Author’s response to reviews

Title: Effect of low dose naloxone on the immune system function of a patient undergoing videoassisted thoracoscopic resection of lung cancer with sufentanil controlled analgesia — a randomized controlled trial.

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Author’s response to reviews:

Dear editors,

Thank you for arranging a timely review for our manuscript. We have carefully evaluated the reviewers’ critical comments and thoughtful suggestions, responded to these suggestions point-by-point, and revised the manuscript accordingly. All changes made to the text are in red so that they can be easily identified. With regard to the reviewers’ comments and suggestions, we would like to reply as follows:

Jose Navas, MD (Reviewer 1):

Q1. Initially the abstract depicts that the 2 groups have 35 patients, when in reality one of the dropped out due to loss in follow-up. I think it should be stated in the abstract the number of patients that actually underwent the analysis (35 and 34).

A1. We agree with the reviewer’s point. According to the suggestion, the actual number of participants in the summary has been modified (Abstract section, page 2).
Q2. Page 4, Method section, ASA status: I just wanted further clarification, how exactly someone who has a suspected lesion for lung cancer, in his/her mid-50s, could be classified as ASA 1? Even the ASA 2. The ASA clearly states that ASA 2 is "mild lung disease". Please provide further clarification, seems to be that this should have been ASA 2s and 3s.

A2. We greatly accept the reviewer’s suggestions. When we classified the patients, the patients who were highly suspected of carcinoma in situ before operation and without any other disease were classified as ASA 1. After consulting the ASA clarification, the "mild lung disease" was ignored. The patients who were classified as ASA 1 has been changed. TABLE 1 has been modified. (TABLE 1, Page 20).

Q3. Page 5, Method section, second paragraph, line 16: Please replace Sauteralgyl, for its generic version, Meperidine.

A3. Sorry about this mistake. We have replace Sauteralgyl with Meperidine (Methods section, line 13, page 5).

Q4. Page 5, Method section, last paragraph: Regarding the nausea and vomiting score, please provide a citation to support this scoring system or if you introduced this score in this study please provide details regarding the validation of this scale.

A4. We thank the reviewer to raise this important issue. At first, we adopted PONV's VAS score, and then we were suggested that it will be better if we separate the nausea and vomiting scores, so we adopted a new scoring criteria. But we did not find the Sufficient evidence support this scoring system through consulting literatures, finally we reused the VAS score standard for PONV. Modify results were showed in (TABLE4, Page 23). At the same time, relevant parts of the article were modified.

Q5. Page 7, Result section, paragraph 2: Please fix "figure 3" spelling.

A5. Sorry for the mistake. Spelling was modified. (figure 3, page 24).

Cheng Ni (Reviewer 2):

Q1. "CD4+/CD8+ T cell ratio" would be better than just "CD4+/CD8+".

A1. Thank you for this suggestion. Modifications have been completely made as required.
Q2. Please explain why the visual analog scales for pain at rest and while coughing are different, for the pain at rest, the difference happens at 12 and 24 hours after surgery, but for the pain at coughing, the difference happens at 48 hours after surgery.

A2. We thank the reviewer to raise this important issue. Relevant parts of the article were modified. (Discussion section, fourth paragraph, page 10)

1) The primary goal of this experiment is to study the effect of naloxone on immune function and the sample size was estimated by NK cells, the difference of the pain VAS scores results may be related to the shortage of samples.

2) The coughing VAS scores were at a higher level than that of rest VAS scores after operation, and the patients in the two groups all showed low tolerance while coughing. It may be the reason for patients not to feel obviously relief at higher level of pain due to the subjectivity of VAS scores. This may explain why the difference happened at 48 hours after surgery for the pain on coughing, but for the pain at rest, the difference happened at 12 and 24 hours after surgery.

Q3. In the first paragraph of discussion, the author has mentioned that the NK cells activity is important for the immune-regulation and tumor development. Thus, if the activities of the NK cells in both groups are provided, the results would be better.

A3. Sorry for the confusion. This is caused by the wrong vocabulary used. We actually agree with the editor that it is meaningful to detect the activity of NK cells. I have modified the relevant parts of the article. Activity was replaced with number. (Methods section, first paragraph, page 8).

Q4. The authors should discuss the relationship between NK cells and CD4+/CD8+ T cells during perioperative context, as well as their effects on postoperative tumor development.

A4. We accepted professor Cheng Ni’s comment. Relevant contents have been added as required. (Discussion section, first and second paragraph, page 7 and 8)
NK cells distinguish healthy cells from abnormal cells by measuring the net input of activating and inhibitory signals perceived from target cells through NK cells surface receptors. Acquisition of activating ligands in combination with reduced expression of major histocompatibility complex (MHC) class I molecules on virus-infected and cancer cells activates NK cells cytotoxicity and release of immunostimulatory cytokines like interferon gamma (IFN-γ). The survival of lung cancer patients was positively correlated with the degree of NK cells infiltration in lung cancer. For example, there was a significant correlation between survival rates and the number of infiltrating NK cells in patients with primary squamous cell lung carcinoma.

CD4+ is a T helper cell with the function of immune regulation. CD8+ is a cytotoxic T lymphocyte, which can eradicate virally infected cells and cancer cells and induce apoptosis by release of cytotoxins (perforin, granulysin and granzymes) and directly cell–cell contacting. T helper cells recognize the antigens secreted by tumor and prevent cancer by activating other immune cells, such as NK cells and cytotoxic T lymphocyte. NK cells also play a role in activation of cytotoxic T cell. In addition, cytokines produced by NK cells influence T helper cell polarization. The maintenance of normal immune function depends on the cooperation or restriction between various immune cells (especially all kinds of T cell subsets and NK cells).

Q5. There are multiple spelling mistakes, need to be revised.

A5. We reviewed and revised spelling mistakes.

Vojislava Neskovic, PhD, MD (Reviewer 3):

A1. Obviously authors present that low dose naloxone affects levels of OGF which is positive outcome of this study. However, there is a bit of a confusion regarding NK cells: Authors declare that NK cells significantly increase at 48 hours after surgery, however, we see from the tables that indeed, there is a decrease of NK cells comparing to the time before surgery, that the number increase in the naloxone group while still decreasing in the non naloxone group, but over all there is no significant difference between the groups. This may be better explained.

Q1. We thank the reviewer to raise this important issue. Relevant contents have been added as required. (Discussion section, third paragraph, page 9).
The postoperative immune function may be related to operation, anesthesia, postoperative pain, body temperature and blood transfusion in the operation, etc. Some studies indicated that the operation itself and stress responses induced by operation could result in a reduction of the postoperative NK cells. The effects of anesthesia on immune function have been widely discussed in recent years, but the result is still controversial. Opioid drugs may cause postoperative immunosuppression by reducing the number of NK cells, but it is difficult to control the stress and pain caused by surgical stimulation without the use of opioids, and the acute pain could activate the hypothalamus-pituitary-adrenal (HPA) axis, which in turn has an effect on the number of NK cells. There are other non-opioid drugs that affect immune function. The combination of these factors may result in our results that number of NK cells in the naloxone group was higher than that of non-naloxone group after operation, but the number of NK cells in the both of two groups were lower compared to the time before surgery.

Q 2. It is good to discuss a clinical relevance of the results. Authors present less nausea in naloxone group, however, non naloxone group was also rather well managed (0 vs 1 class). What do authors think, should this be reason to indicate low dose naloxone?

Same goes with VAS scores, although significantly different, VAS scores were low for both groups and the pain well managed. Would this be enough to include low dose naloxone in the protocol of everyday practice? What was shown as a clear benefit?

A2. We thank the reviewer to raise this important issue. Relevant contents have been added as required. (Discussion section, sixth paragraph, page10)

We have always attached great importance to postoperative analgesia management. Our results showed that postoperative pain in rest can be well controlled, but the pain scores on coughing were overall on the high level. Patients were encouraged to mobilize out of bed early and cough after the thoracoscopic surgery in order to reduce postoperative complications. This promote us to control the VAS scores at a lower level during coughing and activities to ensure patient's favorable prognosis and satisfaction. At the same time, we also found that opioids had effective analgesia on coughing but the frequency of postoperative nausea and vomiting after operation was higher. Hence, with respect to the better postoperative management, we would like to find a way to control the occurrence of postoperative nausea and vomiting in patients, not just the postoperative acute pain. And we hope to find a good balance between the control of postoperative nausea and pain. Low-dose naloxone may be a good choice from the experimental results, but more experiments are needed to prove this possibility.
With best regards,

Yours sincerely,

Qingping Wen