PEER REVIEW HISTORY

ARTICLE DETAILS

TITLE (PROVISIONAL)	The effects of subanaesthetic S-ketamine on postoperative delirium and cognitive function in elderly patients undergoing non-cardiac thoracic surgery: a protocol for a randomised, double-blinded, placebo- and positive-controlled, non-inferiority trial
AUTHORS	WEI, WEI; Zhang, Anyu; Liu, Lv; Zheng, Xi; Tang, Chunlin; Zhou, Ming; Gu, Yu; Yao, Yonghua

VERSION 1 – REVIEW

REVIEWER	Mahanna-Gabrielli , Elizabeth University of Miami Miller School of Medicine, Anesthesiology, Perioperative Medicine and Pain Management
REVIEW RETURNED	10-Mar-2022

GENERAL COMMENTS	The authors are commended on a thorough and scientifically sound protocol that is well written overall. I have a few comments in order to improve the clarity of the manuscript. I look forward to seeing the results of the study. Major Comments 1. In Paragraph 2, P5L7, the extensive discussion of dexmedetomidine is unexpected for the reader as the Title of the study is on S-ketamine. I suggest adding an opening sentence that states that there is limited pharmacological methods to reduce the incidence of delirium. Thus far, dexmedetomidine has shown the most promise etc. I also suggest to greatly reduce the length of this paragraph. 2. The manuscript states the study start date is March 2022. However, the Chinese Clinical Trial registration states the study execute time is from 12/01/2021 to 12/31/2022. Please clarify, which is correct and modify the appropriate document that is incorrect. 3. For randomization, why did the authors choose the website www.randomization.com and not another statistical program? 4. S-ketamine dose. What is the conversion between racemic ketamine and S-ketamine? How much more potent is it? It would be useful to the reader to put this conversion either in the introduction when comparing racemic to S-ketamine or the methods or both. 5. P13L13, I believe the authors mean emergence agitation or delirium and not emergency? 6. P20L35: Please modify this sentence to “We believe that the results will be one of the following:…”. This allows the reader to better understand the authors do not believe it will be all of the following listed outcomes. Minor comments 7. P4 Line 37 – please reference this statement. 8. Table 1. Emergency delirium should be Emergence delirium.

	9. P8 L23, ASA stands for the American Society of Anesthesiologists 10. P9 L 10, Instead of saying alcohol or drug abuser, please state Alcohol or Illicit drug misuse disorder 11. P10 L29, please write out arterial line and either central venous line or peripheral venous line as appropriate 12. Figure 1: please change “Emergency delirium“ to “Emergence Delirium”

REVIEWER	Sigaut, Stéphanie Hôpital Beaujon, Anesthesiology and Intensive Care
REVIEW RETURNED	18-Mar-2022

GENERAL COMMENTS	The authors present the protocol of a single-center RCT aiming to determine if intraoperative S-ketamine is non-inferior to Dexmedetomidine as compared to placebo to reduce the incidence of postoperative delirium and improve other secondary measures associated with delirium. The authors present their protocol well and I believe this study will be a good contribution to the body of literature on the prevention of postoperative delirium. However, I have serious concerns about the fact that dexmedetomidine administration as planned in this protocol, with a bolus, in elderly patients will often induce bradycardia and hypotension compromising the blinding. I strongly suggest the authors to revise their protocol of drug administration to use one without boluses. I have other comments for areas of improvement below. All along the manuscript Please change « emergency delirium » for « emergence delirium » in the abstract, manuscript text, tables and figures. Abstract P2 L40 Please add the advert events in the list of secondary outcomes, as it is planned by the authors and is an important outcome in every trial about medications. P2 L41 I suggest changing « designated timepoint » by the actual timepoints i.e. just before induction, at the end of surgery, and D4 Introduction P4 L38 Authors state that delirium typically occurs during the first 96 hours after surgery. I suggest changing this sentence to be in line with the actual nomenclature stating that delirium is defined as that which occurs in hospital up to 1 week postprocedure or until discharge (whichever occurs first)(Recommendations for the Nomenclature of Cognitive Change Associated with Anaesthesia and Surgery-2018. Anesthesiology. 2018 Nov;129(5):872-879.). Then, the authors can add that the highest incidence is observed during the first 72 hours, with an adequate reference. P4 L42 please include more updated references for the rate of delirium after high risk surgeries. P4 L49 physiopathological mechanisms of postoperative delirium are multiples, please use plural P5 L4 I suggest rewriting this sentence as it is not clear, do the authors mean to write that no recommendation can be made on the use of a specific drug as a prophylactic agent due to lack of data ?

	P5 L11 I suggest adding « in a recent network meta-analysis » at the end of the sentence before [6] to clarify the use of « highest-ranking possibility » P5 L8 >L53 I suggest rewriting the paragraph to separate data on cardiac and non-cardiac surgery, as physiopathology and incidence of delirium is quite different between these types of surgery (cerebral embolism frequent in cardiac surgery), and thus it is difficult and makes little sense to do a literature analysis mixing both. I also suggest adding a sentence about the heterogenous ways dexmedetomidine is administrated in the literature (per or postoperative or both, bolus, continuous…) which complexify the analysis even more. P5 L37 replace « commenced » by « started » P5 L 52 I suggest emphasizing more on side effects of dexmedetomidine in the elderly and adding a recent citation to illustrate this important issue (for exemple Pan H, Liu C, Ma X, Xu Y, Zhang M, Wang Y. Perioperative dexmedetomidine reduces delirium in elderly patients after non-cardiac surgery: a systematic review and meta-analysis of randomized-controlled trials. Can J Anaesth. 2019 Dec;66(12):1489-1500.) P6 L57 Please rewrite, I don't understand if the authors state that s-ketamine is non-inferior to dexmedetomidine as their hypothesis or as a fact here. If it's the last, add a reference and thus justify what your study will add to these results. P6 L60 Please rewrite to make it clearer that if s-ketamine is out of favor it is because of racemic ketamine negative results in podcast and pride P7 L8 Change « far from revealed » by « lack of good quality evidences » P7 L 15 Change « compared with » by « compared to ». I suggest adding that comparison with dexmedetomidine is justified by recent literature data making it the intraoperative drug with the higher potential for prevention of postoperative delirium I suggest that the author add a paragraph about the rationale for their biomarkers analysis. Participant recruitment P8 L19 why have an upper limit for age? Participant consent P9 L 22 Will the participants be given written informed consent or oral consent only? Please clarify. Randomisation and blindness P9 L45 The author state that the anesthetic nurse will not be involved in follow-up, but will she be involved in intraoperative patient management? if yes, it is a serious limitation for blinding.

	I have serious concerns about the fact that dexmedetomidine administration as planned in this protocol, with a bolus, in elderly patients will often induce bradycardia and hypotension compromising the blinding. I strongly suggest the authors to revise their protocol of drug administration to use one without boluses. If the authors disagree about this bias, they should explain it in their manuscript with accurate references about safety in the elderly population. Standard anesthetic management P10 L26 I suggest removing the use of atropine, as limiting secretion at induction is of little interest, and on the opposite, anticholinergic agents are well-known precipitating factors for delirium and thus should be avoided in a high-risk population. P10 L29 Please write fully A-line and V-line P10 L31 I suggest removing the use of midazolam, as literature suggest it has no or little effect on anxiety or memorization, and on the opposite, benzodiazepines are well-known precipitating factors for delirium and thus should be avoided in a high-risk population. P10 L35 Why use a rapid sequence induction dose of rocuronium in a standard induction sequence with mask ventilation? P11 L19 Why use an intercostal block as locoregional anesthesia technic, whereas its effects are modest compared to epidural anesthesia or paravertébral block? Study drug administration Will a dedicated venous line not used for fluid loading be used for study drug administration? This is a major safety point, as inadvertent boluses of dexmedetomidine are at high risk of bradycardia that could lead to cardiac arrest. Once again, I insist on the risk of compromised blinding if a bolus is used at induction. Intraoperative data collection I suggest adding data collection of BIS value, for example, time spend with RS >0 or with BIS <40, as the literature suggests that too deep anesthesia may be associated with postoperative delirium. Postoperative data collection I suggest adding data collection of physiological perturbations like metabolic abnormalities, hypoxia, electrolyte imbalance, infection, which are well-known precipitating factors of postoperative delirium. Primary outcome P13 L51 change « at 4 h.. » for « between 4 h after surgery and the 4th postoperative day » Measurement of delirium

	P14 L20 Will you use a validated translation of the 3D-CAM and CAM-ICU ? If yes, specify which one with reference, if not, cite this as a limitation. P14 L50 Will the 2 investigators in charge of the delirium assessment be able to do them 7/7, even on weekend? P14 L56 I appreciate the authors included a specific training for delirium assessment, as sensitivity is highly dependent on this training. P14 L60 Can the authors specify if a discussion with the bedside nurse will be added to the 3D-CAM evaluation and to the chart review? Indeed, the reported incidence of delirium in the elderly after surgery is highly dependent on how it is diagnosed and screened. In a study for example, the incidence of delirium using chart review only was 3%, noted during routine clinical care was 8%, using interviews with nurses was 9%, and using daily mental status testing and application of a validated diagnostic algorithm was 53%. (Rudolph JL, Marcantonio ER: Review articles: Postoperative delirium: Acute change with long-term implications. Anesth Analg 2011; 112:1202–11) The family interview will also be of great value to add sensitivity to the assessment. Please specify if you planned to do it. Table 3 P16 L18-22 Multiple definitions for hypotension are specified in the table, please explain which one is used in which circumstances. Will noradrenaline be the only way to treat hypotension? Its use in hypovolemic patients can lead to cardiac or renal complications, thus it is important before using it to assess patient volemic status by testing fluid loading impact on systolic ejection volume before starting noradrenaline, which then is not a first-line drug. Moreover, will you use only boluses or is it possible to continue with infusion? If yes, please specify in the table. P16 L50 Atropine 40mg is written as a treatment of tachycardia, please correct to « esmolol 40 mg ». Sample size calculation P17 L 32 A dropout rate of 5% seems a bit low as advert events due to dexmedetomidine may be higher than that (see Balanced Opioid-free Anesthesia with Dexmedetomidine versus Balanced Anesthesia with Remifentanil for Major or Intermediate Noncardiac Surgery. Anesthesiology. 2021 Apr 1;134(4):541-551., which was stopped prematurely because of these advert events.) Discussion P20 L4 please remove « miserable » P20 L35 please rewrite the sentence « we believe that the results will be as follows ». Indeed, after that, the authors stated 4 hypotheses with opposite results. Thus, more than a hypothesis presentation, it should be introduced as possible results, that will be interesting even in the case of negativity.

	P21 L4 This study is not designed and power to assess this 4th hypothesis, please remove it from the manuscript. Supplementary data Please provide the ethical approval form

REVIEWER	Stones, Martyn Cardiff University, Psychiatry
REVIEW RETURNED	24-Mar-2022

GENERAL COMMENTS	Valid study for potentially beneficial interventions not previously compared in a single study why mix 3-D CAM and CAM-ICU, could use CAM-ICU alone? What are safety considerations for halting trial? Rational for the intervention dosage? Does the different loading dose volume un-blind the study? Consider baseline/pre-op delirium assessment and delirium as an exclusion criteria. Rescue medications: Propofol 30mg and haloperidol 10mg should only be used if patient is distressed or for safety reasons. Miserable orthopaedics? spelling? Reference 25: this sentence does not make scientific sense. The plasma amino acids compete for entry to the brain, plasma serotonin does not enter the brain. What is the rational for the choice of plasma biomarkers. why are they measured only at baseline and 96 hours, what about the times in between?

REVIEWER	Diaz, Francisco University of Kentucky, Department of Biostatistics
REVIEW RETURNED	29-Apr-2022

GENERAL COMMENTS	This paper presents the protocol of a 3-arm design to examine the non-inferiority of S-ketamine with respect to dexmedetomidine as prophylactic treatment to prevent postoperative delirium after thoracic surgery. The 1:1:1 ratio design will also include a placebo-controlled group. In general, the description of the design is clear, and the paper is very well written. I have, however, some concerns about uncontrolled potential sources of bias. 1) My main concern is that the PI is not blinded to the randomization protocol and the CRFs during recruitment and data entry. This is a potential source of bias. Please address how this bias will be prevented. Ideally, the PI should be blinded to the randomization protocol and an independent team should conduct the data storage and management. The above concern arouse from line 46 in page 9 which says “The randomization protocol will be kept secure by the primary investigator”. And from lines 35-37 on page 13 that say “Paper case report forms (CRF) will be stored by the primary investigator and entered into the Epidata V4.6 database protected by password only accessible to authorised users.” 2) Another concern is that it is unclear who will conduct the data management and data analyses and whether these two will be conducted in a way that guarantees relative independence from the clinicians collecting the data and the PI. The PI, and the clinicians collecting the data, should be allowed to see the randomization

	protocol only when both recruitment and the database are closed, and an independent statistics team should be in charge of the data management and analysis of the primary outcome to prevent biases. The authors must address these concerns in the paper. 3) The paper does not mention a Data Safety and Monitoring Committee (DSMB). This independent committee is crucial in case the randomization protocol needs to be unblinded to address a safety issue, among many other reasons. Please describe whether such committee exists and how it will interact with the researchers.

VERSION 1 – AUTHOR RESPONSE

Point by point response to reviewer 1#
Dear Dr. Elizabeth Mahanna-Gabrielli
We sincerely appreciate your recognition and suggestions on the SKED protocol, which will play an extremely important guiding role in the following research. Hereby, I will provide detailed explanations of your comments.
Major comments
1. In Paragraph 2, P5L7, the extensive discussion of dexmedetomidine is unexpected for the reader as the Title of the study is on S-ketamine. I suggest adding an opening sentence that states that there is limited pharmacological methods to reduce the incidence of delirium. Thus far, dexmedetomidine has shown the most promise etc. I also suggest to greatly reduce the length of this paragraph.
As you mentioned, the dexmedetomidine is the most well-recognized drug for postoperative delirium (POD) prevention. However, there are still some concerns and uncertainties due to its cardiovascular inhibition, especially in elderly patients. The purpose of this non-inferiority trial is to explore the efficacy and safety of S-ketamine on POD prevention, which may provide a more stable intraoperative hemodynamic effects in view of its mild sympathetic excitatory effect. We drafted the Paragraph 2 which is based on four high-level RCTs and one meta-analysis to give readers a summarized description on the current situation of dexmedetomidine for POD prevention.
So, the Paragraph 2 serves as a connecting role in the full text to propose the necessity of finding a new drug, such as S-ketamine. As you suggested, adding an opening sentence is considerably essential, so we place the sentence “there is limited pharmacological methods to reduce the incidence of delirium. Thus far, dexmedetomidine has shown the most promise” at the end of Paragraph 1. Thanks for your suggestions.
2. The manuscript states the study start date is March 2022. However, the Chinese Clinical Trial registration states the study execute time is from 12/01/2021 to 12/31/2022. Please clarify, which is correct and modify the appropriate document that is incorrect.
Sorry for my unclearly expression. The trial has been registered on the Chinese Clinical Trial Registry (identifier: ChiCTR2100052750; registry date: 11/05/2022) and ClinicalTrials.gov (identifier: NCT05242692; registry date: 03/08/2022) respectively. The anticipated study execute time documented on the Chinese Clinical Trial Registry is from 02/09/2022 to 02/29/2024 and on the ClinicalTrials.gov is 03/10/2022 to 02/29/2024, respectively. The actual study start date was on 03/10/2022 when both registries were approved.
3. For randomization, why did the authors choose the website www.randomization.com and not another statistical program?
We chose the website www.randomization.com to perform randomization by referring to some reliable literature [1-3]. Furthermore, balance between groups can be achieved by simple block randomization because of the single-center study. Of course, STATA and SAS are better choices.
References
1 Shin HJ, Do SH, Lee JS, et al. Comparison of Intraoperative Sedation With Dexmedetomidine Versus Propofol on Acute Postoperative Pain in Total Knee Arthroplasty Under Spinal Anesthesia: A

Randomized Trial. Anesth Analg. 2019;129(6):1512-1518.
2 Jo JY, Jung KW, Kim HJ, et al. Effect of Total Intravenous Anesthesia vs Volatile Induction With Maintenance Anesthesia on Emergence Agitation After Nasal Surgery: A Randomized Clinical Trial. JAMA Otolaryngol Head Neck Surg. 2019;145(2):117-123.
3 Karalapillai D, Weinberg L, Peyton P, et al. Effect of Intraoperative Low Tidal Volume vs Conventional Tidal Volume on Postoperative Pulmonary Complications in Patients Undergoing Major Surgery: A Randomized Clinical Trial. JAMA. 2020;324(9):848-858.
4 S-ketamine dose. What is the conversion between racemic ketamine and S-ketamine? How much more potent is it? It would be useful to the reader to put this conversion either in the introduction when comparing racemic to S-ketamine or the methods or both.
S-ketamine is the S (+) enantiomer of ketamine, which has a higher affinity with aspartate receptor and μ opioid receptor. The anaesthetic potency of S-ketamine is two-fold higher than that of racemic ketamine, and it has higher in vivo clearance rate characterized by lower incidence of adverse reactions. [1] I have put this conversion in the introduction when comparing racemic with S-ketamine, according to your instruction. Thanks for your valuable suggestions. For details, P5L23-26.
1 Krystal JH, Charney DS, Duman RS. A new rapid-acting antidepressant. Cell. 2020;181(1):7.
5. P13L13, I believe the authors mean emergence agitation or delirium and not emergency?
Sorry for my typos. I have revised all.
6. P20L35: Please modify this sentence to “We believe that the results will be one of the following:…”. This allows the reader to better understand the authors do not believe it will be all of the following listed outcomes.
Sorry for my unclearly expression. I have revised this sentence according to your instructions. For details, P20L13.
Minor comments
7. P4 Line 37 – please reference this statement.
Thanks for your reminder. I have supplemented the reference accordingly. For details, P4L2.
8. Table 1. Emergency delirium should be Emergence delirium.
Sorry for my typos. I have revised.
9. P8 L23, ASA stands for the American Society of Anesthesiologists
Sorry for my unclearly expression. I have revised this sentence according to your instructions. For details, P7L6.
10. P9 L 10, Instead of saying alcohol or drug abuser, please state Alcohol or Illicit drug misuse disorder
Sorry for my unclearly expression. I have revised this sentence according to your instructions. For details, P8L16.
11. P10 L29, please write out arterial line and either central venous line or peripheral venous line as appropriate
Sorry for my unclearly expression. I have revised this sentence according to your instructions. For details, P9L28.
12. Figure 1: please change “Emergency delirium“ to “Emergence Delirium”
Sorry for my typos. I have revised.

Point by point response to reviewer 2#
Dear Dr. Stéphanie Sigaut
We attach great importance to your professional comments, which is of practical significance and value for the improvement of the study. Hereby, I will provide detailed explanations of your comments.
1. I have serious concerns about the fact that dexmedetomidine administration as planned in this protocol, with a bolus, in elderly patients will often induce bradycardia and hypotension compromising the blinding. I strongly suggest the authors to revise their protocol of drug administration to use one without boluses.
We can’t agree with your prospective views more. Due to the limitation of previous protocol, we found we found that a loading dose of 0.4 μg/kg dexmedetomidine lead to obvious bradycardia and transient

hypertension events in the preliminary trial. Therefore, we modified the loading dose of dexmedetomidine to 0.2 μg/kg; In addition, in order to ensure blindness, the infusion speed of dexmedetomidine is consistent with that of S-ketamine, which also reduces the side effects of dexmedetomidine.
If dexmedetomidine was administered without a loading dosage, we consider that it may be insufficient to clarify the effect of dexmedetomidine on POD prevention, which may also contribute to selection bias. As far, we have recruited 67 patients after the protocol was modified, and no obvious bradycardia and hypertension events occur. Accordingly, we have submitted the change of the protocol to the Chinese Clinical Trials Registry website and Clinical trials. Gov.
2. Please change « emergency delirium » for « emergence delirium » in the abstract, manuscript text, tables and figures.
Sorry for my typos. I have revised all.
3. P2 L40 Please add the advert events in the list of secondary outcomes, as it is planned by the authors and is an important outcome in every trial about medications.
Thanks for your precious comment. I have added the advert events in the list of secondary outcomes according to your instruction. For details, P2L19.
4.P2 L41 I suggest changing « designated timepoint » by the actual timepoints i.e. just before induction, at the end of surgery, and D4.
We removed «designate timepoints» because excessive time points will confuse the readers in the Abstract part. Furthermore, we presented detailed explanation in the Outcome part. Thanks for your comment. For details, P2L20.
5. P4 L38 Authors state that delirium typically occurs during the first 96 hours after surgery. I suggest changing this sentence to be in line with the actual nomenclature stating that delirium is defined as that which occurs in hospital up to 1 week postprocedure or until discharge (whichever occurs first) (Recommendations for the Nomenclature of Cognitive Change Associated with Anaesthesia and Surgery-2018. Anesthesiology. 2018 Nov;129(5):872-879.). Then, the authors can add that the highest incidence is observed during the first 72 hours, with an adequate reference.
Thanks for your precious comment. I have changed the sentence accordingly in line with your instruction. For details, P3L20-22.
6. P4 L42 please include more updated references for the rate of delirium after high risk surgeries.
Thanks for your comment. I have added three updated refences for the incidence of delirium after high risk surgeries. For details, P3L22.
7. P4 L49 physiopathological mechanisms of postoperative delirium are multiples, please use plural
Sorry for my grammatic error. I have revised it. «The pathophysiological mechanisms of delirium have not been well-elucidated, and neuroinflammation remains a topic of mainstream research interest». For details, P3L27.
8. P5 L4 I suggest rewriting this sentence as it is not clear, do the authors mean to write that no recommendation can be made on the use of a specific drug as a prophylactic agent due to lack of data?
I have rephrased this sentence as « there is limited pharmacological methods to reduce the incidence of delirium ». For details, P4L4.
9. P5 L11 I suggest adding « in a recent network meta-analysis » at the end of the sentence before [6] to clarify the use of « highest-ranking possibility »
Thanks for your precious comment. I have added « in a recent network meta-analysis » at the end of the sentence before reference [6]. For details, P4L7.
10. P5 L8 >L53 I suggest rewriting the paragraph to separate data on cardiac and non-cardiac surgery, as physiopathology and incidence of delirium is quite different between these types of surgery (cerebral embolism frequent in cardiac surgery), and thus it is difficult and makes little sense to do a literature analysis mixing both. I also suggest adding a sentence about the heterogenous ways dexmedetomidine is administrated in the literature (per or postoperative or both, bolus, continuous…) which complexify the analysis even more.
I reorganized this paragraph according to your comment. For details, P4-5.

11. P5 L37 replace « commenced » by « started »
Thanks for your precious comment. I have revised. For details, P4L26.
12. P5 L 52 I suggest emphasizing more on side effects of dexmedetomidine in the elderly and adding a recent citation to illustrate this important issue (for exemple Pan H, Liu C, Ma X, Xu Y, Zhang M, Wang Y. Perioperative dexmedetomidine reduces delirium in elderly patients after non-cardiac surgery: a systematic review and meta-analysis of randomized-controlled trials. Can J Anaesth. 2019 Dec;66(12):1489-1500.)
Thanks for your precious comment. I have cited this reference in the text according to your instruction. For details, P4L16-19.
13. P6 L57 Please rewrite, I don't understand if the authors state that s-ketamine is non-inferior to dexmedetomidine as their hypothesis or as a fact here. If it's the last, add a reference and thus justify what your study will add to these results.
It is a hypothesis, so I have modified as "Furthermore, we hypothesize that the sympathomimetic and analgesic properties of S-ketamine might partially explain its non-inferior property for delirium prevention compared to dexmedetomidine". For details, P6L9-11.
14. P6 L60 Please rewrite to make it clearer that if s-ketamine is out of favor it is because of racemic ketamine negative results in podcast and pride
Sorry for my unclearly expression. Since psychiatric side effects and negative conclusions from PODCAST and PRIDE studies of racemic ketamine, anesthesiologists may have concerns when they consider S-ketamine as a prophylactic drug in POD. Though S-ketamine has stronger potency and lower incidence of adverse reactions, the evidence that it reduces the incidence of postoperative delirium is far from enough. I have revised this sentence. For details, P6L11-13.
15. P7 L8 Change « far from revealed » by « lack of good quality evidences »
Thanks for your suggestion. I have revised. For details, P6L14.

16. P7 L 15 Change « compared with » by « compared to ». I suggest adding that comparison with dexmedetomidine is justified by recent literature data making it the intraoperative drug with the higher potential for prevention of postoperative delirium
Thanks for your suggestion. I have revised. For details, P6L18.
17. I suggest that the author add a paragraph about the rationale for their biomarkers analysis.
I have attached the rationales for biomarkers analysis in the supplementary material.
The rational for the choice of TNF-α
Surgery activates the innate immune system resulting in release of proinflammatory mediators (TNF-α, IL-1 and IL-6). However, ketamine could suppress nuclear factor-κB expression involved in the transcription of genes encoding the proinflammatory cytokines tumour necrosis factor (TNF-α). [1]
The rational for the choice of BDNF
BDNF has a role in increasing synaptic plasticity and synaptic function. Reviews have suggested that brain-derived neurotrophic factor (BDNF) improved memory function, reversed age-related changes in brain and prevented cell death. [2] Furthermore, ketamine requires brain-derived neurotrophic factor (BDNF) signals to exert antidepressant effects. [3]
The rational for the choice of acetylcholine
Acetylcholine is thought to be involved in the neuroplasticity, and is present in several neural pathways responsible for arousal, attention and memory. [4] However, ketamine could increase cholinergic tone that may contribute to the improvement of cognition. [5]
[1] Choudhury D, Autry AE, Tolias KF, et al. Ketamine: Neuroprotective or Neurotoxic?. Front Neurosci. 2021;15:672526.
[2] Kotekar N, Shenkar A, Nagaraj R. Postoperative cognitive dysfunction - current preventive strategies. Clin Interv Aging. 2018;13:2267-2273.
[3] Himmelseher S, Kochs EF. Ready for a "breakthrough" with ketamine? A look at recent pharmacological insights!. Curr Opin Anaesthesiol. 2021;34(4):393-401.
[4] Jin Z, Hu J, Ma D. Postoperative delirium: perioperative assessment, risk reduction, and management. Br J Anaesth. 2020;125(4):492-504.

[5] Hambrecht-Wiedbusch VS, Li D, Mashour GA. Paradoxical Emergence: Administration of Subanesthetic Ketamine during Isoflurane Anesthesia Induces Burst Suppression but Accelerates Recovery. Anesthesiology. 2017;126(3):482-494.

18. P8 L19 why have an upper limit for age?

We set an upper limit for age because there are few patients aged over 90 years undergoing thoracic surgery. It is a limitation for this protocol.

19. P9 L 22 Will the participants be given written informed consent or oral consent only? Please clarify.

The participants will be informed and signed the written informed consent.

20. P9 L45 The author state that the anesthetic nurse will not be involved in follow-up, but will she be involved in intraoperative patient management? if yes, it is a serious limitation for blinding.

The anesthetic nurse will be not only involved in follow-up but also intraoperative patient management.

21. I have serious concerns about the fact that dexmedetomidine administration as planned in this protocol, with a bolus, in elderly patients will often induce bradycardia and hypotension compromising the blinding. I strongly suggest the authors to revise their protocol of drug administration to use one without boluses. If the authors disagree about this bias, they should explain it in their manuscript with accurate references about safety in the elderly population.

As mentioned above, we found that a loading dose of 0.4 μg/kg dexmedetomidine would lead to obvious bradycardia and transient hypertension in the preliminary trial (2 out of 7 patients). Therefore, we modified a loading dose of dexmedetomidine to 0.2 μg/kg; In addition, in order to ensure blindness, the infusion speed of dexmedetomidine is consistent with that of S-ketamine, which also reduces the side effects of dexmedetomidine. For details, P11L14-19.

If dexmedetomidine was administered without bolus dosage, we consider that it may be insufficient to clarify the effect of dexmedetomidine on POD prevention, which may also contribute to selection bias. As far, we have recruited 67 patients after the protocol was modified, and no obvious bradycardia events occurs. Accordingly, we have submitted the change of the protocol to the Chinese Clinical Trials Registry website and Clinical trials. Gov.

22. P10 L26 I suggest removing the use of atropine, as limiting secretion at induction is of little interest, and on the opposite, anticholinergic agents are well-known precipitating factors for delirium and thus should be avoided in a high-risk population.

Thanks for your precious comment. Based on a previous study in our center, there would be a little more secretion after the patient was placed in the lateral decubitus position without preoperative anticholinergic agents, which causes inconvenience for anesthesia management and may also unblind study.

23. P10 L29 Please write fully A-line and V-line

I have revised accordingly. For details, P6L9-11.

24. P10 L31 I suggest removing the use of midazolam, as literature suggest it has no or little effect on anxiety or memorization, and on the opposite, benzodiazepines are well-known precipitating factors for delirium and thus should be avoided in a high-risk population.

Thanks for your professional comment. We use midazolam as a preoperative medication based on two reasons. Firstly, midazolam can reduce the psychiatric side effects of ketamine [1]; Secondly, we insert the arterial line and central venous line when the patients are awake, so midazolam is used to relieve their anxiety.

1 Schwenk ES, Viscusi ER, Buvanendran A, et al. Consensus Guidelines on the Use of Intravenous Ketamine Infusions for Acute Pain Management From the American Society of Regional Anesthesia and Pain Medicine, the American Academy of Pain Medicine, and the American Society of Anesthesiologists. Reg Anesth Pain Med. 2018;43(5):456-466.

25. P10 L35 Why use a rapid sequence induction dose of rocuronium in a standard induction sequence with mask ventilation?

Sorry for my typo. The standard induction dose of rocuronium is 2 ED95 (0.6 mg/kg)

26. P11 L19 Why use an intercostal block as locoregional anesthesia technic, whereas its effects are

modest compared to epidural anesthesia or paravertébral block?
The intercostal nerve block is a routine technic performed by surgeons in my center under direct thoracoscopic view before placing a chest tube. On the one hand, intercostal nerve block is easy to perform and safe under VATS. On the other hand, high-level epidural analgesia is a high-risk procedure, which needs to be operated by experienced anesthesiologists; Parathoracic vertebral block is relatively time-consuming and reduces the speed of surgical turnover. However, epidural block analgesia is the best provided technique in thoracic surgery.
27. Will a dedicated venous line not used for fluid loading be used for study drug administration? This is a major safety point, as inadvertent boluses of dexmedetomidine are at high risk of bradycardia that could lead to cardiac arrest.
Yes, there is a dedicated venous line used for fluid loading for study drug administration.
28. I suggest adding data collection of BIS value, for example, time spend with RS >0 or with BIS <40, as the literature suggests that too deep anesthesia may be associated with postoperative delirium.
Thanks for your precious comment. I have added BIS value to intraoperative data collection part. Each patient under general anesthesia has BIS monitor in our center, and we collect the BIS value every 15 minutes documented in the Anesthesia Information Management System (AIMS).
30. I suggest adding data collection of physiological perturbations like metabolic abnormalities, hypoxia, electrolyte imbalance, infection, which are well-known precipitating factors of postoperative delirium.
Thanks for your precious comment. Actually, we have collected the arterial blood gas analysis indexes (hemoglobin, electrolyte, lactate, PH, oxygen partial pressure, et al) at the following timepoints: Two-lung ventilation after intubation, one-lung ventilation, and two-lung ventilation before chest closure.
31. P13 L51 change « at 4 h.. » for « between 4 h after surgery and the 4th postoperative day »
Thanks for your precious comment. I have changed « at 4 h.. » for « between 4 h after surgery and the 4th postoperative day ». For details, P13L17-18.
32. P14 L20 Will you use a validated translation of the 3D-CAM and CAM-ICU ? If yes, specify which one with reference, if not, cite this as a limitation.
We used a validated translation of 3D-CAM referring to the literature by Mu DL « Cross-cultural adaptation and validation of the 3D-CAM Chinese version in surgical ICU patients. BMC Psychiatry. 2020;20(1):133 ». I have added the reference in the manuscript. For details, P14L3.
33. P14 L50 Will the 2 investigators in charge of the delirium assessment be able to do them 7/7, even on weekend?
At first, when only two investigators were responsible for follow-up, the workload was substantially heavy, so we expanded the follow-up team to four investigators, all of whom were trained by professional psychiatrists. The four investigators are interns who are on duty 24h every 4 days. For details, P14L18.
34. P14 L56 I appreciate the authors included a specific training for delirium assessment, as sensitivity is highly dependent on this training.
We all appreciate your interest and affirmation for the protocol, and we will try our best to improve it.
35. P14 L60 Can the authors specify if a discussion with the bedside nurse will be added to the 3D-CAM evaluation and to the chart review?
We don’t add this item to the 3D-CAM evaluation and the chart review. The investigators inquired the bedside nurse whether the patients had symptoms, such as inattention and altered consciousness, in order to pay more attention to the potential delirious patients.
36. Indeed, the reported incidence of delirium in the elderly after surgery is highly dependent on how it is diagnosed and screened. In a study for example, the incidence of delirium using chart review only was 3%, noted during routine clinical care was 8%, using interviews with nurses was 9%, and using daily mental status testing and application of a validated diagnostic algorithm was 53%. (Rudolph JL, Marcantonio ER: Review articles: Postoperative delirium: Acute change with long-term implications. Anesth Analg 2011; 112:1202–11). The family interview will also be of great value to add sensitivity to the assessment. Please specify if you planned to do it.

I absolutely agree with your opinion. The incidence of delirium varied with assessment methods, which caused confounding bias to a great extent. Investigators give the caregivers educational pamphlet interpreting POD preoperatively, and keep in touch with caregivers by phone during follow-up intervals. If the caregivers found that the patient have abnormal manifestations in attention, consciousness and thinking, investigators will be informed and perform more frequent follow-ups, so as to improve the sensitivity and specificity of diagnosis.

Table 3

38. P16 L18-22 Multiple definitions for hypotension are specified in the table, please explain which one is used in which circumstances.

We place more emphasis on mean arterial pressure (MAP) intraoperatively to keep it within ± 80% of the baseline level. Intraoperative low MAP and changes in the level of SBP or DBP that result in a decrease of MAP will be defined as hypotension.

39. Will noradrenaline be the only way to treat hypotension? Its use in hypovolemic patients can lead to cardiac or renal complications, thus it is important before using it to assess patient volemic status by testing fluid loading impact on systolic ejection volume before starting noradrenaline, which then is not a first-line drug. Moreover, will you use only boluses or is it possible to continue with infusion? If yes, please specify in the table.

Thanks for your comment. The intraoperative hemodynamic management is based on goal-directed fluid therapy. We detect the reasons for hypotension initially using ProAQT/Pulsionflex (GETINGE), including preload decrease detected by the change of SVI after fluid loading, afterload decrease by SVRI or cardiac contractility decrease by CPI or dPmx.

Generally speaking, we will give a bolus of noradrenaline followed by continuous infusion with 0.01-0.1 μg/kg/min when necessary. I have specified in the table note. For details, P16L4-5.

40. P16 L50 Atropine 40mg is written as a treatment of tachycardia, please correct to « esmolol 40 mg ».

Sorry for my typo. I have revised. For details, P16.

Sample size calculation

41. P17 L 32 A dropout rate of 5% seems a bit low as advert events due to dexmedetomidine may be higher than that (see Balanced Opioid-free Anesthesia with Dexmedetomidine versus Balanced Anesthesia with Remifentanil for Major or Intermediate Noncardiac Surgery. Anesthesiology. 2021 Apr 1;134(4):541-551., which was stopped prematurely because of these advert events.)

Thanks for your precious comment. The attrite rate of 5% originated from an ongoing multi-center study of elderly patients undergoing thoracic surgery. Furthermore, we have recruited 67 patients since the loading dose of dexmedetomidine was modified, and there is no dropout due to adverse event of dexmedetomidine. However, if the result were what you predicted, we would enlarge the sample size upon approval from the IRB.

Discussion

42. P20 L4 please remove « miserable »

Sorry for my unclearly expression. I have removed it. For details, P19L27.

43. P20 L35 please rewrite the sentence « we believe that the results will be as follows ». Indeed, after that, the authors stated 4 hypotheses with opposite results. Thus, more than a hypothesis presentation, it should be introduced as possible results, that will be interesting even in the case of negativity.

I have changed the sentence to «We believe that the possible results will be one of the following». For details, P20L14.

44. P21 L4 This study is not designed and power to assess this 4th hypothesis, please remove it from the manuscript.

Thanks for your guidance. I have removed the 4th hypothesis.

Supplementary data

45. Please provide the ethical approval form

I have attached the ethical approval form in the supplementary data.

Point by point response to reviewer 3#

Dear Dr. Martyn Stones

We sincerely appreciate your recognition and suggestions on the SKED protocol, which will play an extremely important guiding role in the following research. Hereby, I will provide detailed explanations of your comments.

1. why mix 3-D CAM and CAM-ICU, could use CAM-ICU alone?

Thanks for your comment. 3-D CAM is used for patients without tracheal intubation; However, CAM-ICU is used for patients intubated. Exactly, we consider CAM-ICU alone is feasible.

2. What are safety considerations for halting trial?

The trial will be halted in the case of serious adverse events (life-threatening events that may be associated with the study drugs or perioperative incidents, such as death or serious cardio-cerebral vascular events) that in the opinion of attending anesthesiologist contraindicates study drug administration.

3. Rational for the intervention dosage?

Rational for dexmedetomidine dosage

Due to the limitation of previous protocol, we found we found that a loading dose of 0.4 μg/kg dexmedetomidine would lead to obvious bradycardia and transient hypertension in the preliminary trial. Therefore, we modified the loading dose of dexmedetomidine to 0.2 μg/kg; In addition, in order to ensure blindness, the infusion speed of dexmedetomidine is consistent with that of S-ketamine, which also reduces the side effects of dexmedetomidine.

Rational for S-ketamine dosage

The rational for S-ketamine dosage referred to the studies that ketamine was used as a prophylactic agent to reduce the incidence of postoperative delirium and depression.

S-ketamine is the S (+) enantiomer of ketamine, which has a higher affinity with aspartate receptor and μ opioid receptor, so the anaesthetic potency of S-ketamine is two-fold higher than that of racemic ketamine, and it has higher in vivo clearance rate characterized with lower incidence of adverse reactions. [1] A loading dose of 0.5 mg/kg ketamine was used in both PRIDE and PODCAST study, so we deem that a loading dose of 0.25 mg/kg S-ketamine in the protocol is rational. [2,3] In a randomized, prospective, double-blind placebo-controlled study, patients undergoing orthopedic surgery received ketamine, 0.5 mg/kg (0.05 ml/kg) at induction of anesthesia, followed by 0.25 mg/kg/h (0.025 ml/kg/h) continuous infusion for 30 min and were reported to have improved scores for depressed mood after surgery. [4]

1 Krystal JH, Charney DS, Duman RS. A new rapid-acting antidepressant. Cell. 2020;181(1):7.

2 Hollinger A, Rüst CA, Riegger H, et al. Ketamine vs. haloperidol for prevention of cognitive dysfunction and postoperative delirium: A phase IV multicentre randomised placebo-controlled double-blind clinical trial. J Clin Anesth. 2021;68:110099.

3 Avidan MS, Maybrier HR, Abdallah AB, et al. Intraoperative ketamine for prevention of postoperative delirium or pain after major surgery in older adults: an international, multicentre, double-blind, randomised clinical trial [published correction appears in Lancet 2017;390:267–75.

4 Jiang M, Wang MH, Wang XB, et al. Effect of intraoperative application of ketamine on postoperative depressed mood in patients undergoing elective orthopedic surgery. J Anesth 2016;30(2):232–7.

4. Does the different loading dose volume un-blind the study?

Due to the limitation of previous protocol, we found we found that a loading dose of 0.4 μg/kg dexmedetomidine would lead to obvious bradycardia and transient hypertension in the preliminary trial. Therefore, we modified the loading dose of dexmedetomidine to 0.2 μg/kg; In addition, in order to ensure blindness, the loading dose volume and continuous infusion speed of dexmedetomidine are consistent with that of S-ketamine, which also reduces the side effects of dexmedetomidine.

5. Consider baseline/pre-op delirium assessment and delirium as an exclusion criteria.

Thanks for your precious suggestion. Actually, we have performed the baseline delirium assessment after enrollment.

6. Rescue medications: Propofol 30mg and haloperidol 10mg should only be used if patient is

distressed or for safety reasons.
Thanks for your precious comment. Propofol 30mg and haloperidol 10mg should only be used if patient is distressed or for safety reasons.
7. Miserable orthopaedics? spelling?
Sorry for my unclearly expression. I have removed miserable. For details, P19L27.
8. Reference 25: this sentence does not make scientific sense. The plasma amino acids compete for entry to the brain, plasma serotonin does not enter the brain.
Sorry for my unclearly expression. S-ketamine decreases the levels of circulating branched chain amino acids which inhibit the synthesis and release of serotonin and noradrenaline in the brain. Thus, S-ketamine could, in theory, increase the effects of serotonin and noradrenaline in the brain, and contribute to the improvement of depression and cognitive impairment. I have revised. For details, P6L5-9.
9. What is the rational for the choice of plasma biomarkers. why are they measured only at baseline and 96 hours, what about the times in between?
The rational for the choice of TNF-$\boldsymbol{\alpha}$
Surgery activates the innate immune system resulting in release of proinflammatory mediators (TNF-$\boldsymbol{\alpha}$, IL-1 and IL-6). However, ketamine could suppress nuclear factor-$\boldsymbol{\kappa}$B expression involved in the transcription of genes encoding the proinflammatory cytokines tumor necrosis factor (TNF-$\boldsymbol{\alpha}$). [1]
The rational for the choice of BDNF
BDNF has a role in increasing synaptic plasticity and synaptic function. Reviews have suggested that brain-derived neurotrophic factor (BDNF) improved memory function, reversed age-related changes in brain and prevented cell death. [2] Furthermore, ketamine requires brain-derived neurotrophic factor (BDNF) signals to exert antidepressant effects. [3]
The rational for the choice of acetylcholine
Acetylcholine is thought to be involved in the neuroplasticity, and is present in several neural pathways responsible for arousal, attention and memory. [4] However, ketamine could increase cholinergic tone that may contribute to the improvement of cognition. [5]
[1] Choudhury D, Autry AE, Tolias KF, et al. Ketamine: Neuroprotective or Neurotoxic?. Front Neurosci. 2021;15:672526.
[2] Kotekar N, Shenkar A, Nagaraj R. Postoperative cognitive dysfunction - current preventive strategies. Clin Interv Aging. 2018;13:2267-2273.
[3] Himmelseher S, Kochs EF. Ready for a "breakthrough" with ketamine? A look at recent pharmacological insights!. Curr Opin Anaesthesiol. 2021;34(4):393-401.
[4] Jin Z, Hu J, Ma D. Postoperative delirium: perioperative assessment, risk reduction, and management. Br J Anaesth. 2020;125(4):492-504.
[5] Hambrecht-Wiedbusch VS, Li D, Mashour GA. Paradoxical Emergence: Administration of Subanesthetic Ketamine during Isoflurane Anesthesia Induces Burst Suppression but Accelerates Recovery. Anesthesiology. 2017;126(3):482-494.

Point by point response to reviewer 4#
Dear Dr. Francisco Diaz
We attach great importance to your methodological comments, which is of practical significance and value for the improvement of the study. Hereby, I will provide detailed explanations and revision according to your comments.
1. My main concern is that the PI is not blinded to the randomization protocol and the CRFs during recruitment and data entry. This is a potential source of bias. Please address how this bias will be prevented. Ideally, the PI should be blinded to the randomization protocol and an independent team should conduct the data storage and management. The above concern arouse from line 46 in page 9 which says “The randomization protocol will be kept secure by the primary investigator”. And from lines 35-37 on page 13 that say “Paper case report forms (CRF) will be stored by the primary investigator and entered into the Epidata V4.6 database protected by password only accessible to authorised users.”

Thanks for your precious comment. It is a limitation that the randomization protocol was kept by the PI, so we have transferred the randomization protocol to the anesthetist nurse who is responsible randomization and not involved in the anesthetic management and delirium assessment. Furthermore, paper case report forms (CRF) will be stored by a member of Data Safety and Monitoring Board (DSMB) and entered into the Epidata V4.6 database protected by password only accessible to DSMB.
The modifications above are revised in the protocol.
2. Another concern is that it is unclear who will conduct the data management and data analyses and whether these two will be conducted in a way that guarantees relative independence from the clinicians collecting the data and the PI. The PI, and the clinicians collecting the data, should be allowed to see the randomization protocol only when both recruitment and the database are closed, and an independent statistics team should be in charge of the data management and analysis of the primary outcome to prevent biases. The authors must address these concerns in the paper.
Thanks for your comments. The PI, investigators, and the clinicians collecting the data, are allowed to unmask the randomization protocol only when both recruitment and the database are closed. Furthermore, the statistical analyses will be performed by biostatisticians independent of the study.
3. The paper does not mention a Data Safety and Monitoring Committee (DSMB). This independent committee is crucial in case the randomization protocol needs to be unblinded to address a safety issue, among many other reasons. Please describe whether such committee exists and how it will interact with the researchers.
Data Safety and Monitoring Committee (DSMB) is consist of three senior anesthesiologists and one surgeon who are independent of the trial. The DSMB will provide independent oversight of the SKED trial and will review the study data for the participant safety as well as CRF storage.

VERSION 2 – REVIEW

REVIEWER	Mahanna-Gabrielli , Elizabeth University of Miami Miller School of Medicine, Anesthesiology, Perioperative Medicine and Pain Management
REVIEW RETURNED	31-May-2022

GENERAL COMMENTS	I believe the authors have addressed all my comments/suggestions for their protocol. Given that major changes to the protocol are not easily performed as the trial is partly undertaken, I feel they have appropriately addressed concerns that can be addressed by the other reviewers.

REVIEWER	Sigaut, Stéphanie Hôpital Beaujon, Anesthesiology and Intensive Care
REVIEW RETURNED	12-May-2022

GENERAL COMMENTS	The authors extensively respond to my comments and modified their manuscript accordingly, I have no more comments.

REVIEWER	Stones, Martyn Cardiff University, Psychiatry
REVIEW RETURNED	05-Jun-2022

GENERAL COMMENTS	I am satisfied that my original comments have been addressed consider 1) exclusion criteria 1: need only say 'history of severe? psychiatric disorder' (adding depression is confusing)

	2) stating, who will be performing biomarker assay, and if they will they be blinded?

REVIEWER	Diaz, Francisco University of Kentucky, Department of Biostatistics
REVIEW RETURNED	18-May-2022

GENERAL COMMENTS	I want to thank the authors for their careful address of my comments.

VERSION 2 – AUTHOR RESPONSE

Point by point response to reviewer 1#
I believe the authors have addressed all my comments/suggestions for their protocol. Given that major changes to the protocol are not easily performed as the trial is partly undertaken, I feel they have appropriately addressed concerns that can be addressed by the other reviewers.
Dear Dr. Elizabeth Mahanna-Gabrielli
We sincerely appreciate your recognition and suggestions on the SKED protocol, which will play an extremely important guiding role in the following research. We will try our best to perfect the protocol and investigate the effect of subanesthetic S-ketamine on postoperative delirium and cognitive function in elderly patients, so as to provide new clinical evidence for the prevention of postoperative delirium.

Point by point response to reviewer 2#
The authors extensively respond to my comments and modified their manuscript accordingly, I have no more comments.
Dear Dr. Stéphanie Sigaut
We attach great importance to your professional comments, which is of practical significance for the improvement of the study. We will try our best to perfect the protocol and investigate the effect of subanesthetic S-ketamine on postoperative delirium and cognitive function in elderly patients, so as to provide new clinical evidence for the prevention of postoperative delirium.

Point by point response to reviewer 3#
Dear Dr. Martyn Stones
We sincerely appreciate your recognition and suggestions on the SKED protocol, which will play an extremely important guiding role in the following research. Hereby, I will revise some details according to your comments.
I am satisfied that my original comments have been addressed
1) exclusion criteria 1: need only say 'history of severe? psychiatric disorder' (adding depression is confusing)
Thanks for your precious suggestion. We have revised accordingly. P8L4
2) stating, who will be performing biomarker assay, and if they will they be blinded?
The biomarker assay will be performed by a specialist who is blinded to the randomization. P15L18-19
Reviewer: 4
I want to thank the authors for their careful address of my comments.
Dear Dr. Francisco Diaz
We attach great importance to your methodological comments, which is of practical significance and value for the improvement of the study. We will try our best to perfect the protocol and investigate the

effect of subanesthetic S-ketamine on postoperative delirium and cognitive function in elderly patients, so as to provide new clinical evidence for the prevention of postoperative delirium.