

Peer Review File

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**Reviewer A**

The original article entitled “A cervical compartment syndrome impairs cerebral circulation in post thyroidectomy hemorrhage data from an animal model” using 6 pigs to evaluate hypoxemic brain damage and underlying pathophysiology in an established animal model for post thyroidectomy hemorrhage.

The followings are my comments:

1. The animal study related to post thyroidectomy hemorrhage are specific and important to provide a theoretical basis for evaluating clinical postoperative hemorrhage patients.

Reply: -

2. The intention to reduce pig usage deserves to be commended, however, the allocation of 12 experiments and 5 pigs should be described, and the resulting difference in initial conditions and bias should be discussed.

Reply: In results section we further describe the allocation of the n=12 experiments to the n=5 pigs. We added some statistics for comparison of baseline vital signs and outcome measure between first and repeated experiments.

In methods section we added some details about possible difference in initial conditions in repeated experiments, but repeated experiments have only been performed if vital signs returned to baseline values. Regarding baseline vital signs and outcome measures like cerebral or peripheral oxygenation and cervical compartment pressure there is no statistically significant difference between first and repeated experiments. Furthermore, we added some discussion of possible bias performing repeated experiments in the discussion section

Changes in the text:

Methods: "When respiration recovered and vital signs remained stable at baseline levels, the experiment was repeated." (page 8, line 159/160)

"Friedmann's ANOVA for repeated measures (FA) was used with Bonferroni correction for multiple comparisons and Wilcoxon signed-rank test (Wilcoxon) for comparison of metric data between groups as normal distribution could not be assumed." (page 8/9, line 175-178)

Results: "In two animals one experiment, in two animals three experiments and in one animal four experiments could be performed." (page 10, line 185-187)

"There was no significant difference in baseline vital signs (heart rate:  $p=0.068$ ; mean arterial blood pressure:  $p=0.066$ ), cervical compartment pressure ( $p=0.102$ ), peak

systolic flow velocity ( $p=0.138$ ) and peripheral ( $p=0.786$ ) or cerebral oxygenation ( $p=0.180$ ) at beginning of the experiments between first and repeated experiments (Wilcoxon).” (page 10, line 191-195)

Discussion: “We did not detect relevant differences in the baseline vital signs or outcome measures between first and repeated experiments in the animals. The number of experiments differed between the animals, as we only repeated the experiments in an animal if respiratory function recovered and the vital signs as well as outcome measures like cervical compartment pressure and oxygenation returned to baseline values.” (page 17, line 335-340)

3. In Line 149 to 164, many experimental steps are omitted. “transferring blood into the cervical transferring blood into the cervical”, what is the source of blood? How to evaluate the volume or velocity? The definition of apnea (how many seconds/cycles)? Please describe the experimental steps in more detail and define more clearly to make it easy for readers to understand.

Reply: We revised the section about experimental setting and experimental procedure and added some information as requested. Overall, we report the necessary details relevant for this experimental setting, but not all details because this will impair the readability of this manuscript. In this manuscript our focus was not on the mechanisms necessary to induce the apnea by artificial increase of the cervical compartment pressure, as we proved before that the apnea is pressure dependent and described the experimental setting and the induction of a pressure dependent apnea in detail in our previous work (Schopf S et al. Surgery. 2018 Sep;164(3):518-524. doi: 10.1016/j.surg.2018.05.022). In this manuscript we want to present and discuss our results on the temporal relationship between increased cervical compartment pressure, cerebral perfusion and oxygenation and apnea to gain further insight into the pathophysiologic mechanisms. Therefore, we focused on reporting all relevant details for measurements of cerebral oxygenation, doppler ultrasound measurement of the cerebral perfusion and observation of the vital signs as well as the cervical compartment pressure. We ask the reviewer and the editorial board members to consider this aspect in their decision.

Changes in the text:

“Via a retroperitoneal approach, one central venous catheter was brought into the inferior vena cava for measurement of central venous pressure. A multi-lumen catheter was placed into the aorta abdominalis for blood pressure monitoring and to establish a direct connection to the neck using three-way stopcocks and an extension line with Luer Lock connection.” (page 6, line 126-130)

“First, post thyroidectomy hemorrhage was simulated by transferring blood into the cervical compartment using the established direct connection between the catheters in

the aorta abdominalis and the neck to increase the cervical compartment pressure until apnea was evident (11). Blood was slowly pumped artificially from the aorta to the neck using a 50 mL syringe until apnea occurred. Apnea was defined clinically as a loss of respiratory function for at least 30 sec followed by peripheral hypoxemia. After apnea occurred, cervical compartment pressure was reduced by extracting blood from the cervical compartment. When respiration recovered and vital signs remained stable at baseline levels, the experiment was repeated.” (page 7, line 152-160)

4. The readability of the results can be further improved, the figures and tables presentation make the readers easily confused. The citations in Line 184, 185 are inappropriate and also confusing. The description of time, such as 3:20 min, is not intuitive to the reader, and it is clearer to describe it directly in seconds, such as 200 sec.

Reply: As suggested, we improved the readability of the results section. We removed the citation in Line 184/185. In methods section there is a clear definition of apnea. Furthermore, we changed the descriptions of time as suggested by the reviewer and removed some redundant references to tables/figures.

Changes in the text:

Methods: “Apnea was defined clinically as a loss of respiratory function for at least 30 sec followed by peripheral hypoxemia.” (page 7, line 156-157)

Results: changes in line 203, 205, 211, 212, 213, 232,

5. All pigs in this study were intubated, the postoperative hemorrhage scenario without intubation after surgery were not discussed. Compartment syndrome may cause acute mucosal edema, which is also a major reason for the need of tracheostomy during revision surgery. Please revise the arbitrary statement in abstract (Line 71-73).

Reply: We added a section about the importance of the laryngo-pharyngeal edema and its clinical implications in the discussion section to take this aspect into account and furthermore revised the “conclusion” section of our abstract.

Changes in the text:

Discussion: “In our experimental setting with by intubation secured airways we cannot address a laryngo-pharyngeal mucosal edema, which can be caused by the impaired cervical venous and lymphatic drainage due to the elevated cervical compartment pressure (20). In our data we only could demonstrate the relevant increase in pressure of the internal jugular vein as a correlate to this impaired venous drainage. This laryngo-pharyngeal edema is of utmost clinical relevance as it may complicate intubation attempts to secure the patient’s airway for revision surgery and even lead to the need

for a tracheostomy (20). Furthermore, it might result in prolonged intubation and ventilation therapy (20).” (page 16/17, line 325-333)

Abstract-Conclusion: “... With this evidence of impaired cerebral perfusion and cerebral hypoxemia due to an increased cervical compartment pressure we can disprove the historic theory of tracheal collapse due to a compressive hematoma in post thyroidectomy hemorrhage. A cervical compartment syndrome seems to be causal, not only for brain hypoxemia but also an additional laryngo-pharyngeal mucosal edema.“

6. Strengths of this manuscript include the good research designs, careful statistics, and effective discussion to explain their findings. Overall, this article helps surgeons to better understand the pathophysiology of post thyroidectomy hemorrhage.

Reply: -

#### Reviewer B

This experimental study was undertaken in the established model for post thyroidectomy hemorrhage in n=6 pigs. Authors found that peripheral hypoxemia occurred with relevant delay in time after decrease of cerebral perfusion and cerebral hypoxemia, leading to a central apnea as a result. With this evidence of impaired cerebral perfusion and cerebral hypoxemia due to an increased cervical compartment pressure Author were able to disprove the historic theory of tracheal collapse caused by compression in post thyroidectomy hemorrhage as a predominant mechanism leading to apnea.

The study is well-designed and clearly reported.

Outcomes of this study are of interest in the field and have some potential clinical implications.

Reply: -

#### Reviewer C

The authors reported that cerebral hypoxemia might be the main cause of central apnea using an animal model. This manuscript is well written, and the topic is interesting to me. I recommend to accept this manuscript after minor revision.

1. Although the authors suggested a clinical classification system for post-thyroidectomy hemorrhage (Table 3), results of the present study did not test or validate this classification. In my opinion, the paragraph about Table 3 seems irrelevant.

Reply: With our experiments we were able to gain further insight into the

pathophysiology of post-thyroidectomy hemorrhage, especially in acute arterial bleeding. But we have also to take into account that venous bleedings have a differential dynamic and therefore may be less dangerous. Based on the data we referred to in our manuscript and our pathophysiologic model, taking into account even venous bleeding sources and the formation of hematomas without acute clinical relevance, we tried to structure the available information and well-known clinical findings and to sum up a comprehensive explanation for different scenarios of bleeding complications occurring in thyroid surgery. Our data do not explicitly validate this classification, but in our opinion the pathophysiologic knowledge obtained by our experiments allow this classification of differentially hazardous events to place our results in the context of clinical evidence. In our opinion, this classification is the logical consequence of our results with relevance in everyday clinical practice.

2. In the conclusion section, the notion about continuous pressure measurement is not supported by your result.

Reply: We changed this text in the conclusion to a more general statement

Changes in the text: “Due to the correlation of increased pressure and impairment of perfusion and function, new strategies for postoperative patients’ surveillance are necessary to avoid major morbidity (6,14).” (page 18, line 358-361)