

Peer Review File

Article information: <https://dx.doi.org/10.21037/jtd-22-1687>

Reviewer A

**Comment 1:** Patient selection: Were pediatric patients intentionally excluded in the cohort? Were there any patients with congenital (complete rings) tracheal stenosis in the cohort? This should be stated.

**Reply 1:** We thank the reviewer for this important comment and for giving us the opportunity to clarify that point. We did not exclude pediatric patients intentionally. Due to the fact that pediatric surgery is not performed in our department, we didn't see any pediatric patients. Therefore, we had no cases to report.

**Changes in the text:** We added the sentence "Since we do not treat patients under the age of 16 in our department, no pediatric patients were included in our case series." in the *Materials and Methods* section. (See page 5, line 124-125)

**Comment 2:** Was this planned as a retrospective or prospective study? State in the methods section what kind of study this is.

**Reply 2:** The current study was planned in a retrospective study design. We added this information in the *Materials and Methods* section.

**Changes in the text:** In the light of reviewer's comment, we added the sub heading "*Study design*" in the *Material and Methods* section and included "The current study was designed as a retrospective, single-center analysis comparing ITS and PTTS as control group" (See page 5, line 116-118)

**Comment 3:** Was there IRB approval for this study? Was this IRB separate from the IRB for maintaining a biorepository of the resected specimens?

**Reply 3:** We thank the reviewer for giving us the opportunity to add this information to the manuscript. Local ethics group was consent with the analyses. Due to the retrospective study design, according to Bavarian and Saxonian law in Germany, no official IRB was necessary for the present analyses. However, all patients gave informed consent for the analyses of the specimens and clinical data. We added this important information in the *Material and Methods* section.

**Changes in the text:**

We added the sub heading "Ethical aspects" in the *Material and Methods* section and included "The study was conducted according to the Declaration of Helsinki with informed consent of all patients. Local ethics group was consent with the analyses." (See page 7, line 171-173).

**Comment 4:** Line 135-136: What is meant by 'Vocal cord function was postoperatively controlled as well as respiratory and swallowing function'? This sentence does not read well and needs to be edited.

**Reply 4:** We thank the reviewer for this valuable comment. In light of the reviewer's comment, we changed the sentence in the revised version of the manuscript (*Materials and Methods* section).

**Changes in the text:** “Vocal cord function was postoperatively controlled by laryngoscopy. Respiratory and swallowing function was evaluated clinically.” (see page 6, line 146-148)

**Comment 5:** Table 1: History of oral contraception: what does n.s. stand for? provide a key for abbreviations. Similarly OSAS.

**Reply 5:** We thank the reviewer for this important comment. In the light of reviewer's comment we changed the abbreviation “n.s.” into “information not available” and “OSAS” into “Obstructive sleep apnea”.

**Changes in the text:** We changed “n.s.” in “Information not available” and “OSAS” in “Obstructive sleep apnea” see (page 16, table 1a). Additionally, we deleted OSAS in the table 1a legend (see page 16, line 415-416)

**Comment 6:** Table 1: Tracheal stenosis %. What does this mean?

**Reply 6:** We thank the reviewer for giving us the opportunity to clarify this point. “Tracheal stenosis %” aims to display the percentage of obstruction of the tracheal lumen.

**Changes in the text:** We changed “Tracheal stenosis” in “Percentage of obstruction of the tracheal lumen” within the former table 1b. However, according to Reviewer C (comment 4b) we removed table 1b and provided the relevant information of this table within the text

“According to the Myer-Cotton classification of tracheal stenoses n=4 patients presented with grade I, n=13 patients with grade II and n=6 patients with grade III. There was no significant difference between both groups. Due to missing data Myer-Cotton classification was not possible in n=4 patients. Median length of tracheal stenosis was 2cm (range 1-2.5cm) with distance to vocal cords of median 1cm (range 1-4cm) also with no significant difference between the two groups. Neither the diameter of the normal trachea (Median 15 vs. 17 mm) nor the remaining diameter in the stenosis (Median 5,2 vs. 5,5 mm) differed significantly. (Supplement Table A). “ (see page 8, line 206-210 and page 9, line 211-214).

In addition, former table 1b is available as supplement data: Supplement table A (see page 22, Supplement table A).

**Comment 7:** The figures do not have any legends

**Reply 7:** We thank the reviewer for this important comment. We have to apologize. We were not aware that some mistakes had occurred during the submitting process and all submitted figure legends were deleted.

**Changes in the text:** We added the figure legends (see page 19-21).

“Figure 1: (a): Formalin embedded sections using IHC with antibodies against progesterone receptors on fibroblasts in tracheal specimens in the ITS group. Magnification 200x. (b):

Formaline embed sections using IHC with antibodies against estrogen receptors on fibroblasts in tracheal specimens in the ITS group. Magnification 200x.

Figure 2: (a): Formaline embed sections using IHC with antibodies against progesterone receptors on fibroblasts in tracheal specimens in the PTTS group. Magnification 200x. (b): Formaline embed sections using IHC with antibodies against estrogen receptors on fibroblasts in tracheal specimens in the PTTS group. Magnification 200x. “

## **Reviewer B**

The authors reported that “Two hormone receptors may link to the genesis of idiopathic tracheal stenosis”.

**Comment 1:** Ambiguous title is not desirable as a scientific paper.

**Reply 1:** We thank the reviewer for the valuable comment. Despite the existing data, including our study, there is still no clear evidence for the hormone induced pathogenesis of tracheal stenosis and we have chosen this ambiguous title.

**Changes in the text:** In the light of reviewer’s comment, we have modified the title of the manuscript into “The role of local expression of hormone receptors in the genesis of idiopathic tracheal stenosis” (see page 1, line 1-2).

**Comment 2:** Including “Abstract Line 1”, proofreading is required by native speaker.

**Reply 2:** We thank the reviewer for giving us the chance to improve the manuscript. The whole manuscript was revised by a native speaker.

**Changes in the text:** All language-related changes after proofreading by a native speaker are in “Green” within the revised version of the manuscript.

**Comment 3:** The number of cases in the paper is smaller than in previous reports, and I can find no novel knowledge.

**Reply 3:** We thank the reviewer for giving us the chance to clarify that point. Even though, larger case series are existing, the whole number of cases with specific analyses of hormone receptors reported in the literature remains comparably small. Consequently, we think it is worth it to add our findings.

**Comment 4:** It would not make sense to compare ITS and PTTS, because the cause and pathology is clearly different between them.

**Reply 4:** We thank the reviewer for giving us the opportunity to clarify that point as well. The current study, the PTTS subgroup serves mainly as the negative control group for the hormone receptor expression. Therefore, we think it makes sense to include them in the analysis.

**Changes in the text:** In the light of reviewer’s comment, we added the subheading “*Study design*” in the Material and Methods section and included “The current study was designed as

a retrospective, single-center analysis comparing ITS and PTTs as control group” (See page 5, line 116-118)

**Comment 5:** Please describe the criteria for immunohistochemistry. (About positive criteria.)

**Reply 5:** In the light of reviewer’s comment, we added the definition for positive hormone receptor reaction of the immunohistochemistry by introducing the immunoreactive score as defined by Remmele (Remmele und Stegner 1987). The added changes are marked in the revised version of the manuscript. The reference was added to the literature.

**Changes in the text:** We have modified the text of the section Histopathological/immunohistochemical analysis and added the following changes: “All tissue specimens were analyzed by standardized Hematoxylin/Eosin (HE) staining in formalin fixed sections. In addition, immunohistochemical staining using antibodies against progesterone receptor (Progesteron Receptor, Clone PgR636, Mouse; Dako/Agilent) and estrogen receptor (Estrogen Receptor alpha, Clone EP1, Rabbit; Dako/Agilent) was performed” (see page 6, line 151-155).

“A positive reaction was defined as at least weak nuclear staining specific to fibroblast nuclei, analogous to the IRS (immunoreactive score) as defined by Remmele [13]” (page 6, line 157-159).

**Added literature:**

Remmele, W. and H.E. Stegner, *[Recommendation for uniform definition of an immunoreactive score (IRS) for immunohistochemical estrogen receptor detection (ER-ICA) in breast cancer tissue]*. Pathologe, 1987. 8(3): p. 138-40. (see page 16, line 385-387).

**Reviewer C**

The Authors describe that local expression of female sexual hormone receptors occurs only in idiopathic but not in posttraumatic tracheal stenosis and may therefore play a role in the genesis of this rare disease.

I have some questions and suggestions:

**Comment 1:** According to the authors, could there be a correlation between the hormonal status of the patient and the tracheal stenosis? Did the authors investigate the levels of estrogen and progesterone in the blood? Indeed, idiopathic tracheal stenosis have been described in cases of pregnant women, as in the manuscript by Tapias LF et al. “Pregnancy-associated idiopathic laryngotracheal stenosis: presentation, management and results of surgical treatment” (Eur J Cardiothorac Surg. 2021 Jan 4;59(1):122-129. doi: 10.1093/ejcts/ezaa296. PMID: 33038218), which should be cited and added in discussion section.

**Reply 1:** We thank the reviewer for this valuable comment. We totally agree that this is an important question. Due to the retrospective study design, levels of estrogen and progesterone were unfortunately not examined at the time of surgery. However, the subjective impression is that there might be a correlation. This should be further investigated. We will adapt our

standards towards routinely measuring hormone levels in those patients. We thank the reviewer for this precious literature advice and added this interesting paper to our discussion.

**Changes in the text:** We added the sentence “Due to the retrospective study design, neither levels of estrogen and progesterone in patients’ blood nor local hormone receptor expression in initial tracheal biopsies were examined.” (see page 5, line 133-135).

We included the mentioned paper in our discussion section: “Currently, Tapias et al. described the onset and /or exacerbation of ITS during pregnancy in 15 patients. In comparison to their non-pregnancy associated cases (n=248) the expression of estrogen receptors tend to be more frequent in the pregnancy associated group (100 vs. 75%) without statistical significance while the progesterone receptor expression was exactly the same (71,4 vs. 72,1%) [19].” (see page 13, line 314-318).

**Added literature:**

Tapias, L.F., et al., *Pregnancy-associated idiopathic laryngotracheal stenosis: presentation, management and results of surgical treatment*. Eur J Cardiothorac Surg, 2021. **59**(1): p. 122-129. (see page 16, line 399-401)

**Comment 2:** Do you have other female patients with ITS who were treated only with bronchoscopy and dilation or stenting without surgery at your center during the same period? Did the authors study the expression of female sex hormone receptors in tracheal biopsy specimen?

**Reply 2:** We thank the reviewer for giving us the opportunity to clarify this important point. After consultation with the department of pulmonology, there were no additional ITS patients treated bronchoscopically in our hospital. All patients presenting with symptomatic high grade ITS were discussed interdisciplinary and in most cases transferred to surgery. Due to the retrospective design of our study, there was no focus on hormone expression in tracheal specimens at the time of the initial biopsies. These data are unfortunately not available.

**Changes in the Text:** “Due to the retrospective study design, neither levels of estrogen and progesterone in patients’ blood nor local hormone receptor expression in initial tracheal biopsies were examined.”.” (see page 5, line 133-135)

“All patients of the present study were discussed interdisciplinary. There was a consent on primary surgery in most cases due to good surgical long-term results and missing good endoscopic or interventional options. Especially in the PTTS group, we mostly had an additional instability of the tracheal wall with no good interventional options.”.” (see page 5, line135-137 and page 6, line 138-139)

**Comment 3:** The authors wrote that only nine patients in the entire cohort had previous operations. What were the indications for the other (16) patients undergoing primary surgical treatment?

**Reply 3:** We thank the reviewer for this comment. As mentioned above, all cases in our hospital were discussed interdisciplinary. Due to the good surgical (long-term) results, patients were transferred to surgery often primarily, depending on age and general condition.

Especially in the PTTS group, we mostly had an additional instability of the tracheal wall with no good interventional options.

**Changes in the text:** “All patients of the present study were discussed interdisciplinary. There was a consent on primary surgery in most cases due to good surgical long-term results and missing good endoscopic or interventional options. Especially in the PTTS group, we mostly had an additional instability of the tracheal wall with no good interventional options.” (see page 5 , line135-137 and page 6, line 138-139)

**Comment 4:** There are too many tables with too much information. I suggest harmonizing the tables and reducing the items:

a. I suggest removing some comorbidities in Table 1a (for example glaucoma, or polyneuropathy or “state after pulmonary embolism”) that are not directly related to the pathology being treated.

**Reply 4a:** We thank the reviewer for this valuable comment. We strongly agree with this advice and shortened table 1a.

**Changes in the text:** We deleted the comorbidities “Glaucoma, Hypothyreosis, Polyneuropathy, Depression, State after pulmonary embolism” (see page 16, table 1a)

b. Table 1b: I think it might be better to describe these findings only in the text

**Reply 4b:** In the light of reviewer’s comment, we included relevant findings displayed in table 1b in the text in the result section. As some modifications of table 1b were desired by Reviewer A (comment 7), we provide **former table 1b now as Supplement Table A**. According to the preferences of the reviewers and editors, this table can also be completely removed.

**Changes in the text:** We removed table 1b from the manuscript and added the relevant information in the result section. The text was modified as follows:

“According to the Myer-Cotton classification of tracheal stenoses n=4 patients presented with grade I, n=13 patients with grad II and n=6 patients with grade III. There was no significant difference between both groups. Due to missing data Myer-Cotton classification was not possible in n=4 patients. Median length of tracheal stenosis was 2cm (range 1-2.5cm) with distance to vocal cords of median 1cm (range 1-4cm) also with no significant difference between the two groups. Neither the diameter of the normal trachea (Median 15 vs. 17 mm) nor the remaining diameter in the stenosis (Median 5,2 vs. 5,5 mm) differed significantly. (Supplement Table A).“ (see page 8, line 205-210 and page 9, line 211-214).

**Comment 5:** The Legend of Figures is necessary to better understand the findings

**Reply 5:** As mentioned above (Reviewer A, Comment 8) we have to excuse for this mistake during the submitting process.

**Changes in the text:** We added the figure legends (see page 19-21).

“Figure 1: (a): Formaline embed sections using IHC with antibodies against progesterone receptors on fibroblasts in tracheal specimens in the ITS group. Magnification 200x. (b): Formaline embed sections using IHC with antibodies against estrogen receptors on fibroblasts in tracheal specimens in the ITS group. Magnification 200x.

Figure 2: (a): Formaline embed sections using IHC with antibodies against progesterone receptors on fibroblasts in tracheal specimens in the PTTS group. Magnification 200x. (b): Formaline embed sections using IHC with antibodies against estrogen receptors on fibroblasts in tracheal specimens in the PTTS group. Magnification 200x. “