

PEER REVIEW HISTORY

BMJ Open publishes all reviews undertaken for accepted manuscripts. Reviewers are asked to complete a checklist review form (<http://bmjopen.bmj.com/site/about/resources/checklist.pdf>) and are provided with free text boxes to elaborate on their assessment. These free text comments are reproduced below.

ARTICLE DETAILS

TITLE (PROVISIONAL)	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
AUTHORS	Yuan, Yi; Song, Yanan; Wang, Geng; Jia, Yunyang; Zhou, Yang; Mi, Xinning; Jia, Xixi; Wang, Xiaoxiao; Liu, Chang; Li, Yue; Shi, Chengmei; Han, Yongzheng; Guo, Xiangyang; Zhang, Wenchao; Li, Zhengqian

VERSION 1 – REVIEW

REVIEWER	Shen Qihong The First Hospital of Jiaxing, China.
REVIEW RETURNED	11-Oct-2020

GENERAL COMMENTS	<p>I am glad to have this opportunity to read this manuscript. This is a well conducted study protocol. Postoperative delirium (POD) is prevalent among elderly patients, and is associated with neurological dysfunction, prolonged hospital stay, and high medical costs.</p> <ol style="list-style-type: none">1. Please further clarify how the investigators handle patients who have failed spinal or epidural anesthesia.2. Please describe in more detail how to calculate the association between melatonin circadian rhythm and postoperative delirium.
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REVIEWER	Qiujun Wang The Third Hospital of Hebei Medical University, China
REVIEW RETURNED	09-Nov-2020

GENERAL COMMENTS	<ol style="list-style-type: none">1. During preoperative management, were all subjects placed in the same room with the standard light cycle and room temperature for the same amount of time? Whether the different time will affect the melatonin cycle formation?2. Whether intraoperative use of different anesthetic drugs will lead to intra-group differences and affect the test results? Is the time for pain intensity assessment during postoperative rest and activity consistent with that of POD? If not, when to evaluate?3. Melatonin is affected by many aspects. In the design of this experiment, only light was strictly required before surgery, however, light after surgery will also affect the secretion of melatonin, and the light after discharge is uncontrollable.4. There are no "results" part in the whole paper. The incidence of delirium and subtypes and the changes in the sleep quality, such as melatonin, were not statistically compared and analyzed, which could not reflect the objectives of this trial and could not judge the use of statistical methods because there was no statistical result.
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	<p>5. Part of the discussion is weak and is not discussed in combination with the results of this trial. This study is innovative, but it does not elaborate on the logical relationship between the study content and the index.</p> <p>6. The flowchart in Figure 1 is only about the general flow. It does not elaborate on the number of samples that subjects were removed due to the withdrawal of the study during the perioperative period or even the absence of contact with patients after discharge.</p>
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VERSION 1 – AUTHOR RESPONSE

Reviewer 1: Comments to the Author

Q1 : Please further clarify how the investigators handle patients who have failed spinal or epidural anesthesia.

Reply: Thanks for your insightful suggestions. The patients who failed spinal anesthesia will withdraw from this trial to avoid the influence of lumbar puncture. A detailed description of this point has been added in the “METHODS AND ANALYSIS” section (Page 9, line 195-196, highlighted in red).

Q2. Please describe in more detail how to calculate the association between melatonin circadian rhythm and postoperative delirium.

Reply: Thank you for your suggestion. The association between melatonin parameters and sleep quality as well as POD will be validated by Pearson or Spearman analysis using SPSS software. To better demonstrate the relationship between rhythm markers of melatonin and sleep quality as well as POD, we will also perform a linear regression analysis using GraphPad Prism software (GraphPad Software, Inc). These descriptions have been added in the “METHODS AND ANALYSIS - Statistical analysis” section (Page 16, line 341-344, highlighted in red).

Reviewer 2: Comments to the Author:

Q1. During preoperative management, were all subjects placed in the same room with the standard light cycle and room temperature for the same amount of time? Whether the different time will affect the melatonin cycle formation?

Reply: Thanks for your careful reading. A set of environmental factors (light, meal, activity, temperature) are known to influence melatonin¹. Thus, we will control these factors by standard private room with light cycle and room temperature, to avoid interference with melatonin secretion. More importantly, not just before surgery, but throughout the study period, we will try to make sure that the patient's environment is consistent. (Page 9, line 179-182, highlighted in red).

Furthermore, the start time of anesthesia and surgery can influence the internal clock and melatonin secretion¹. Anesthesia during the active period of an animal is more frequently associated with disrupted rest/activity rhythms than anesthesia during the rest period, resulting in activity during the normal rest period or decreased activity during the active period. Meanwhile, the magnitude and direction of the shifts depend on the different types of anesthetics¹. Mihara et al.² found ketamine-induced anesthesia resulted in a large phase advance when administered during an active period and a large phase delay when administered during the rest period. In our study, the patients will also be assigned to morning or afternoon groups depending on the start time of anesthesia to further observe the effect of different times on the circadian rhythm of melatonin.

Reference:

1. Coppola S, Caccioppola A, Chiumello D. Internal clock and the surgical ICU patient. Curr Opin

Anaesthesiol. 2020 Apr;33(2):177-184.

2. Mihara T, Kikuchi T, Kamiya Y, et al. Day or night administration of ketamine and pentobarbital differentially affect circadian rhythms of pineal melatonin secretion and locomotor activity in rats. *Anesth Analg.* 2012 Oct;115(4):805-13.

Q2. Whether intraoperative use of different anesthetic drugs will lead to intra-group differences and affect the test results? Is the time for pain intensity assessment during postoperative rest and activity consistent with that of POD? If not, when to evaluate?

Reply: Thanks for your suggestion. We totally agree with you that intraoperative anesthetic drugs (GABA receptor agonists, NMDA receptor antagonists, or others) will affect the melatonin secretion and the incidence of POD. The patients will receive a standard anesthesia regimen and pain management to avoid the effects of anesthetics and analgesics on melatonin. This has been added in the "METHODS AND ANALYSIS- Anesthesia and analgesia" section (Page 10, line 198-215, highlighted in red).

Concerning postoperative assessment, each participant will be followed up twice daily (8:00 and 20:00) for delirium screening and assessment of pain and sleep (Page 12, line 243, highlighted in red).

Q3. Melatonin is affected by many aspects. In the design of this experiment, only light was strictly required before surgery, however, light after surgery will also affect the secretion of melatonin, and the light after discharge is uncontrollable.

Reply: Thank you very much for reminding me of that. The light received by patients in the ward was controlled throughout our study. Specifically, the lights will be turned off between 20:00 and 06:00 even at the time of blood sample collection, and light exposure (controlled to be < 10 lux) will be measured during a night-time sleep period (Page 9, line 179-182, highlighted in red). Furthermore, we agree with you that the light after discharge is difficult to control, and we plan to acknowledge this in the paper after the completion of this study.

Q4. There are no "results" part in the whole paper. The incidence of delirium and subtypes and the changes in the sleep quality, such as melatonin, were not statistically compared and analyzed, which could not reflect the objectives of this trial and could not judge the use of statistical methods because there was no statistical result.

Reply: Thanks for the suggestion. BMJ Open is encouraging the submission of protocol manuscripts at an early stage of the study. The protocol papers should report planned or ongoing studies. Manuscripts that report work already carried out will not be considered as protocols. Therefore, we did not present the "result" part in this protocol manuscript. Nevertheless, your kind advice can help us to perform and report the study better.

Q5. Part of the discussion is weak and is not discussed in combination with the results of this trial. This study is innovative, but it does not elaborate on the logical relationship between the study content and the index.

Reply: Our trial is still in progress. So the discussion section of the current protocol manuscript is weak and lacks a combination with the results. Nevertheless, we believe that your review comments have improved the quality of our manuscript. As your kind advice, the logical relationship between the study content and the index should be addressed in the manuscript after the completion of this study.

Q6. The flowchart in Figure 1 is only about the general flow. It does not elaborate on the number of samples that subjects were removed due to the withdrawal of the study during the perioperative

period or even the absence of contact with patients after discharge.

Reply: Thank you for this valuable suggestion. If the trial is completed, the flowchart in our manuscript will include the number of patients that subjects were removed due to the withdrawal of the study during the perioperative period or even the absence of contact with patients after discharge.

VERSION 2 – REVIEW

REVIEWER	QiuJun Wang The third hospital of hebei medical university, hebei, china
REVIEW RETURNED	25-Dec-2020
GENERAL COMMENTS	The author fully answers and explains the questions raised by the reviewers