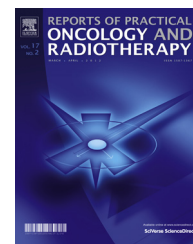


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Original research article

Assessment of cartilage invasion in case of laryngeal cancer by means of longitudinal sectioning for histopathology – Clinical implications



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ABSTRACT

Aim: The aim of the study was to assess the accuracy of radiological diagnosis of laryngeal cartilage infiltration by histopathological examination of laryngeal specimen after total laryngectomy.

Background: Despite the development of new medical technologies and significant clinical advances allowing early diagnosis and treatment of laryngeal cancer, mortality is still on the rise. Neoplastic infiltration of the laryngeal cartilages is the most common source of error in the assessment of cancer staging. Furthermore, cartilage invasion is listed as a contraindication to partial surgical techniques as well as radiotherapy.

Materials and methods: The study was carried out on 21 larynges following total laryngectomy. Before taking the decision to perform surgery, high-resolution CT scans were performed in all cases. An extended histopathological examination was conducted using a unique vertical cross-section of the whole larynx.

Results: Pathology reported 2 cases of arytenoid cartilage invasion, 5 cases of cricoid cartilage invasion, 12 cases of thyroid cartilage penetration, 1 case of internal cortex invasion and 9 cases of extra-laryngeal spread. CT imaging identified 8 of 13 cases (61.5%) of pathologically proven invasion of thyroid cartilage and only 2 cases (2/9, 22%) of extra-laryngeal spread. According to CT results, arytenoid cartilage invasion was correctly identified in 2 cases, cricoid cartilage invasion in 4 (4/5, 80%). The positive predictive values for thyroid, cricoid and arytenoid cartilage invasion and penetration were 80%, 66.7% and 50%, respectively. In case of pre-laryngeal spread the positive predictive value was 100%.

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Conclusion: Despite increasingly advanced methods involved in the diagnosis of laryngeal cancer, many discrepancies may be observed between the radiological and histopathological assessments. CT imaging has limitations especially in thyroid cartilage penetration and extra-laryngeal spread assessment in advanced laryngeal cancer cases. An extended histopathological examination, involving vertical cross-sections of the whole larynx is a very precise study that allows a precise determination of local cancer staging (T).

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1. Background

According to GLOBOCAN 2018 global statistics, cancer of the larynx accounts for 20.89% of all neck cancers and is the second most frequently diagnosed of the head and neck cancers, and the second greatest cause of mortality.¹ Despite the development of new medical technologies and significant clinical advances allowing early diagnosis and treatment of this condition, mortality from cancer of the larynx is on the rise.² Laryngeal cancers are most often diagnosed at the advanced locoregional stage, in stages III and IV.³ In that group of patients, combined treatment modalities are used, including surgery, radiotherapy and chemotherapy. Total laryngectomy remains the procedure of choice in patients with advanced cancer of the larynx and laryngopharynx with infiltration of the cartilages and extra-laryngeal soft tissue, and in patients with recurrence after sparing treatment, such as radio-chemotherapy. The most common imaging test to assess the extent of neoplastic infiltrations in laryngeal and laryngopharyngeal tumors is computed tomography (CT) with contrast which, in combination with direct laryngoscopy, may help to determine the clinical stage of cancer and, consequently, the method of treatment. Infiltration of the peri-laryngeal structures including large cervical vessels and, in particular, the laryngeal cartilages are the most common source of error in the assessment of cancer staging.⁴ This is of key importance in treatment planning.

2. Aim

The aim of the study was to assess the accuracy of radiological diagnosis of laryngeal cartilage infiltration by histopathological examination of laryngeal specimen after total laryngectomy.

3. Materials and methods

The study was conducted on 21 larynges collected from patients after total laryngectomy in the age range of 34–93 years, including 5 women and 16 men, in the years 2016–2018. Before the decision to operate was taken, all patients were examined using high-resolution computed tomography (CT) with contrast enhancement. The interval between CT scanning and surgery ranged from 4 to 7 weeks. All scans were evaluated independently by two radiologists. Also, ultrasonography of the neck, with assessment of the cervical lymphatic system and pre-laryngeal tissue, indirect fiberoptic

laryngoscopy involving NBI light, and direct microlaryngoscopy using a Kleinsasser laryngoscope for the assessment of the extent of the neoplastic infiltration and for the collection of specimens for histopathology were performed. Histopathology showed squamous cell carcinoma in all cases. According to the clinical evaluation and CT scans, the tumor stage was assessed using the 8th edition of the UICC TNM classification. Cases of supraglottic and glottic cancer with invasion (infiltration of the cartilage without signs of external cortex destruction) of the thyroid cartilage were assessed as cT3 as, for example, case number 8 (Table 1). In cases of penetration of the cartilage (infiltration through the cartilage) the T stage was cT4a (case number 9 in Table 1). If there were signs of invasion of cricoid and/or thyroid cartilage(s) in cases of subglottic and glottic cancer, we described that as cT4a. Also, after discussion, we decided to describe cases with signs of cartilage sclerosis as cT3. Finally, in 6 patients cT stage was found to be cT4a. Stage cT3 was determined in 15 patients. For all patients, the therapeutic decisions were taken by interdisciplinary teams.

In order to better reveal the structure of the whole larynx and establish the pT, extended histopathology was used on the pathology specimens and, more importantly, in the laryngeal cartilages, involving longitudinal sectioning of the whole larynx. In all cases, longitudinal sections of the larynx were made, according to a special laboratory procedure. Briefly, on receipt of the specimen in the pathology department, the larynges were opened longitudinally and allowed 10% neutral buffered formalin to be fixed in them for a full day. After initial fixation, slices no greater than 3 mm thick were cut through the entire length of the specimen in order to reveal all the structures from the hyoid bone to the cricoid cartilage, including the thyroid cartilage and vocal muscles. The slices, one from each side of the larynx involved by the tumor, were then demineralized in an aqueous solution of 10% formic acid for 5–8 h prior to be processed by routine methods and embedded in paraffin wax blocks approximately 8 cm in length. Thusly embedded, the full longitudinal slices were thinly sectioned using a microtome and mounted onto oversized glass slides for staining and subsequent examination. By use of the above protocol, the whole laryngeal structure was revealed, allowing a precise identification of infiltrations into the structures of the larynx and adjacent tissues. The above preparations were evaluated independently by two pathologists.

The histopathology results of the excised larynges were compared with the preoperative CT scans for neoplastic cartilage invasion. Cartilages were assessed as being invaded on

Table 1 – Discrepancies in laryngeal cartilages invasion between the findings of the CT and microscopic examinations (n = 21).

| Patient | Age | Sex | Laryngeal cartilages infiltration basing on CT (cT) | Laryngeal cartilages infiltration basing on histopathological exam (pT) |
|---------|-----|-----|---|--|
| 1 | 66 | M | No signs of thyroid cartilage invasion – cT3 | Infiltration through thyroid cartilage, prelaryngeal space invasion – pT4a |
| 2 | 34 | F | Right arytenoid cartilage invasion – cT3 | Right arytenoid cartilage invasion – pT3 |
| 3 | 67 | M | Bilateral thyroid cartilage invasion without destruction of external cortex, left arytenoid cartilage sclerosis – cT3 | Infiltration through thyroid cartilage, prelaryngeal space invasion, no signs of arytenoid cartilage invasion – pT4a |
| 4 | 62 | M | Left arytenoid cartilage sclerosis, sclerosis of the cricoid cartilage on the left – cT3 | Infiltration through thyroid cartilage on the left, prelaryngeal space invasion, no signs of arytenoid and cricoid cartilages invasion – pT4a |
| 5 | 68 | M | Infiltration through thyroid cartilage, perilaryngeal tissue invasion suspected – cT4a | Infiltration through thyroid cartilage, perilaryngeal tissue invasion confirmed – pT4a |
| 6 | 62 | M | Thyroid cartilage invasion, invasion of the cricoid cartilage on the left suspected – cT4a | Infiltration through thyroid cartilage, prelaryngeal space invasion, no signs of cricoid cartilage invasion – pT4a |
| 7 | 64 | M | No invasion of laryngeal cartilages – cT3 | No invasion of laryngeal cartilages – pT3 |
| 8 | 60 | M | Invasion of thyroid cartilage on the right without signs of destruction of external cortex – cT3 | Infiltration through thyroid cartilage, prelaryngeal space invasion – pT4a |
| 9 | 93 | F | Right arytenoid cartilage invasion, sclerosis of cricoid cartilage on the right, infiltration through thyroid cartilage with perilaryngeal tissue invasion – cT4a | Invasion of right arytenoid cartilage and cricoid cartilage on the right, infiltration through thyroid cartilage with perilaryngeal tissue invasion – pT4a |
| 10 | 58 | M | Suspicion of thyroid cartilage invasion without signs of external cortex destruction on the right – cT3 | No signs of thyroid cartilage invasion – pT3 |
| 11 | 62 | M | Infiltration close to the thyroid cartilage without signs of invasion of internal cortex – cT3 | Infiltration through thyroid cartilage, prelaryngeal space invasion – pT4a |
| 12 | 77 | M | Invasion of thyroid and cricoid cartilages on the right – cT4a | Thyroid and cricoid cartilages penetration on the right, prelaryngeal space invasion – pT4a |
| 13 | 62 | M | Cricoid cartilage sclerosis – cT3 | Infiltration through cricoid cartilage – pT4a |
| 14 | 68 | F | No invasion of laryngeal cartilages – cT3 | No invasion of laryngeal cartilages – pT3 |
| 15 | 61 | F | No invasion of laryngeal cartilages – cT3 | No invasion of laryngeal cartilages – pT3 |
| 16 | 78 | M | Suspicion of invasion of the right plate of thyroid cartilage without signs of destruction of external cortex – cT3 | No invasion of thyroid cartilage – pT3 |
| 17 | 64 | F | Infiltration close to the thyroid cartilage without signs of destruction of internal cortex – cT3 | Infiltration through thyroid cartilage – pT4a |
| 18 | 63 | M | No invasion of laryngeal cartilages – cT3 | Invasion of internal cortex of thyroid cartilage – pT3 |
| 19 | 67 | M | No invasion of laryngeal cartilages – cT3 | Infiltration through thyroid cartilage – pT4a |
| 20 | 68 | M | Thyroid and cricoid cartilages penetration – cT4a | Thyroid and cricoid cartilages penetration – pT4a |
| 21 | 68 | M | Infiltration through thyroid cartilage – cT4a | Infiltration through thyroid and cricoid cartilages – pT4a |

CT scans if there was cartilage erosion, if tumor was seen on both sides of the cartilage, if there was asymmetrical cartilage sclerosis with tumor adjacent to the cartilage. Cartilage sclerosis was defined as increased ossification of the medullary cavity of the cartilage or as thickening of the cartilage cortex.⁵ Finally, we assessed the sensitivity, specificity, positive predictive values (PPV) and negative predictive values (NPV) of the information provided by CT.

4. Results

The pre-surgery imaging tests showed the following:

- (a) laryngeal cartilages infiltration in 13 patients (arytenoid cartilage invasion in 4 CT scans, thyroid cartilage invasion (without signs of external cortex destruction) or penetration (infiltration through the cartilage) in 10 CT scans,

Table 2 – Distribution of cartilaginous infiltration basing on histopathology results (n = 21).

| Cartilage | n | % |
|--|----|------|
| Thyroid | 10 | 47.6 |
| Cricoid | 1 | 4.7 |
| Arytenoid | 1 | 4.7 |
| Thyroid and cricoid | 3 | 14.2 |
| Thyroid, cricoid, epiglottis and arytenoid | 1 | 4.7 |
| None | 5 | 23.8 |

cricoid cartilage invasion in 6 CT scans); pre-laryngeal spread was diagnosed in 2 CT scans

- (b) no clear signs of laryngeal cartilages infiltration in CT scans in 8 patients.

For the distribution of cartilaginous infiltration see [Tables 1 and 2](#).

After performing extended histopathological examination with longitudinal sectioning of the whole larynx (according to the procedure presented above) discrepancies between the findings of the radiological and microscopic examinations were found in 13 out of 21 patients. In those cases histopathology revealed wider neoplastic infiltration of the anatomical structures of the larynx than had been suggested by imaging, also with the involvement of extra-laryngeal structures in 7 patients. In two cases, cartilage infiltration was excluded on histopathological examination despite having been suggested in the radiological exam ([Table 1](#)). In the remaining 8 cases, the extended histopathological examination of the laryngeal specimens corresponded to the radiological evaluations from the pre-surgery period.

Pathology reported 2 cases of arytenoid cartilage invasion, 5 cases of cricoid cartilage invasion, 12 cases of thyroid cartilage penetration, 1 case of internal cortex invasion and 9 cases of extra-laryngeal spread. CT imaging identified 8 of 13 cases (61.5%) of pathologically proven invasion of thyroid cartilage and only 2 cases (2/9, 22%) of extra-laryngeal spread. According to CT results, arytenoid cartilage invasion was identified in 2 cases, cricoid cartilage invasion in 4 (4/5, 80%). Sclerosis was of limited value in predicting arytenoid and cricoid cartilage invasion. The positive predictive values for thyroid, cricoid and arytenoid cartilages invasion and penetration were 80%, 66.7% and 50%, respectively. In case of pre-laryngeal spread,

the positive predictive value was 100%. Finally, the T stage was changed from cT3 to pT4a in 8 patients. [Table 3](#) gives an overview of radiologically suspected and histologically confirmed malignant invasion of laryngeal cartilages.

For examples of radiological examinations and histopathological specimens, see the figures below ([Figs. 1 and 2](#)).

5. Discussion

Cartilage infiltration in cancer of the larynx is a very significant issue, very often of decisive importance in the choice of treatment modality. Laryngeal cartilage invasion upstages the primary tumor stage to T4a according to the 8th edition of the UICC TNM classification which usually qualifies patients for total laryngectomy with postoperative radiotherapy. CT is the most common image performed to assess the extent of laryngeal cancer preoperatively. It helps in the assessment of the depth of tumor invasion and its relationship to surrounding structures. Unfortunately, as repeatedly reported in the literature, CT is not always an excellent tool for determination of the extent of laryngeal tumor. If CT scans reveal tumor on the extra-laryngeal side of the cartilage, it is generally accepted as a specific radiological sign of cartilage invasion. According to Becker et al.⁶ the specificity of CT in those situations is 95%. However, this is true only for advanced cases of cartilage destruction and, therefore, the sensitivity is rather low – 44%. In our study, we also found a large divergence in specificity and sensitivity of CT in the assessment of pre-laryngeal tissue involvement (100% and 22%, respectively). Adolphs et al. found, after analysing the literature, that CT imaging is an adequate tool for the evaluation of neoplastic laryngeal cartilage infiltration, in particular that of the thyroid cartilage.⁷ In that case, according to literature, the PPV of CT is reported to be between 44% and 80%.⁸ Our result is in line with those of other researchers (80%). Otherwise, the NPV in our assay was only 37.5%, which may stand for inability to detect early cartilage invasion basing on CT scans. The reason for that might be inhomogenous calcification zones in the cartilage.⁹ Koopmann et al., having retrospectively examined 120 larynges, after total laryngectomy due to cancer of the larynx or laryngopharynx, compared findings from laryngeal specimens with CT and concluded that pre-operative

Table 3 – Comparison of radiologic and histopathologic findings with division into individual laryngeal cartilages.

| Findings | h-p: invasion | h-p: no invasion | PPV (%) | NPV (%) | Sensitivity (%) | Specificity (%) |
|---------------------|---------------|------------------|---------|---------|-----------------|-----------------|
| Thyroid cartilage | | | | | | |
| CT: invasion | 8 | 2 | 80 | 37.5 | 61.5 | 60 |
| CT: no invasion | 5 | 3 | | | | |
| Cricoid cartilage | | | | | | |
| CT: invasion | 4 | 2 | 66.7 | 75 | 80 | 60 |
| CT: no invasion | 1 | 3 | | | | |
| Arytenoid cartilage | | | | | | |
| CT: invasion | 2 | 2 | 50 | 100 | 100 | 60 |
| CT: no invasion | 0 | 3 | | | | |
| Prelaryngeal space | | | | | | |
| CT: invasion | 2 | 0 | 100 | 30 | 22 | 100 |
| CT: no invasion | 7 | 3 | | | | |

CT, computed tomography; h-p, histopathology exam; PPV, positive predictive value; NPV, negative predictive value.

a)



b)

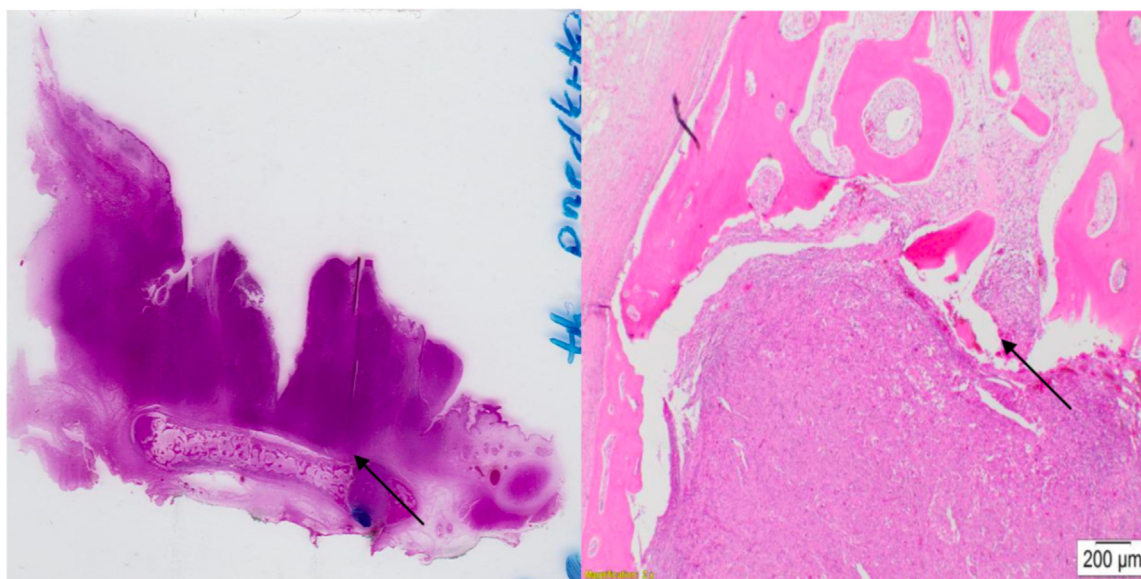
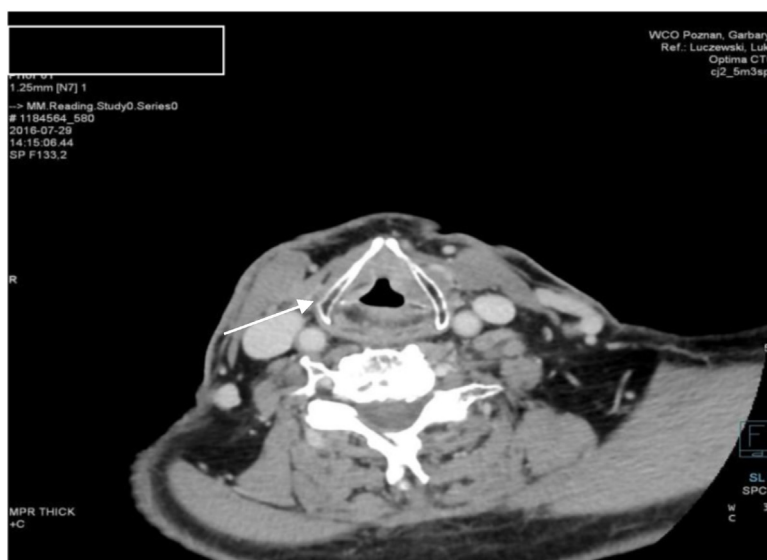


Fig. 1 – Patient aged 62 years (number 11 in [Table 1](#)). Cervical CT showed a focus strongly enhanced in contrast at the level of the glottis and the anterior commissure, the lesion spread toward the subglottic region, touching the surfaces of the thyroid cartilage, but not infiltrating it (a) (cT3). Histopathology: the cancer infiltrated the whole thickness of the thyroid cartilage, focally passed through the cartilage and penetrated the pre-laryngeal tissue (b) (pT4a).

CT is an important tool in the detection of laryngeal cartilage infiltration.¹⁰ In turn, Dadfar et al., when assessing the density and homogeneity of the thyroid cartilage in CT with contrast in individuals with a normal larynx (55 women and 45 men), found that non-calcified cartilage in CT with contrast indicates the homogeneous nature of the enhancement with minor variations that might be confused with neoplastic cartilage infiltration.¹¹ In our series, the PPV for cricoid and arytenoid cartilages invasion, according to CT scans results, were 66.7% and 50%, respectively. The most sensitive criterion of neoplastic invasion of these cartilages is cartilage sclerosis.⁶ However, as showed in our study, cartilage sclerosis does not always stand for neoplastic cartilage invasion. Mumoz et al.⁵

also reported the PPV of cartilage sclerosis for cartilaginous invasion at the level of 46%, which confirms our findings. Furthermore, in a report published by the *American Journal of Neuroradiology*, in January 2018, Kuno et al. compared the efficacy of CT and MRI in the detection of cartilage invasion in cancer of the larynx and laryngopharynx in 55 patients.¹² As a benchmark to compare the assessment of sensitivity and specificity, he used histopathology specimens. Those features were compared using the McNemara test. A higher specificity, and acceptable sensitivity, for the diagnosis of laryngeal cartilage infiltration was reported for CT. We acknowledge the limitations of our study. Our sample size was relatively small and the time passed between tumor imaging and surgery was

a)



b)

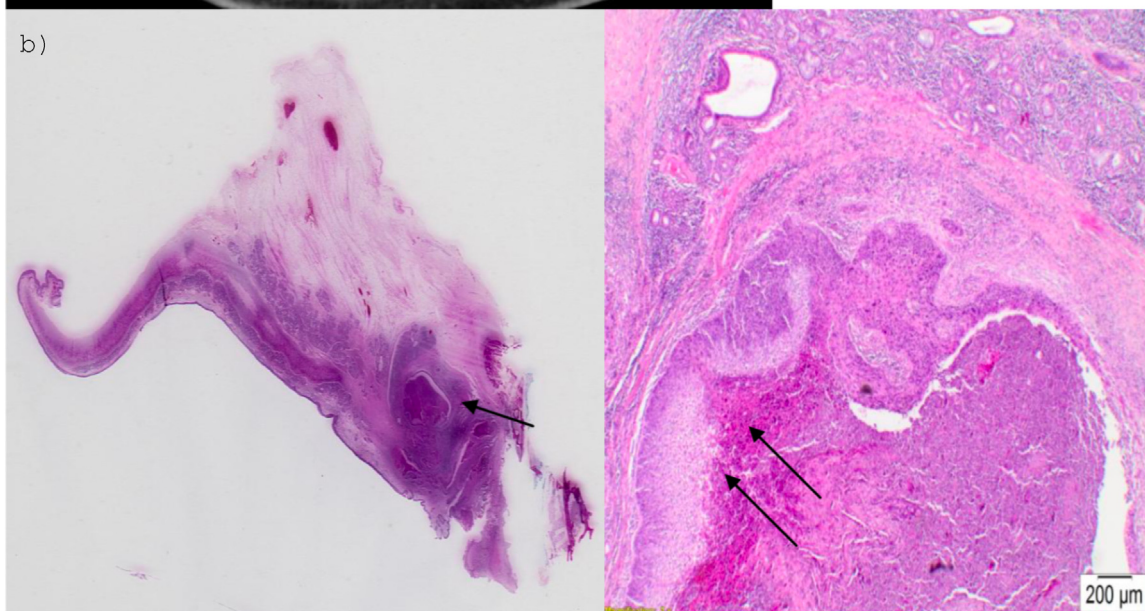


Fig. 2 – Patient aged 80 years (number 8 in [Table 1](#)). CT showed infiltration within the anterior commissure, clearly enhanced in contrast, penetrating both vocal cords to approx. 1/2 of their length. The image of the right plate of the thyroid cartilage was unclear (a) (cT3). Histopathology: microscopically the neoplastic infiltration covered the anterior commissure and glottis and penetrated the thyroid cartilage on the right side (b) (pT4a).

quite long in some cases (approximately 6 weeks), which could affect the final result of the analysis. Nevertheless, our data support findings of previous investigators.

6. Conclusion

Unfortunately, despite increasingly advanced methods involved in the diagnosis of laryngeal cancer, many discrepancies may be observed between the radiological and histopathological assessments, which also concerns CT. The study conducted at our clinic using longitudinal sectioning of the whole larynx shows cartilage infiltration in a

multi-dimensional way. In this way, it allows the invasion in particular cartilage to be assessed more precisely than by pre-operative imaging. In 13 out of 21 specimens assessed, discrepancies between pre-operative imaging and histopathological examinations were found. In our view, this may have been caused by the time passed between tumor imaging and surgery, as well as the tumor pathobiology determining clinical advancement and its histopathological type.

Conflict of interest

None declared.

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REFERENCES

1. Bray F, Ferlay J, Soerjomataram I, Siegel RL, Torre LA, Jemal A. Global cancer statistics 2018: GLOBOCAN estimates of incidence and mortality worldwide for 36 cancers in 185 countries. *CA Cancer J Clin* 2018;**68**(6):394–424, <http://dx.doi.org/10.3322/caac.21492>.
2. Gupta B, Johnson NW, Kumar N. Global epidemiology of head and neck cancers: a continuing challenge. *Oncology* 2016;**91**(1):13–23.
3. Smee R, Williams JR, Kotevski DP. Management of locally advanced T3-4 glottic laryngeal carcinomas. *J Laryngol Otol* 2018;**132**(July (7)):642–50, <http://dx.doi.org/10.1017/S0022215118000993>.
4. Gómez Serrano M, Iglesias Moreno MC, Gimeno Hernández J, Ortega Medina L, Martín Villares C, Poch Broto J. Cartilage invasion patterns in laryngeal cancer. *Eur Arch Otorhinolaryngol* 2016;**273**(July (7)):1863–9.
5. Mumoz A, Ramos A, Ferrando J, et al. Laryngeal carcinoma: sclerotic appearance of the cricoid and arytenoid cartilage – CT – pathologic correlation. *Radiology* 1993;**189**:433–7.
6. Becker M. Neoplastic invasion of laryngeal cartilage: radiologic diagnosis and therapeutic implications. *Eur J Radiol* 2000;**33**:216–29.
7. Adolphs AP, Boersma NA, Diemel BD, et al. A systematic review of computed tomography detection of cartilage invasion in laryngeal carcinoma. *Laryngoscope* 2015;**125**(July (7)):1650–5.
8. Xia C-X, Zhu Q, Zhao H-X, et al. Usefulness of ultrasonography in assessment of laryngeal carcinoma. *Br J Radiol* 2013;**86**:20130343, <http://dx.doi.org/10.1259/bjr.20130343>.
9. Phelps PD. Carcinoma of the larynx – the role of imaging in staging and pre-treatment assessments. *Clin Radiol* 1992;**46**:77–83, [http://dx.doi.org/10.1016/S0009-9260\(05\)80307-1](http://dx.doi.org/10.1016/S0009-9260(05)80307-1).
10. Koopmann M, Weiss D, Steiger M, Elges S, Rudack C, Stenner M. Thyroid cartilage invasion in laryngeal and hypopharyngeal squamous cell carcinoma treated with total laryngectomy. 2016;**273**(November (11)):3789–94 [Epub 2016 June 2].
11. Dadfar N, Seyyedi M, Forghani R, Curtin HD. Computed tomography appearance of normal nonossified thyroid cartilage: implication for tumor invasion diagnosis. *Comput Assist Tomogr* 2015;**39**(March–April (2)):240–3.
12. Kuno H, Sakamaki K, Fujii S, et al. Comparison of MR imaging and dual-energy CT for the evaluation of cartilage invasion by laryngeal and hypopharyngeal squamous cell carcinoma. *Am J Neuroradiol* 2018;**39**(March (3)):524–31, <http://dx.doi.org/10.3174/ajnr.A5530>.