Peer Review File
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First Round Peer Review

Reviewer A

Comment 1: *Thank you for the opportunity to review this interesting paper on tracheal length measurement using 3D imaging.*
Tracheal length is a relevant parameter for pulmologists, anesthesiologists, ENT surgeons, and thoracic surgeons. Its anatomical evaluation provides interesting data for the prediction of possible risk factors of interventions and complications. This is especially true considering sex-related differences. The manuscript reads fluent and comprehensive and is focused on the main results. I have only a few comments and suggestions.

Reply: Thank you for these comments.

Comment 2: *The authors discuss tracheal length in the light of tracheal surgery. This is correct but it may be helpful to note that related parameters (e.g., short body height) may serve as an indirect association of intervention-related complications, tracheal rupture in particular, which has relevance in tracheal intubation under emergency conditions and/or inexperienced operators. I would suggest that the authors discuss this issue to enhance the importance and clinical relevance of the knowledge of tracheal length. One of the most-cited paper regarding this issue is Minambres E Eur J Cardiothorac Surg 2009 and there are some more recent studies from other authors which might be worth to be cited as well.*
One further reference recommendation regarding the management of tracheal injuries could be a review of the literature by Grewal HS et al Chest 2019.

Reply: Thank you for your suggestions. The studies you mentioned regarding postintubation tracheal rupture (PiTR) reported that all patients with post-intubation tracheal rupture (PiTR) had a height of less than 165 cm, possibly indicating short stature as a predisposing factor for PiTR. Furthermore, another study revealed that the endotracheal tube of patients with PiTR was positioned significantly more distantly in the already-smaller trachea, which can be forced under by cuff overpressure. These findings may indicate that short stature increases the risk of , since patients with short statures are managed with distal positioning of the endotracheal tube due to their short tracheal length. Thus, when performing TRR and endotracheal intubation, it is important to be aware of the tracheal length of each patient. We have added three references about PiTR and revised the "Discussion" section according to your suggestions as follows:

Changes in the text: Paragraph 4 in the Discussion section (page 10):

Some studies reported that all patients with postintubation tracheal rupture (PiTR) had their height under 165 cm, and a short stature could be a predisposing factor for PiTR (22, 23).

Another study revealed that the endotracheal tube of patients with PiTR was positioned significantly more distantly in the already-smaller trachea, which can be forced under overpressure of the cuff (24). These findings may indicate that short stature increases the risk of PiTR because patients with short stature are managed with the distal position of the endotracheal tube due to the short tracheal length. However, although rare, there were cases in which the trachea was extremely short relative to the height (Figure 5B). Thus, when performing TRR and endotracheal intubation, it is important to be aware of the tracheal length of each patient

Reviewer B

Comment 1: *I am unable to have complete view of Table 2. Please adjust.*

Reply: We are sorry for presenting Table 2 inadequately. We have properly modified the page layout of Table 2 as follows:

Table 2. Correlation analysis between tracheal length and different parameters

	Total tracheal length				Cervical tracheal length				Thoracic tracheal length			
	Male		Female		Male		Female		Male		Female	
Variable	R	*p* value	r	*p* value	r	*p* value	r	*p* value	r	*p* value	r	*p* value
Age, y	0.02	0.82	-0.24	**0.01**	-0.37	**<.001**	-0.43	**<.001**	0.46	**<.001**	0.30	**<.001**
Height, cm	0.31	**<.001**	0.42	**<.001**	0.24	**0.01**	0.45	**<.001**	-0.01	0.9	-0.14	0.2
Weight, kg	0.02	0.8	0.16	0.1	0.03	0.8	0.04	0.7	-0.02	0.8	0.11	0.3
BMI, kg/m^2	-0.14	0.15	-0.06	0.5	-0.09	0.4	-0.23	**0.02**	-0.02	0.8	0.21	**0.03**
BSA, m^2	0.1	0.3	0.25	**0.01**	0.1	0.4	0.16	0.09	-0.02	0.8	0.05	0.6
Cervical tracheal length, cm	0.56	**<.001**	0.62	**<.001**	-	-	-	-	-0.68	**<.001**	-0.61	**<.001**
Thoracic tracheal length, cm	0.23	**0.02**	0.24	**0.02**	-0.68	**<.001**	-0.61	**<.001**	-	-	-	-

Abbreviations: BMI, body mass index; BSA, body surface area

Comment 2: *Please add reference in discussion part on page 7. I have copied the text needing reference.*

Reply: Thank you for this remark. The reference of the text you pointed out has the same reference as the text before it. We have modified the related text as follows:

Changes in the text: Paragraph 3 in the Discussion section (page 10)
Similarly, Madariaga et al. analyzed 545 patients who underwent TRR and reported that the mean resected tracheal length was 3.5 cm (range, 1–6.3 cm) with tracheal release maneuvers and 3.0 cm (range, 0.8–6.5 cm) without tracheal release maneuvers (21). Interestingly, the patient who underwent only 1 cm of tracheal resection in our study required the tracheal release maneuver, whereas the patient with 6.5 cm of tracheal resection did not undergo the tracheal release maneuver.

Reviewer C

Comment 1: *Title does not correspond with the study. What is the significance of the tracheal length? Where is this question answered in the manuscript?*

Reply: Thank you for these comments. Our study demonstrated that the range of the total tracheal length varied widely from 8.8 to 14.4 cm, and there were individual and gender differences and features in tracheal length depending on the part of the trachea. Furthermore, as we mentioned in the Discussion section, the patient who underwent only 1 cm of tracheal resection required the tracheal release maneuver in tracheal resection and reconstruction (TRR). Conversely, the patient with 6.5 cm of tracheal resection did not undergo the tracheal release maneuver. Although body habitus or kyphosis may be associated with tracheal mobility in TRR, these findings indicate that the total tracheal length in each case is necessary for discussing the resectable tracheal length. Therefore, we considered that the awareness of the tracheal length of each patient is significant in TRR because the resectable tracheal length would vary depending on the tracheal length of each patient, as well as body habitus and kyphosis. We have presented these ideas in the "Discussion" section as follows:

Paragraph 1 in the Discussion section (page 9)
This study demonstrated that the mean total tracheal length was 11.5±1 cm, which is consistent with that of previous studies (4,12), although its range varied widely from 8.8 to 14.4 cm. In our analysis of the relationship between tracheal length and physiological parameters, we found that physiological features differed depending on the parts of the trachea.

Paragraph 3 in the Discussion section (page 10)
Similarly, Madariaga et al. analyzed 545 patients who underwent TRR and reported that the mean resected tracheal length was 3.5 cm (range, 1–6.3 cm) with tracheal release maneuvers and 3.0 cm (range, 0.8–6.5 cm) without tracheal release maneuvers (21). Interestingly, the patient who underwent only 1 cm of tracheal resection in our study required the tracheal release maneuver, whereas the patient with 6.5 cm of tracheal resection did not undergo the tracheal

release maneuver. Even though body habitus or kyphosis may be associated with tracheal mobility in tracheal reconstruction, these findings indicate that the total tracheal length in each case is necessary for discussing resectable tracheal length.

Comment 2: *There are no comparison between the use of 3d reconstruction and normal thoracic CT. Why are the measurements and limits made in normal CT and not in the 3d reconstruction?*

Reply: Thank you for this remark. We believe that 3D imaging would be more accurate than multiplanar 2D imaging in spatial evaluation because the tracheal length in this study was measured by tracing the center of the trachea without the influence of its meandering and kyphosis. We have mentioned this in the Discussion section of the revised manuscript. Furthermore, as you mentioned, there was no comparison between the use of 3D imaging and 2D imaging in our study. We have added the following advantages of 3D imaging, as well as a limitation of the study to the Discussion section:

Changes in the text: Paragraph 1 in the Discussion section (page 9)
This is the first study to focus on tracheal length using a 3D imaging workstation, encompassing normal physiological conditions in comparison with the conditions included in anatomical studies of cadaver specimens with formalin fixation. Three-dimensional imaging would be more accurate than multiplanar 2D imaging in spatial evaluation because the tracheal length in this study was measured by tracing the center of the trachea without the influence of its meandering and kyphosis

Changes in the text: Paragraph 5 in the Discussion section (page 11)
Fourth, our study was unable to show the superiority of 3D imaging measurement because we did not investigate the tracheal length using multiplanar 2D imaging.

Comment 3: *Discussion should be focused on the use of 3d reconstruction and study findings and less about tracheal resection*.

Reply: Thank you for this comment. We agree that the discussion focused on the use of 3D imaging is necessary. As mentioned in our reply to comment 2, we have added this in the Discussion section. However, our study aimed to identify the reference value of tracheal length and its features to apply these findings in tracheal management, such as tracheal resection and reconstruction (TRR). As we mentioned in our reply to comment 1, the resectable tracheal length would vary depending on the tracheal length of each patient, as well as body habitus and kyphosis. However, there was no information on the tracheal length of each patient before TRR. Furthermore, short tracheal length may be a risk factor for postintubation tracheal rupture (PiTR) because the endotracheal tube of patients with PiTR was positioned significantly more distantly in the trachea that is already smaller and can be forced under overpressure of the cuff. Therefore, we have mainly discussed the importance of the awareness of tracheal length for these tracheal managements. Please refer to our reply to your comments 1 and 2, and our reply to reviewer A's comment 2.

Comment 4: *I think it would be interesting a figure or table showing the relation between tracheal length and age*.

Reply: Thank you for this suggestion. Table 2, which has an inadequate layout, presents the relation between tracheal and age. Age was significantly negatively correlated with the cervical tracheal length in both sexes (male, r=−0.34, p<.001; female, r=−0.43, p<.001) and positively correlated with the thoracic tracheal length (male, r=0.46, p<.001; female, r=0.3, p<.001). Therefore, older age significantly contributed to a shorter cervical trachea and longer thoracic trachea. This may result from a reduction in the strength of the annular ligament and fibrous tissue with aging. We have properly modified the page layout of Table 2 as follows:

Table 2. Correlation analysis between tracheal length and different parameters

	Total tracheal length				Cervical tracheal length				Thoracic tracheal length			
	Male		Female		Male		Female		Male		Female	
Variable	R	*p* value	r	*p* value	r	*p* value	r	*p* value	r	*p* value	r	*p* value
Age, y	0.02	0.82	-0.24	**0.01**	-0.37	**<.001**	-0.43	**<.001**	0.46	**<.001**	0.30	**<.001**
Height, cm	0.31	**<.001**	0.42	**<.001**	0.24	**0.01**	0.45	**<.001**	-0.01	0.9	-0.14	0.2
Weight, kg	0.02	0.8	0.16	0.1	0.03	0.8	0.04	0.7	-0.02	0.8	0.11	0.3
BMI, kg/m^2	-0.14	0.15	-0.06	0.5	-0.09	0.4	-0.23	**0.02**	-0.02	0.8	0.21	**0.03**
BSA, m^2	0.1	0.3	0.25	**0.01**	0.1	0.4	0.16	0.09	-0.02	0.8	0.05	0.6
Cervical tracheal length, cm	0.56	**<.001**	0.62	**<.001**	-	-	-	-	-0.68	**<.001**	-0.61	**<.001**
Thoracic tracheal length, cm	0.23	**0.02**	0.24	**0.02**	-0.68	**<.001**	-0.61	**<.001**	-	-	-	-

Abbreviations: BMI, body mass index; BSA, body surface area

Comment 5: *How did the authors avoid the cofounding factor of the different height in male and women group?*

Reply: Thank you for this comment. There are several methods to avoid confounding factors in research, such as randomization, restriction, matching, and statistical control. Among them, we performed the statistical control using multiple linear regression analysis in this study. In the analysis, the total tracheal length was influenced most by height, while cervical and thoracic tracheal lengths were influenced mostly by sex. Furthermore, older age was an independent contributor to a shorter cervical trachea and longer thoracic trachea in both sexes. These detailed results are shown in the Result section and Table 3 as follows:

Result on paragraph 3 (page 8)
Table 3 summarizes the results of the multiple linear regression analyses of the total, cervical, and thoracic tracheal lengths using common physiological parameters (age, sex, height, and weight). Height and weight were significant independent predictors of the total tracheal length, and the contribution of height was greater than that of weight based on the standardized coefficients (height, $\beta=0.69$, $p<.001$; weight, $\beta=-018$, $p=0.03$). In particular, height significantly contributed to the cervical tracheal length (cervical tracheal length, $\beta=0.42$, $p<.001$; thoracic tracheal length, $\beta=0.1$, $p=0.2$). Age was an independent negative and positive contributor to cervical and thoracic tracheal lengths, respectively (cervical tracheal length, $\beta=-0.29$, $p<.001$; thoracic tracheal length, $\beta=0.37$, $p<.001$). Sex had the greatest contribution to the cervical and thoracic tracheal lengths (cervical tracheal length: male, $\beta=-0.49$, $p<.001$; thoracic tracheal length: male, $\beta=0.4$, $p<.001$). These results indicated that the male sex is a contributor to a shorter cervical trachea and longer thoracic trachea. Conversely, the female sex is a contributor to a longer cervical trachea and shorter thoracic trachea.

Table 3. Multiple regression analysis

	Total tracheal length (R^2=0.22)				Cervical tracheal length (R^2=0.32)				Thoracic tracheal length (R^2=0.44)			
Variable	B	SE	B	*p* value	B	SE	β	*p* value	B	SE	β	*p* value
Age, y	0.009	0.007	0.09	0.2	−0.035	0.008	−0.29	**<.001**	0.04	0.007	0.37	**<.001**
Sex, male	0.3	0.207	0.14	0.15	−1.36	0.254	−0.49	**<.001**	1.06	0.219	0.4	**<.001**
Height, cm	0.083	0.014	0.69	**<.001**	0.07	0.017	0.42	**<.001**	0.02	0.015	0.1	0.2
Weight, kg	−0.018	0.008	−0.18	**0.03**	−0.03	0.01	−0.24	**0.003**	0.01	0.009	0.1	0.2

Abbreviations: B, unstandardized coefficients; β, standardized coefficients; SE, standard error

Comment 6: *What is the advantage of 3d reconstruction compared with CT? It has not been established*.

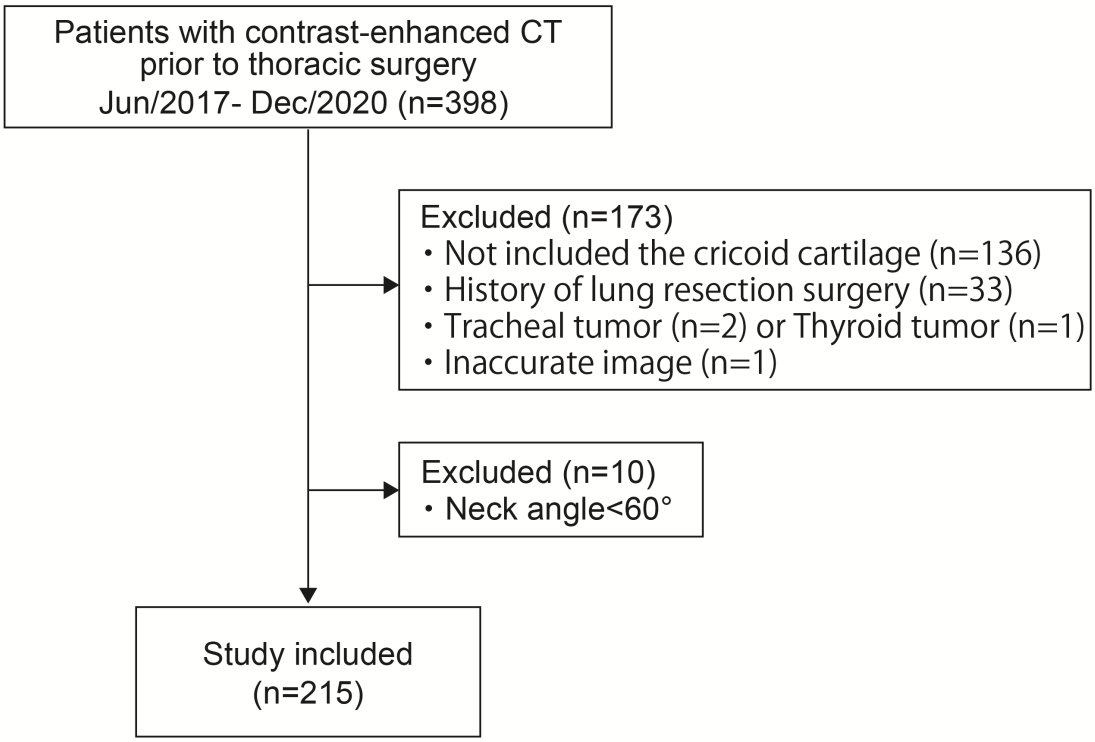
Reply: Thank you for your question. In this study, we measured the tracheal length by tracing the center of the trachea, which would be more accurate than the multiplanar 2D imaging in spatial evaluation because this method was not influenced by tracheal meandering and kyphosis. We have mentioned this in the Discussion section of the revised manuscript. However, as you mentioned, there was no comparison between the use of 3D imaging and 2D imaging in our study. Please refer to our reply to your comment 2.

Reviewer D

Comment 1: *Cases with tracheal or thyroid tumors are excluded*. *However, such cases are where this study is useful*. *Therefore, the 10 excluded cases should also be presented*.

Reply: Thank you for this remark and suggestion. We agree that the tracheal length using 3D imaging is useful for patients with tracheal or thyroid tumors, which can help in tracheal length meandering, expansion, and contraction. In fact, we can easily measure the tracheal length of patients with tracheal or thyroid tumors using the same technique. However, we excluded three patients with these tumors in this study because this study aimed to identify the reference value of tracheal length and its features (Figure 1).

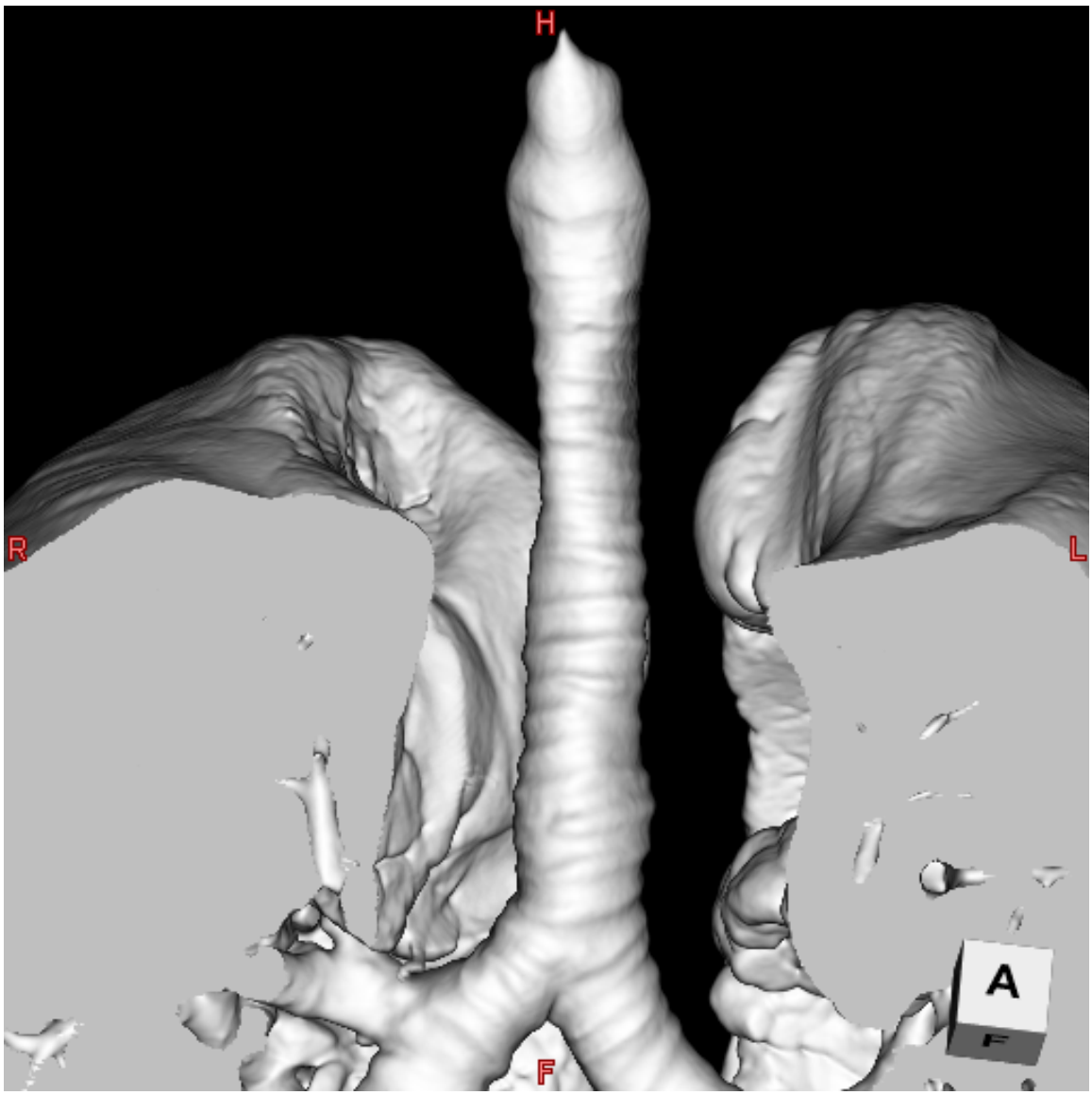
Figure 1



Comment 2: *The authors stated that preoperative bronchoscopy was used to measure the distance from the vocal cords. The number of tracheal cartilages to indicate the distance is also helpful in the surgery, so the number of rings of tracheal cartilages should also be noted.*

Reply: Thank you for this remark and suggestion. We agree that the number of rings of tracheal cartilage is important data in tracheal surgery. However, we could not evaluate the ring of tracheal cartilage in detail due to the blurred 3D imaging. Furthermore, it presents not only a simple ring but also other shaped rings, such as a forked ring, which cannot be quantifiable. Therefore, we did not investigate the number of rings of tracheal cartilage in this study. We have attached a reference figure as follows, which did not accurately reflect the number of rings of tracheal cartilage.

Reference figure:



Comment 3: *The authors stated that it helps in predicting the appropriate resection distance and anastomotic complications, but this paper does not have data on the tension of the anastomosis or laryngeal release maneuver and hilar release maneuvers. Although the distance of the trachea is presented in this paper, there is no data whatsoever to support the distance of the tracheal resection. A review of tracheal surgery data in your institution would be beneficial to show data to support the authors' assertions.*

Reply: We thank you for this comment. As you mentioned, the usefulness of the data on tracheal length in TRR remains unclear, since there are no data on the tracheal length of patients who underwent TRR. We have added this as a limitation to the "Discussion" section. In fact, TRR is an extremely rare surgery, and we have managed only three patients who underwent TRR in our institution from 2015 to 2022. Among them, we experienced a case with adenoid cystic carcinoma, wherein the trachea was resected at 4.5 cm, but we managed to perform TRR using all tracheal maneuvers unexpectedly. We then retrospectively measured the tracheal length using

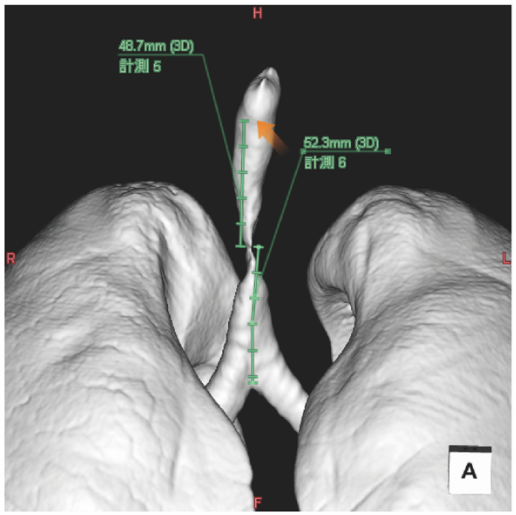
3D imaging and identified that the tracheal length was 10.1 cm. However, we did not know what this meant, which is why we planned to investigate the reference value of the tracheal length and its features. Finally, we found that the tracheal length (10.1 cm) of the patient was short, relative to the height (166 cm), as shown by the red point in the reference figure below.

Changes in the text: Paragraph 6 in the Discussion section (page 11-12)

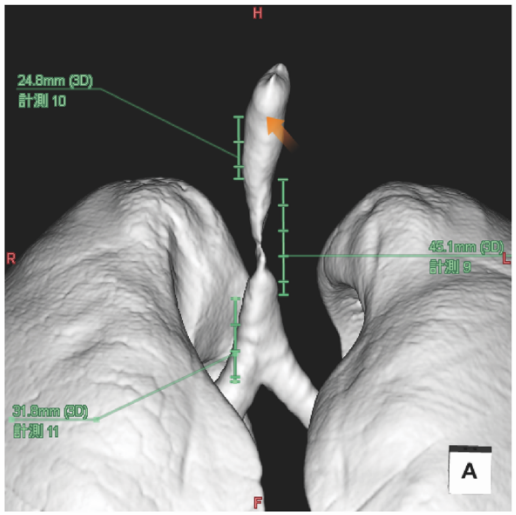
Lastly, the usefulness of data on the tracheal length in TRR remains unclear, since there are no data on the tracheal length of patients who underwent TRR. Further studies using accumulated data on the tracheal length in TRR are necessary to discuss and address this issue.

Reference figure

53 yo, F, 166 cm, 93 kg
Tracheal adenoid cystic carcinoma

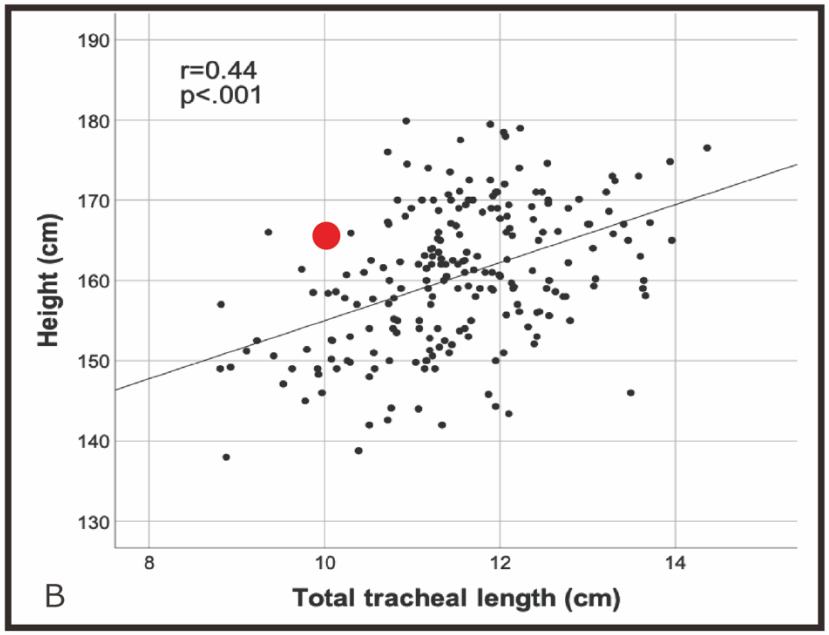


Total tracheal length:
10.1 cm



Resected tracheal length:
4.5 cm

Correlation analysis between heigth and total tracheal length



Second Round Peer Review

Comment 1: *The latest technology, 3D-CT, was used to evaluate the length of the trachea. It was found that the reference values and characteristics of tracheal length vary according to height, age, and gender.*
Although the actual surgery may involve resection by the number of tracheal rings, they could not be discussed in this study. It is unclear whether this study is useful as a treatment for the disease.

Reply: Thank you for this remark. As you have mentioned, the resected number of tracheal cartilage rings is another indicator in tracheal resection and reconstruction, with the length of two rings considered to be 1 cm. However, the number of tracheal cartilage rings also varies in individuals, and tracheal cartilage rings have various shapes, which are divided into 12 patterns. These findings indicate that the length of a tracheal cartilage ring varies depending on the total tracheal length, number of tracheal cartilage rings, and their shapes. We have added these concepts and references to the "Discussion" section as follows:

Changes in the text: Paragraph 3 in the Discussion section (page 10):

Furthermore, the number of resected tracheal cartilage rings is another indicator in TRR (22), with the length of two rings considered to be 1 cm (23,24). Still, the number of tracheal cartilage rings also varies in individuals (mean, 19±3 cm); additionally, tracheal cartilage rings have various shapes, which are divided into 12 patterns (25). These findings indicate that the length of a tracheal cartilage ring varies depending on the total tracheal length, as well as the number and shape of the tracheal cartilage rings.

Comment 2: *In conclusion, the authors stated, "This study may serve as a basis for decision making for tracheal resection or reconstruction. It may be a basis for decision making for tracheal resection or reconstruction," but there is a lack of data related to the disease and surgical techniques that support this. The logical development is incomplete.*
The conclusion needs to be changed.

Reply: Thank you for your suggestion. As you have mentioned, it is unclear whether our data may be a basis for decision making in tracheal resection and reconstruction. We modified the conclusion as follows:

Changes in the text: Conclusions in the Abstract section (page 2):

The total tracheal length ranged from short to long in individuals, and characteristics of tracheal length varied with height, age, sex, and part of the trachea. We should thus be aware of the tracheal length of each patient for appropriate tracheal management.

Changes in the text: in the Conclusion section (page 12):

This study demonstrated the reference values and characteristics of tracheal length, including cervical and thoracic tracheal lengths, using a 3D imaging workstation. The total tracheal length ranged from short to long in individuals, and characteristics of tracheal length varied with height, age, sex, and part of the trachea. We should thus be aware of the tracheal length of each patient for appropriate tracheal management.