XYG obtained funding. ZQL, WCZ, GW, and XYG
23
24 402 contributed to the study design. YY, YNS, YYJ, and YZ performed the trial. XNM ,XXJ and YZH
25
26 403 contributed to data collection. CL, YL, CMS and XXW analyzed the data. YNS and YY drafted the
27
28 404 manuscript. ZQL and WCZ carefully reviewed the manuscript. All authors have read and
29
30 405 approved the final manuscript.

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33
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39
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46 410 **Competing interests:** None declared.

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49
50 411 **Acknowledgments:** Not applicable.

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53 412 **Patient and public involvement statement:** Patients and the public were not
54
55 413 involved in the design, conduct, reporting, and dissemination plans of this study.

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58 414 **Patient consent for publication:** Not required.

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6 548 **Figure legend**

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9 549 **Figure 1 Flow chart of the trial.** ASA, American Society of Anesthesiologists; CAM,
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11 550 Confusion Assessment Method; MMSE, Mini-Mental State Examination; ADL,
12
13 551 activities of daily living; PSQI, Pittsburgh Sleep Quality Index; IL, interleukin; CRP, C-
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15 552 reactive protein; Cor, cortisol; MAP, mean arterial pressure; HR, heart rate; BIS,
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17 553 bispectral index; MDAS, Memorial Delirium Assessment Scale; TICS-m, Modified
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19 554 telephone interview for cognitive status.
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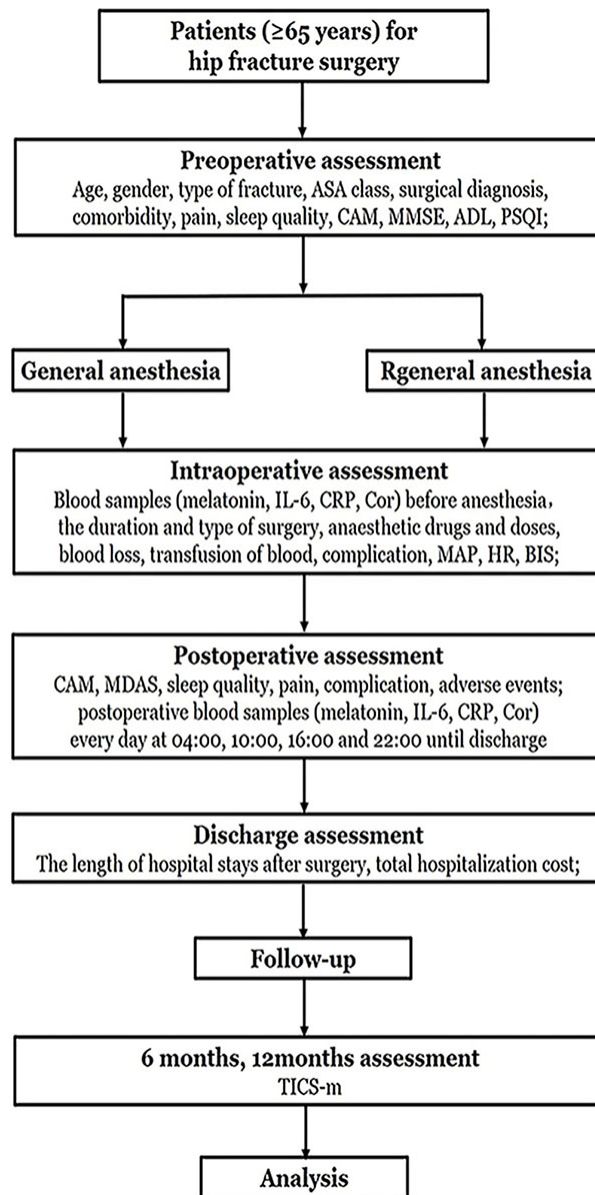


Figure 1

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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	<u>Page 1, line 2-4</u>
Trial registration	2a	Trial identifier and registry name. If not yet registered, name of intended registry	<u>Page 15, line 319</u>
	2b	All items from the World Health Organization Trial Registration Data Set	<u>Page 4, line 71-73</u>
Protocol version	3	Date and version identifier	<u>Page 4, line 72-73</u>
Funding	4	Sources and types of financial, material, and other support	<u>Page 19, line 410</u>
Roles and responsibilities	5a	Names, affiliations, and roles of protocol contributors	<u>Page 19, line 402</u>
	5b	Name and contact information for the trial sponsor	<u>Page 19, line 409</u>
	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	<u>Page 19, line 402</u>
	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)	<u>Page 16, line 329</u>

1	Background and	6a	Description of research question and justification for undertaking the trial, including summary of relevant	<u>Page 5, line 93-</u>
2	rationale		studies (published and unpublished) examining benefits and harms for each intervention	<u>132</u>
3				
4		6b	Explanation for choice of comparators	<u>Page 5, line 119-</u>
5				<u>132</u>
6				
7	Objectives	7	Specific objectives or hypotheses	<u>Page 6, line 127-</u>
8				<u>129</u>
9				
10	Trial design	8	Description of trial design including type of trial (eg, parallel group, crossover, factorial, single group),	<u>Page 7, line 135</u>
11			allocation ratio, and framework (eg, superiority, equivalence, noninferiority, exploratory)	
12				
13				
14	Methods: Participants, interventions, and outcomes			
15				
16	Study setting	9	Description of study settings (eg, community clinic, academic hospital) and list of countries where data will	<u>Page 7, line 140</u>
17			be collected. Reference to where list of study sites can be obtained	
18				
19	Eligibility criteria	10	Inclusion and exclusion criteria for participants. If applicable, eligibility criteria for study centres and	<u>Page 8, line 153-</u>
20			individuals who will perform the interventions (eg, surgeons, psychotherapists)	<u>163</u>
21				
22	Interventions	11a	Interventions for each group with sufficient detail to allow replication, including how and when they will be	<u>Page 7, line 136-</u>
23			administered	<u>139</u>
24				
25		11b	Criteria for discontinuing or modifying allocated interventions for a given trial participant (eg, drug dose	<u>Page 9, line 195-</u>
26			change in response to harms, participant request, or improving/worsening disease)	<u>196</u>
27				
28		11c	Strategies to improve adherence to intervention protocols, and any procedures for monitoring adherence	<u>Page 15, line 307</u>
29			(eg, drug tablet return, laboratory tests)	
30				
31		11d	Relevant concomitant care and interventions that are permitted or prohibited during the trial	<u>Page 9, line 191-</u>
32				<u>192</u>
33				
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35	Outcomes	12	Primary, secondary, and other outcomes, including the specific measurement variable (eg, systolic blood	<u>Page 7, line 142-</u>
36			pressure), analysis metric (eg, change from baseline, final value, time to event), method of aggregation (eg,	<u>150</u>
37			median, proportion), and time point for each outcome. Explanation of the clinical relevance of chosen	
38			efficacy and harm outcomes is strongly recommended	
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1	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
2				
3				
4	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
5				
6				
7	Recruitment	15	Strategies for achieving adequate participant enrolment to reach target sample size	Page 13, line 275-291
8				
9				

11 **Methods: Assignment of interventions (for controlled trials)**

13 Allocation:

15	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
16				
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21	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
22				
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25	Implementation	16c	Who will generate the allocation sequence, who will enrol participants, and who will assign participants to interventions	Page 12, line 247-251
26				
27				
28	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
29				
30				
31		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
32				
33				

35 **Methods: Data collection, management, and analysis**

37	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
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1		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	<u>Page 10, line 218-247</u>
2				
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4	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	<u>Page 15, line 310</u>
5				
6				
7				
8	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	<u>Page 16, line 332-346</u>
9				
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11		20b	Methods for any additional analyses (eg, subgroup and adjusted analyses)	<u>Page 16, line 332-346</u>
12				
13				
14				
15		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)	<u>Page 16, line 343-344</u>
16				
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18				
19	Methods: Monitoring			
20				
21	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	<u>Page 16, line 330 - 332</u>
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26		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	<u>Page 16, line 329 - 331</u>
27				
28				
29	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	<u>Page 14, line 300-304</u>
30				
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33	Auditing	23	Frequency and procedures for auditing trial conduct, if any, and whether the process will be independent from investigators and the sponsor	<u>Page 15, line 324-325</u>
34				
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37	Ethics and dissemination			
38				
39	Research ethics approval	24	Plans for seeking research ethics committee/institutional review board (REC/IRB) approval	<u>Page 15, line 318</u>
40				
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1	Protocol	25	Plans for communicating important protocol modifications (eg, changes to eligibility criteria, outcomes,	<u>Page 15, line 325</u>
2	amendments		analyses) to relevant parties (eg, investigators, REC/IRBs, trial participants, trial registries, journals,	
3			regulators)	
4				
5	Consent or assent	26a	Who will obtain informed consent or assent from potential trial participants or authorised surrogates, and	<u>Page 15, line 323-</u>
6			how (see Item 32)	<u>324</u>
7				
8		26b	Additional consent provisions for collection and use of participant data and biological specimens in ancillary	<u>Page 15, line 321-</u>
9			studies, if applicable	<u>323</u>
10				
11	Confidentiality	27	How personal information about potential and enrolled participants will be collected, shared, and maintained	<u>Page 15, line 310-</u>
12			in order to protect confidentiality before, during, and after the trial	<u>312</u>
13				
14	Declaration of	28	Financial and other competing interests for principal investigators for the overall trial and each study site	<u>Page 19, line 413</u>
15	interests			
16				
17				
18	Access to data	29	Statement of who will have access to the final trial dataset, and disclosure of contractual agreements that	<u>Page 15, line 312</u>
19			limit such access for investigators	
20				
21	Ancillary and post-	30	Provisions, if any, for ancillary and post-trial care, and for compensation to those who suffer harm from trial	<u>Page 14, line 303-</u>
22	trial care		participation	<u>304</u>
23				
24	Dissemination policy	31a	Plans for investigators and sponsor to communicate trial results to participants, healthcare professionals,	<u>Page 15, line 325-</u>
25			the public, and other relevant groups (eg, via publication, reporting in results databases, or other data	<u>327</u>
26			sharing arrangements), including any publication restrictions	
27				
28				
29		31b	Authorship eligibility guidelines and any intended use of professional writers	<u>Page 16, line 329</u>
30				
31		31c	Plans, if any, for granting public access to the full protocol, participant-level dataset, and statistical code	<u>Page 16, line 329</u>
32				
33	Appendices			
34				
35	Informed consent	32	Model consent form and other related documentation given to participants and authorised surrogates	<u>Page 15, line 323-</u>
36	materials			<u>324</u>
37				
38	Biological	33	Plans for collection, laboratory evaluation, and storage of biological specimens for genetic or molecular	<u>Page 13, line 263-</u>
39	specimens		analysis in the current trial and for future use in ancillary studies, if applicable	<u>274</u>
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1 *It is strongly recommended that this checklist be read in conjunction with the SPIRIT 2013 Explanation & Elaboration for important clarification on the items.
2 Amendments to the protocol should be tracked and dated. The SPIRIT checklist is copyrighted by the SPIRIT Group under the Creative Commons
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