Oral Oncology 133 (2022) 106023

Contents lists available at ScienceDirect

Oral Oncology

journal homepage: www.elsevier.com/locate/oraloncology

Application and accuracy of ultrasound-guided resections of tongue cancer

Klijs J. de Koning [a], Robert J.J. van Es [a], Reinoud J. Klijn [b], Gerben E. Breimer [c], Jan Willem Dankbaar [d], Weibel W. Braunius [a], Ellen M. van Cann [a], François J. Dieleman [a], Johannes A. Rijken [a], Bernard M. Tijink [a], Remco de Bree [a], Rob Noorlag [a,*]

[a] Department of Head and Neck Surgical Oncology, University Medical Center Utrecht, Utrecht, The Netherlands
[b] Department of Oral and Maxillofacial Surgery, Medisch Spectrum Twente, Enschede, The Netherlands
[c] Department. of Pathology, University Medical Center Utrecht, Utrecht, The Netherlands
[d] Department of Radiology, University Medical Center Utrecht, Utrecht, The Netherlands

ARTICLE INFO

Keywords:
Clinical study
Ultrasound
Image-guided surgery
Tongue cancer
Oral cancer
Resection margin
Adjuvant treatment

ABSTRACT

Objectives: Surgical removal of squamous cell carcinoma of the tongue (SCCT) with tumour-free margin status (≥5 mm) is essential for loco-regional control. Inadequate margins (<5 mm) often indicate adjuvant treatment, which results in increased morbidity. Ultrasound (US)-guided SCCT resection may be a useful technique to achieve more adequate resection margins compared to conventional surgery. This study evaluates the application and accuracy of this technique.

Methods: Forty patients with SCCT were included in a consecutive US cohort. During surgery, the surgeon aimed for a 10-mm echographic resection margin, while the tumour border and resection plane were captured in one image. Ex-vivo US measurements of the resection specimen determined whether there was a need for an immediate re-resection. The margin status and the administration of adjuvant treatment were compared those of with a consecutive cohort of 96 tongue cancer patients who had undergone conventional surgery. A receiver operating characteristic analysis was done to assess the optimal margin of ex-vivo US measurements to detect histopathologically inadequate margins.

Results: In the US cohort, the frequency of free margin status was higher than in the conventional cohort (55% vs. 16%, $p < 0.001$), and the frequency of positive margins status (<1 mm) was lower (5% vs. 15%, respectively, $p < 0.001$). Adjuvant radiotherapy was halved (10% vs. 21%), and the need for re-resection was comparable (10% vs. 9%). A cut-off value of 8 mm for ex-vivo measurements prevented histopathologically inadequate margins in 76%.

Conclusion: US-guided SCCT resections improve margin status and reduce the frequency of adjuvant radiotherapy.

Introduction

Surgery is the first choice of treatment for squamous cell carcinoma of the tongue (SCCT) [1]. After surgery, margin status is assessed through histopathological examination of the resection specimen. A free margin status, generally when the smallest histopathological margin is ≥ 5 mm, is essential for local control. However, in daily practice an inadequate margin, which can be a close margin (1–5 mm) or a positive margin (<1 mm), is not uncommon. In a retrospective analysis, we found that at our centre 74% of all SCCT resections had a close margin status and 10% had a positive margin status [2]. These numbers are in line with those of other studies in which up to 45% close margins and 43% positive margins in oral cancer [3] are reported.

After histopathological examination of the resection specimen, adjuvant treatment is indicated when positive margins are found or when close margins are found in combination with unfavourable histopathological parameters, that is non-cohesive, perineural, or vaso-invasive growth. The type of adjuvant treatment—that is, re-resection (RR) or (chemo)radiotherapy ((C)RT)—depends on several factors, such as whether the insufficient margin can be found in the wound bed, the occurrence of neck metastases and the patient's preferences.

Adjuvant treatment for oral cancer has multiple disadvantages. Local

* Corresponding author at: Department of Head and Neck Surgical Oncology, University Medical Center Utrecht, Heidelberglaan 100, P.O. Box 85500, 3508 GA Utrecht, The Netherlands.
E-mail address: r.noorlag@umcutrecht.nl (R. Noorlag).

https://doi.org/10.1016/j.oraloncology.2022.106023
Received 19 February 2022; Received in revised form 7 July 2022; Accepted 10 July 2022
Available online 25 July 2022

RT on the oral cavity has several side effects (e.g. mucositis, xerostomia and osteoradionecrosis [4]), while the major issue with RRs is that inadequate margins must be retraced from the initial operation site. Hence, an inadequate RR may result in uncertainty about definitive margin status [5]. Moreover, RRs may require extra operating time and anaesthesia. Previous data from our centre showed that RRs and local RT were performed for 26% and 21% of oral cancer patients with an inadequate margin status [6]. These numbers would have been lower if a higher frequency of free margin status was achieved during the initial surgical treatment.

Intraoral US is an accurate method to predict histopathological tumour thickness (TT) of SCCT [7,8] and is a better predictor than manual palpation [9]. Hence, US can visualize the deep tumour border reliably. At our centre, US-guided SCCT resections are performed using a small hockey stick-shaped probe. The tumour border and resection plane are captured in one image, as described by Tarabichi et al. [10]. The resection specimen is directly examined, ex-vivo, using a high-resolution US probe to visualise resection margins and indicate immediate RRs. Our group performed a feasibility study that evaluated this technique in 10 SCCT patients. The study showed that the frequency of free margin status increased from 17% to 70% ($p = 0.005$) compared to a cohort of 91 conventionally treated SCCT patients and that adjuvant treatment was prevented in one patient (10%)[2].

In this study, we evaluate the application and accuracy of the US-guided SCCT resection technique as described previously [2] in a larger cohort. We compare the final margin status and the margins of five specific areas (anterior, posterior, craniomedial, caudolateral and central) of the resection specimens to those of a cohort of conventionally treated SCCT patients. We also compare the frequency of adjuvant treatments between both cohorts. Finally, we compare US measurements with histopathological measurements to assess the accuracy of the technique.

Patients and methods

This study was performed in accordance with the 1964 Declaration of Helsinki and the guidelines for Good Clinical Practice. Our institute's local independent Medical Ethics Review Board approved the study protocol (trial ID: NL8336).

US cohort

Patient inclusion

Forty SCCT patients were consecutively enrolled between November 2019 and June 2021. Patients were eligible when: 1) SCCT was diagnosed and 2) the surgical treatment was performed under general anaesthesia. Exclusion criteria were: 1) a clinically staged T4 tumour (*Tumour, nodes and metastases (TNM) Classification of Malignant Tumours,* 8th *edition* [11]) that extends to structures other than the tongue, 2) a tumour that expanded to such an extent towards the contralateral side of the tongue that US was not able to define the deeper tumour margin and 3) a final resection specimen in which no tumour cells were found (e.g. in the case of a previous excisional biopsy with a positive margin status).

Surgical procedure: in-vivo imaging

Under general anaesthesia, the TT was measured in-vivo using a US system (TE7, Mindray Bio-Medical Electronics, Shenzhen, China) in combination with a small 16 MHz hockey stick-shaped US probe (L16-4Hs, Mindray), as can be seen in Fig. 1**A-C**. A mucosal margin of 10 mm was marked around the lesion (Fig. 1**D**). Next, the surgeon started the

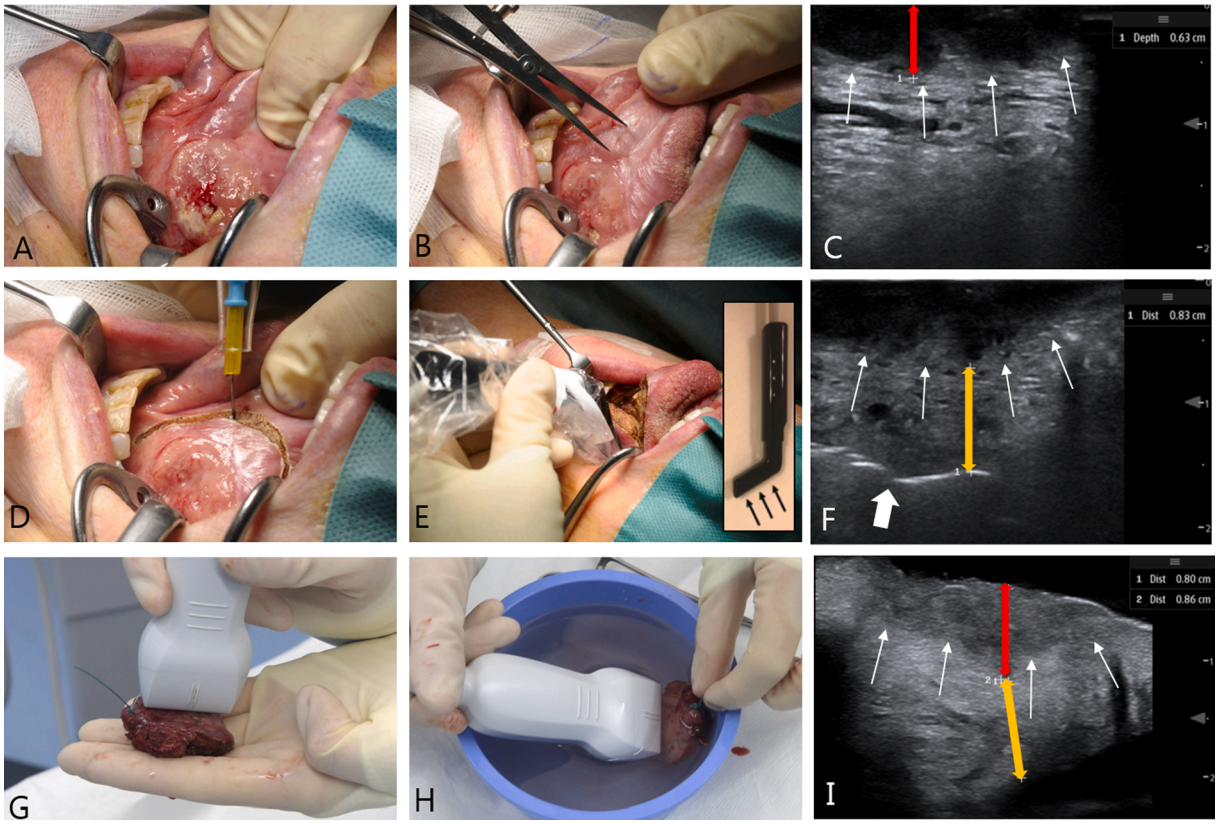

Fig. 1. US-guided resection of SCCT. Red double arrows define echographical TT, yellow double arrows define the margin, and white arrows define the tumour border. A: SCCT of the tongue. B: Demarcation of a mucosal margin of 10 mm. C: Intraoral US for in-vivo determination of TT. D: Resection of the anterior part of the TT. E–F: Intraoral US for in-vivo measurement of the central margin with a 16 MHz hockey stick-shaped probe (indent, transducer demarcated with black arrows). G: Hand-based ex-vivo control with a high-resolution 20 MHz symmetrically shaped probe. Note the sUS mark indicating the smallest echographic margin. H–I: Ex-vivo US control of the central resection margin using the water-based method.

resection of the tumour from the anterior side using a monopolar diathermic knife or a thulium laser. When the resection plane reached under the anterior mucosal border of the tumour, a technical physician (KJK) captured the tumour border and anterior part of the resection plane in one US image. The resection plane was made visible after performing haemostasis and by placing the partially resected specimen back in its original location. In this way, a layer of air was created that was visible as a hyperechoic border. In case the border became not visible, a small surgical instrument was placed under the resection plane, comparable with the methods of Songra et al. [12] (Fig. 1**E**). The distance between the tumour border and the resection plane was measured and was used as a basis to aim for a 10-mm echographic resection margin (Fig. 1**F**). The same procedure was repeated when the plane reached under the central and posterior parts of the tumour.

Surgical procedure: ex-vivo imaging

The resection specimen was marked with sutures for orientation. The TT and echographical margins were measured ex-vivo for five specific areas of the resection specimen (anterior, posterior, craniomedial, caudolateral and central) by sweeping a high-resolution, symmetrically shaped 20 MHz US probe (L20-5 s, Mindray) over all five areas and measuring the smallest margin for those areas (Fig. 3**B**). Resection specimens were assessed by the technical physician and were either examined while being held in a gloved hand (hand-based) or while in a bath of saline (water-based), as can be seen in Fig. 1**G-I**. When an inadequate (i.e. $<$ 5 mm) margin was suspected, the surgeon decided whether an immediate intraoperative RR (iRR) was indicated. iRR specimens were either sutured or marked on the resection specimen, depending on the size and the location of the iRR or the surgeon's preference. If no iRR was performed, the smallest US-measured tumour-free margin (sUS) was localised and indicated by an extra suture (sUS mark).

Adjuvant treatment

After surgery, patients were advised about whether to undergo adjuvant treatment after a multidisciplinary team discussion. Decisions were primarily based on resection margins and the histopathological growth pattern of the tumour. For this study, we recorded how many patients underwent adjuvant treatment and whether this was RR or local RT.

Conventional cohort

A consecutive cohort of 96 T1-3 SCCT patients conventionally treated between July 2014 and September 2018 was retrospectively analysed. Excisional biopsies and resections without curative intention were excluded. The results of frozen-section analysis were not included, as this type of analysis was performed in only 2% of the cases.

Histopathology

Specimen fixation and HE coupe preparation

In both cohorts, resection specimens and iRRs (if applicable) were cut into slices of ~3–5 mm from anterior to posterior. Slice thickness was estimated by dividing the reported length of the specimens by the reported number of slices. The thickness of the iRR specimens was measured macroscopically with a ruler.

For the US cohort, the location and size of the iRR specimen were marked on the resection specimen with dye (Fig. 2). The same colour dye was applied on one side of the iRR specimen's location to indicate its orientation with respect to the resection specimen. If no iRR was performed, the location of the sUS mark was indicated by a small dot of dye. A 4-µm section of the slices was obtained, and each was stained with hematoxylin-eosin (HE) and digitalised according to the methods described by Stathonikos et al. [13].

Microscopic examination

TT and tumour growth patterns (non-cohesive, perineural and vaso-invasive) were recorded for both cohorts. Histopathological margins at the five specific areas (anterior, posterior, craniomedial, caudolateral and central, Fig. 3) were determined by a dedicated head and neck pathologist. Margins at the craniomedial, caudolateral and central parts of the specimen were measured digitally [13] (Fig. 2). Margins at the anterior and posterior parts were calculated by multiplying the number of tumour-free slices, as determined by microscopical images, by the mean slice thickness (Fig. 2). For the US cohort, additional attention was given to the areas of the iRRs and sUS marks in relation to the resection specimen; both were assigned to one of the following categories:

1) Correct: The sUS mark or iRR was situated at the exact same location of an inadequate (i.e. $<$ 5 mm) margin. The margin of that location was re-calculated by adding the macroscopically determined thickness of the iRR specimen. If that also changed the smallest margin at one of the five specific areas (Fig 3**B**), the margin of that area was redefined.
2) Justified: The sUS mark or iRR was situated at approximately the same location of an inadequate margin, but in the case of an iRR it was too small to contribute to a change in margin status.
3) Incorrect: Either the sUS mark or iRR was at the position of a $\geq$ 5 mm margin.

Statistical analyses

US vs. conventional cohort

For both cohorts, the histopathological margins at the specific areas of the resection specimen (anterior, posterior, craniomedial, caudolateral and central) were categorised as 'free' ($\geq$5 mm), 'close' (1–5 mm) or 'positive' ($<$1 mm). The smallest margin of all these areas determined

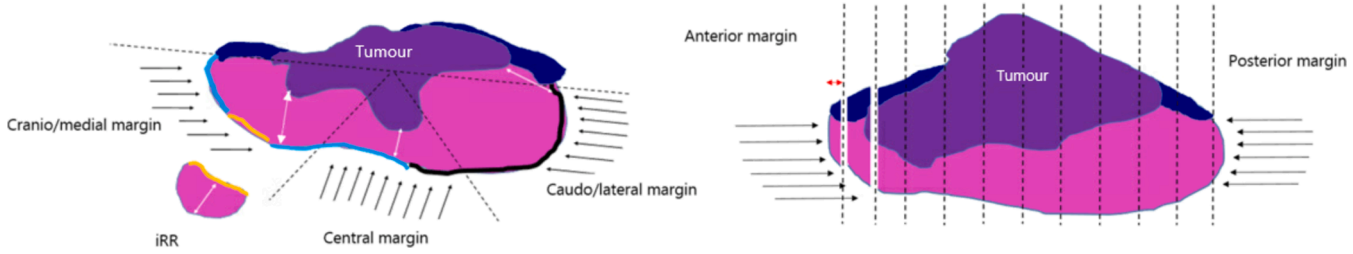

Fig. 2. Schematic representation of histopathological assessment of the resection specimen. Pink: normal HE-stained tissue. Purple: HE-stained tumour. Dark blue: HE-stained mucosa. A: HE section with light-blue ink demarcating the craniomedial side and black ink demarcating the caudolateral side of the resection specimen. The central location is between the craniomedial and caudolateral sides of the specimen. Its area is defined as between the lines of 45 degrees originating from a line parallel with the specimen's mucosa. The yellow area indicates the location of the iRR. The white double arrows indicate the histopathological margins. Note that the iRR is not included, as it does not change the smallest distance at the craniomedial location. B: Assessment of the anterior margin. The red double arrow depicts the defined histopathological margin by calculating the thickness of the slices and multiplying it by the amount of tumour-free slices.

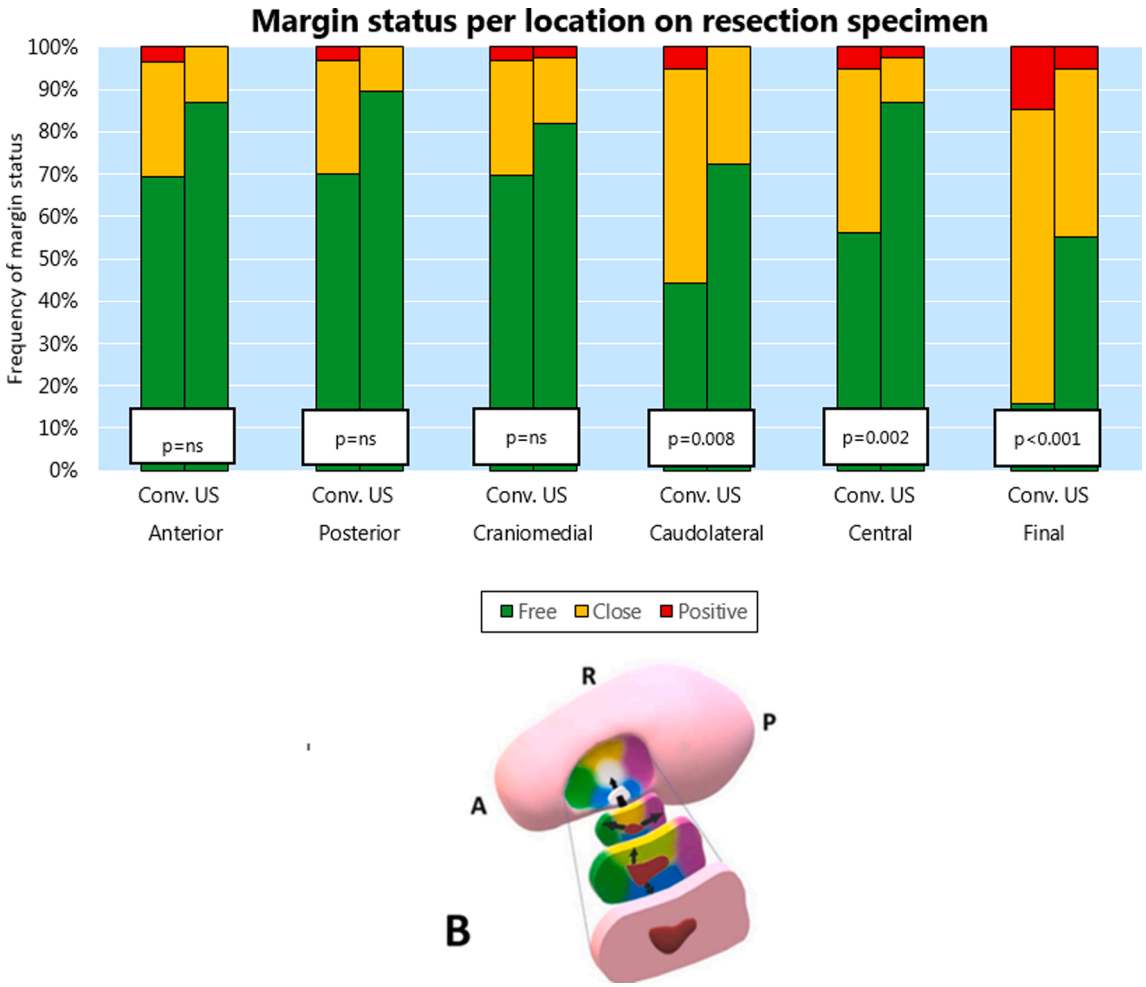

Fig. 3. A: Margin status of all analysed locations of the resection specimen and final margin status of both cohorts. P-values are representative of all comparisons (found using a Fisher's exact test) in the black indents. **B:** The analysed locations are schematically depicted in a virtually sliced resection specimen at the lower-left corner. Green: anterior; purple: posterior; yellow: craniomedial; blue: caudolateral; white: central. Black arrows indicate the examples of the measured margins in all directions. A: anterior; P: posterior; R: right side of the tongue.

the final margin status. The mean margin of the five specific areas was calculated and considered as the 'overall margin' of a resection specimen. Patient characteristics, histopathological margins at the analysed areas, final margin status, overall margin, smallest margin and frequency of adjuvant treatment (local (C)RT or a RR) were compared (Tables 1 and 2 and Fig. 3). For continuous variables, statistically significant differences were determined with an independent *t*-test. For categorical variables, statistically significant differences were determined with a Chi-square test or with a Fisher's exact test if the minimum expected count was less than five.

Table 1
Demographical data and tumour characteristics US- and conventional cohort.

	Conventional cohort (n = 96)	*US cohort (n = 40)*	*P-value*
Gender (n)			
Male (%)	56 (58)	23 (58)	ns
Female (%)	40 (42)	17 (43)	
Age (years)			
Mean (SD)	67.0 (12.7)	58.9 (15.0)	**0.004**[a]
Depth of invasion			
Mean (SD)	7.8 (5.3)	7.2 (5.5)	ns
Pathological tumour stage (n)[b]			
pT1 (%)	33 (34)	15 (38)	ns
pT2 (%)	37 (39)	18 (45)	
pT3 (%)	26 (27)	7 (18)	
Growth pattern (n)			
Non-cohesive (%)	47 (49)	31 (78)	**0.002**[b]
Perineural (%)	28 (29)	14 (35)	ns
Vaso-invasive (%)	6 (6)	5 (13)	ns

[a] determined with independent *t*-test.
[b] determined with Chi square test.

Table 2
Results of US- and conventional cohort.

	Conventional Cohort (n = 96)	*US Cohort (n = 40)*	*P-value*
Margin status (n)			
Free (%)	15 (16)	22 (55)	**<0.001**[a]
Close (%)	67 (70)	16 (40)	
Positive (%)	14 (15)	2 (5)	
Margin distances (mm) – *Mean (SD)*			
Smallest	3.4 (2.0)	4.9 (2.6)	**0.002**[b]
Overall	6.8 (2.4)	8.6 (2.5)	**<0.001**[b]
Adjuvant treatment (n)			
Local adjuvant RT (%)	20 (21)	4 (10)	ns
Adjuvant re-resection (%)	9 (9)	4 (10)	ns
Total (%)	29 (30)	8 (20)	ns

ns: not significant.
[a] Statistical significance was determined with Fisher's exact test.
[b] Statistical significance was determined with independent *t*-test.

Accuracy of US-guided resections

Modified Bland–Altman plots were made to compare in-vivo and ex-vivo US measurements of TT with histopathological TT as the reference standard. One-sample t-tests were performed to examine whether the mean differences between histological and US-based measurements were statistically significant non-zero. The frequency of iRR and sUS marks classified as 'correct', 'justified' and 'incorrect' was analysed (Table 3). A receiver operating characteristic (ROC) curve was made (using all areas—anterior, posterior, craniomedial, caudolateral and central) to assess the diagnostic ability of ex-vivo US to find inadequate (i.e. < 5 mm) margins.

Results

US vs. conventional cohort

Forty-four patients were initially included for this study in our outpatient clinic. Four drop-outs were reported. One patient had a tumour that was re-staged as a T4 tumour during surgery because it appeared to extend towards the base of the tongue. Another patient appeared to have a tumour that extended extensively towards the contralateral side of the tongue resulting in an echographically undefinable tumour border. In two patients, no tumour cells were found in the resection specimen. Considering the demographic data and tumour characteristics, the only significant differences between both cohorts were in age and non-cohesive growth pattern (Table 1). As shown in Table 2 and Fig. 3, the US cohort had more than a threefold increase in free margin status and a threefold decrease in positive margin status compared to the conventional cohort ($p < 0.001$). The smallest margins and overall margins were significantly smaller in the conventional cohort ($p = 0.002$ and $p < 0.001$, respectively); see Table 2. Although not statistically significant, the frequency of local RT in the US cohort was half that in the conventional cohort. There was no difference in the frequency of RR as adjuvant treatment (Table 2). Histopathological margin status per analysed location on the resection specimen (anterior, posterior, craniomedial, caudolateral and central) and final margin status are depicted in Fig. 3. For every location, the frequency of free margins was higher in the US cohort than in the conventional cohort, while the frequency of positive and close margins was lower. This difference was statistically significant at the caudolateral and central locations.

Accuracy of US-guided resections

Modified Bland–Altman plots are shown in Fig. 4. There was a mean difference in TT of 0.4 (SD 2.4) mm between in and vivo measurements on US and upon histopathologic examination, indicating a small over-estimation of TT by US. For ex-vivo US, the measurement was 0.9 (SD 2.6) mm, indicating an over-estimation. Neither of the mean difference are statistically significant non-zero.

Fifteen patients received one iRR and five received two iRRs, resulting in 25 iRRs in total. The analysis of three iRRs failed due to unclear localisation of the iRR specimens. Therefore, only 22 iRRs could be analysed (Table 3). Twelve iRRs and 10 sUS marks were incorrectly placed and were not at the location of the smallest margin, although three of the 12 incorrectly placed iRRs were placed at the location of the smallest margin with a margin distance of 5.5 mm, 5.1 mm and 5.2 mm. Five iRRs and five sUS marks were classified as 'justified'. Five iRRs and six sUS marks were correctly executed in four patients. Because one of the four patients had a close margin elsewhere in the resection specimen, the five correctly placed iRRs contributed to the changed margin status in three of the 21 (14%) patients with an initial close and positive margin status.

Table 3
Success of iRRs and sUS-marks.

	Correct	*Justifiable*	*Incorrect*
iRR (%) (n = 22)	5 (23)	5 (23)	12 (55)
sUS-marks (%) (n = 21)	6 (29)	5 (24)	10 (48)
Total (%) (n = 43)	11 (26)	10 (23)	22 (51)

Fig. 5 shows the ROC curve, which depicts the ability of ex-vivo US to identify histopathologically inadequate (i.e. < 5 mm) margins. The area under the curve is 0.633, which is statistically significantly different from an area of 0.5 ($p = 0.009$, 95% CI: 0.54–0.74). An echographical cut-off value of 8.1 mm yields a sensitivity of 76% and a specificity of 43% in detecting histopathologically inadequate margins.

Discussion

Although earlier studies have demonstrated the advantages of US-guided SCCT resections [2,10,12,14–19], to our knowledge this is the first study that compares the resection margins of a large prospectively analysed US cohort with those of a retrospectively analysed conventional cohort. We identified echographic and histopathologic margins in five specific areas of the resection specimen, which provided us detailed insight in terms of the accuracy and utility of this technique. In our experience, US-guided surgery for SCCT is an accessible, relatively inexpensive technique because it provides a good overview of the deep and submucosal tumour margins. Moreover, pre-excisional US for TT estimation gives insight about the tumour's extent, although one should be careful regarding the setting of an exophytic tumour where TT might overestimate the depth of invasion.

Although there is no follow-up data about survival and quality of life (QoL) available yet, we assume that the current results will lead to more favourable patient outcomes. The most important outcome of this study is that US-guided SCCT resections contributed to a statistically significant more than threefold increase in free margin status and a statistically significant threefold decrease in positive margin status compared to conventional treatment. There is ongoing debate about the definition of close margin status; different studies search for a cut-off margin distance that significantly reduces the chance of recurrence without excessive removal of healthy tongue tissue [6,20–25]. However, there is a consensus that a positive margin status as defined in this study (i.e. < 1 mm) negatively impacts disease-free survival and local recurrence [24–27]. Although the total frequency of the adjuvant treatment discussed did not significantly differ between the two cohorts, the frequency of adjuvant local RT in the US-guided cohort was half that of the conventional cohort (i.e. 21% vs. 10%). It is well known that adjuvant RT in the oral cavity diminishes QoL [28] and tongue function (e.g. mobility and sensory function [4,28]). In a multiple regression analysis, Yang et al. [28] found that adjuvant RT has the most negative effect on QoL in tongue cancer patients (B = -9.595). In addition, Jehn et al. [4] described specific physical impairments (e.g. xerostomia and pain) that were significantly associated with mucositis as a result of local adjuvant RT after surgery.

One could argue that the larger size of free margins might result in overtreatment. Indeed, the mean overall histopathological margin was 1.8 mm higher in the US cohort (8.6 mm vs. 6.8 mm, $p < 0.001$; see Table 2). This might be a logical effect of the surgeon's aim to achieve an intra-operative echographical deep/submucosal margin of 10 mm. Although we do not expect clinically significant differences in oral function and QoL due to this small enlargement, this issue will be addressed in a future analysis.

At every analysed area (Fig. 3), a higher frequency in free margin status was achieved, while the frequency of close and positive margin status decreased. Significant improvements are seen at the caudolateral and central areas, whereas the smallest margins were measured at these areas in the conventional cohort (Fig. 3). Regarding the caudolateral area, this might be due to intra-operative margin overestimation because during conventional treatment the muscular tongue tissue might be overstretched to reveal the caudolateral portion of the tumour.

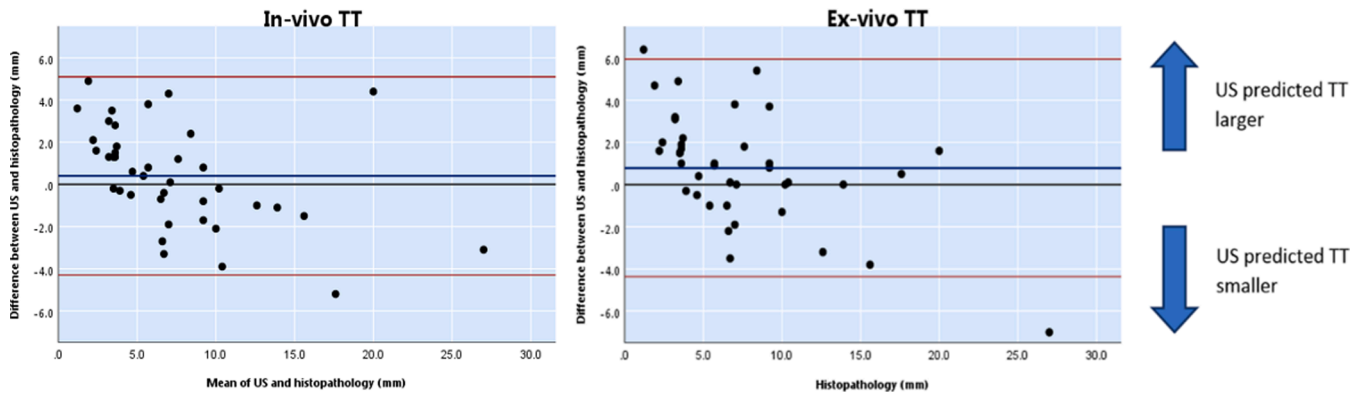

Fig. 4. Modified Bland–Altman plots depicting the differences between in- and ex-vivo TT measurements on US and upon histopathology. The blue line depicts the mean difference between US and histopathology. For in-vivo US, it is 0.4 mm; for ex-vivo US, it is 0.9 mm. Red lines and blue arrows depict the upper and lower 95% level of agreement. For in-vivo US, it is −4.3–5.1 mm; for ex-vivo TT, it is −4.4–5.9 mm.

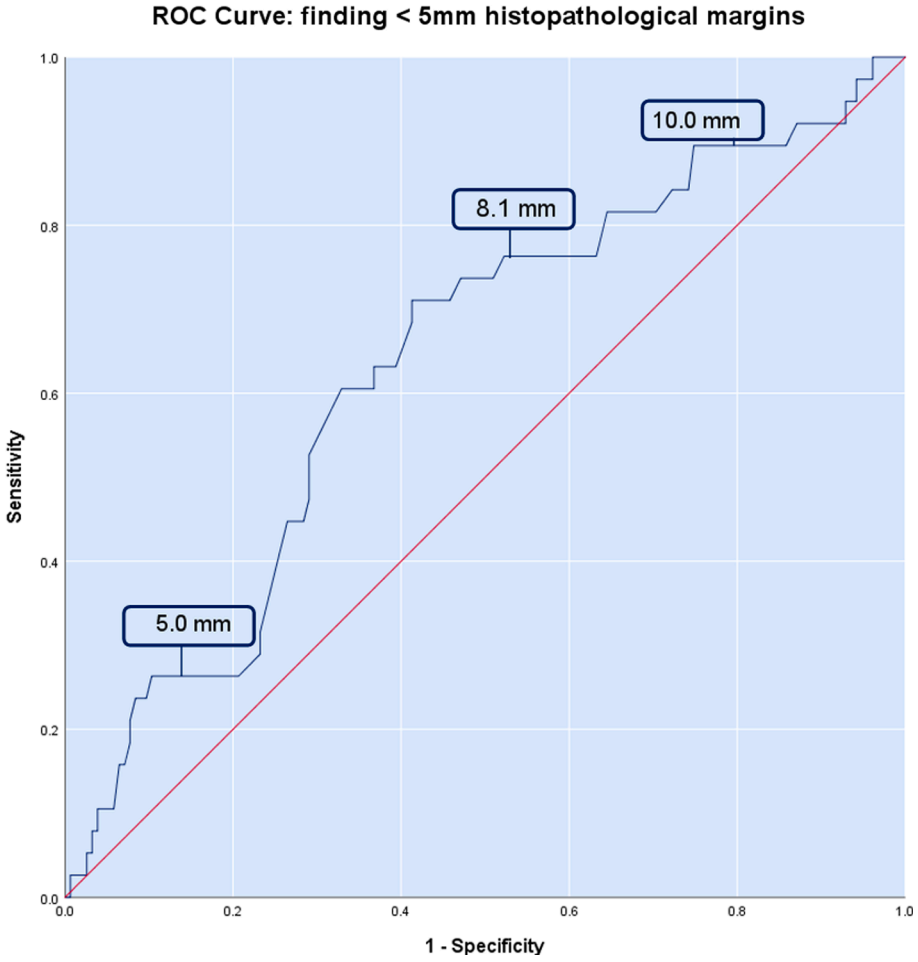

Fig. 5. ROC curve (blue) depicting the diagnostic ability of US to identify < 5 mm margins (area under the curve: 0.633). The red line is a reference line for a method with no diagnostic value (area under the curve: 0.5). All margins measured by ex-vivo US and histopathology (anterior, posterior, craniomedial, caudolateral and central) are analysed. Data points corresponding with a margin of 5.0 mm, 8.1 mm and 10.0 mm are shown with respect to the ROC curve.

Thus, US guidance seems to provide better margin control at the caudolateral area. Regarding the improvement at the central area, this might be because this part of the resection is generally situated directly under the thickest part of the tumour. The fact that in-vivo TT measurements on US represent a fairly good predictor of histopathological TT (Fig. 4) might explain this improvement. Indeed, if TT is well estimated, the surgeon has a better chance of achieving a 10 mm central margin on US.

TT measured by in-vivo and ex-vivo US showed approximately the same difference in histopathological TT (Fig. 4). The slightly higher mean difference and larger interval of the 95% limits of agreement might be because both hand- and water-based measurements were done. We cannot conclude from this data that the tumour itself shrinks after resection; neither of the mean differences were statistically significant

non-zero because there was also a considerable amount of TT underestimation. Indeed, both the in-vivo and ex-vivo results are very much in line with the *meta*-analysis by Klein Nulent et al. [7] in which the difference between US-predicted invasion depth and histopathological TT in oral cancer was assessed (mean 0.5 mm, 95% limits of agreement −5.5–6.5 mm).

Ex-vivo US is able to identify inadequate margins but is moderate, as can be concluded from the area under the ROC curve (0.633) (Fig. 5) and the number of misplaced iRRs (41%) and sUS marks (48%) (Table 3). Microscopic infiltrative growth that is too small for the US probe's resolution might cause underestimation of tumour thickness and overestimation of resection margins, while differences in muscle density or salivary gland tissue close to the floor of the mouth might cause the opposite effect [2]. Hence, these factors can hamper the diagnostic accuracy of ex-vivo US. Although we prefer SCCT surgery without the need for an iRR, we still believe ex-vivo US can play an important role in achieving a higher frequency of free margin status. First, as described by others [2,16], ex-vivo US control of the resection specimen prolongs surgical time by only 5–10 min but allows sampling the resection specimen as a whole. This is in contrast to frozen sections, the sensitivity of which is limited because only 0.1%–1% of the resection specimen is sampled [3]. Second, if an iRR was performed with 8 mm as an ex-vivo echographical cut-off value (instead of 5 mm, which was the case in this study), it would have detected 76% of the inadequate (i.e. < 5 mm) histopathological margins, which is an acceptable sensitivity (Fig. 5). Although this alternative cut-off value would have led to a decrease in specificity, we expect that this would not have led to an increase in overtreatment and loss of tongue function. Third, the orientation of iRRs should be better harmonised with histopathology. In this study, we added the macroscopically determined thickness of the iRR specimen to prevent further loss of information about its relationship to the resection specimen because the iRR specimen must also be sliced for microscopic examination. As shown in Table 3, **23**% of the iRRs would have been placed correctly if they were larger or better orientated. This would have led to a change in margin status in more patients. The use of 'parallel tagging' as described by Van Lanschot et al. [29], that is, placing corresponding tags on the side of the wound bed and resection specimen, might be a potential solution to prevent relocation errors.

Several limitations of this study are worth mentioning. First, this study compares a prospective US cohort with a retrospectively analysed conventional surgery cohort. A randomised controlled trial could produce more reliable outcomes because two groups will be compared, while the same surgeons perform SCCT resections and the same pathologists examine the resection specimen. Currently, a multicentre randomised controlled trial is being conducted at several centres of the Dutch Head and Neck Society (NWHHT). Second, no data about overall survival and recurrence-free survival is available yet. Although we expect a better patient outcome in the US cohort because more patients have free margin status, we do not know the effect of iRR on survival. iRRs might be prone to 'relocation errors', which is also seen in frozen section analysis [30]. As iRRs are not anatomically oriented [31], it is challenging to translate an echographically inadequate margin's location on the resection specimen to its corresponding location on the tumour bed [5]. Several studies have paradoxically reported that iRRs, indicated by frozen sections, are predictors for local recurrence [26,27]. We expect that 'parallel tagging'[29], next to harmonisation of the resection specimen with histopathology, can solve this problem. Third, not every suspected close or positive margin was followed by an iRR during ex-vivo US control. In some cases, it was decided to spare tongue function when the close margin corresponded to the location of the lingual nerve or the lingual artery. This might have influenced the results presented in Table 3. Fourth, we used two different techniques to assess margins and TT during the ex-vivo US, hand- and water-based measurements. In case hand-based measurements required too much pressure on the resection specimen, water-based measurements were done to prevent specimen deformation. A paired comparison between both methods might provide more insight about their reliability. Finally, we did not correct for post-excision or post-fixation specimen shrinkage. Although this phenomena is described in the literature for the surface and mucosal margins of oral tumours [32,33], the effect of specimen shrinkage at deep and submucosal levels is unknown. We do not believe that US is accurate enough to identify shrinkage at these levels, as it cannot capture microscopic infiltrative non-cohesive growth or other histopathological growth factors (i.e. vasoinvasive and perineural growth) that may affect margin distances as well.

In conclusion, US-guided resection of SCCT is a surgical technique able to increase the frequency of free margin status and decrease the frequency of positive margin status when compared to conventional treatment. Ex-vivo US control of the resection specimen makes it possible to generate additional free margins. However, effort must be made to orientate the resection specimen in the same manner during surgery as during histopathological examination. Nevertheless, the results of this study suggest that this technique may improve disease-specific survival and QoL. This will be assessed in a Dutch multicentre randomised controlled trial.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Acknowledgments

The authors would like to thank Carleen Adriaansens, MD (Department of Head and Neck Surgical Oncology, University Medical Center Utrecht) for providing us the photographs used in this paper.

Funding

This work was supported by the KWF Dutch Cancer Society (grant number 11906).

References

[1] Chinn SB, Myers JN. Oral cavity carcinoma: current management, controversies, and future directions. J Clin Oncol 2015;33(29):3269–76. https://doi.org/10.1200/JCO.2015.61.2929.
[2] de Koning KJ, Koppes SA, de Bree R, Dankbaar JW, Willems SM, van Es RJJ, et al. Feasibility study of ultrasound-guided resection of tongue cancer with immediate specimen examination to improve margin control – comparison with conventional treatment. Oral Oncol 2021;116:105249.
[3] Smits RWH, Koljenović S, Hardillo JA, et al. Rection margins in oral cancer surgery: room for improvement. Head Neck 2016;38(S1):E2197–203.
[4] Jehn P, Stier R, Tavassol F, Dittmann J, Zimmerer R, Gellrich N-C, et al. Physical and psychological impairments associated with mucositis after oral cancer treatment and their impact on quality of life. Oncol Res Treat 2019;42(6):342–9.
[5] Kubik MW, Sridharan S, Varvares MA, Zandberg DP, Skinner HD, Seethala RR, et al. Intraoperative margin assessment in head and neck cancer: a case of misuse and abuse? Head Neck Pathol 2020;14(2):291–302.
[6] Dik EA, Willems SM, Ipenburg NA, Adriaansens SO, Rosenberg AJWP, van Es RJJ. Resection of early oral squamous cell carcinoma with positive or close margins: relevance of adjuvant treatment in relation to local recurrence. Oral Oncol 2014;50(6):611–5. https://doi.org/10.1016/j.oraloncology.2014.02.014.
[7] Klein Nulent TJW, Noorlag R, Van Cann EM, Pameijer FA, Willems SM, Yesuratnam A, et al. Intraoral ultrasonography to measure tumor thickness of oral cancer: a systematic review and meta-analysis. Oral Oncol 2018;77:29–36.
[8] Tarabichi O, Bulbul MG, Kanumuri VV, Faquin WC, Juliano AF, Cunnane ME, et al. Utility of intraoral ultrasound in managing oral tongue squamous cell carcinoma: systematic review. Laryngoscope 2019;129(3):662–70.
[9] Narayana HM, Panda NK, Mann SBS, Katariya S, Vasishta RK. Ultrasound versus physical examination in staging carcinoma of the mobile tongue. J Laryngol Otol 1996;110(1):43–7.
[10] Tarabichi O, Kanumuri V, Juliano AF, Faquin WC, Cunnane ME, Varvares MA. Intraoperative ultrasound in oral tongue cancer resection: feasibility study and early outcomes. Otolaryngol - Head Neck Surg 2018;158(4):645–8. https://doi.org/10.1177/0194599817742856.
[11] Amin MB, Edge SB. AJCC cancer staging manual. x: Springer; 2017.
[12] Songra AK, Ng SY, Farthing P, Hutchison IL, Bradley PF. Observation of tumour thickness and resection margin at surgical excision of primary oral squamous cell

carcinoma - assessment by ultrasound. Int J Oral Maxillofac Surg 2006;35(4):324–31. https://doi.org/10.1016/j.ijom.2005.07.019.
[13] Stathonikos N, Nguyen TQ, Spoto CP, Verdaasdonk MAM, van Diest PJ. Being fully digital: perspective of a Dutch academic pathology laboratory. Histopathology 2019;75(5):621–35. https://doi.org/10.1111/his.13953.
[14] Helbig M, Flechtenmacher C, Hansmann J, Dietz A, Tasman AJ. Intraoperative B-mode endosonography of tongue carcinoma. Head Neck 2001;23(3):233–7. https://doi.org/10.1002/1097-0347(200103)23:3<233::AID-HED1024>3.0.CO;2-P.
[15] Kodama M, Khanal A, Habu M, Iwanaga K, Yoshioka I, Tanaka T, et al. Ultrasonography for intraoperative determination of tumor thickness and resection margin in tongue carcinomas. J Oral Maxillofac Surg 2010;68(8):1746–52.
[16] Brouwer de Koning SG, Karakullukcu MB, Lange CAH, Schreuder WH, Karssemakers LHE, Ruers TJM. Ultrasound aids in intraoperative assessment of deep resection margins of squamous cell carcinoma of the tongue. Br J Oral Maxillofac Surg 2020;58(3):285–90. https://doi.org/10.1016/j.bjoms.2019.11.013.
[17] Bekedam NM, Smit JN, de Koekkoek - Doll PK, van Alphen MJA, van Veen RLP, Karssemakers LHE, et al. Intra-operative resection margin model of tongue carcinoma using 3D reconstructed ultrasound. Adv Oral Maxillofac Surg 2021;4:100154. https://doi.org/10.1016/j.adoms.2021.100154.
[18] Bulbul MG, Tarabichi O, Parikh AS, Yoon BC, Juliano A, Sadow PM, et al. The utility of intra-oral ultrasound in improving deep margin clearance of oral tongue cancer resections. Oral Oncol 2021;122:105512. https://doi.org/10.1016/j.oraloncology.2021.105512.
[19] Baek C-H, Son Y-I, Jeong H-S, Chung MK, Park K-N, Ko Y-H, et al. Intraoral sonography-assisted resection of T1–2 tongue cancer for adequate deep resection. Otolaryngol - Head Neck Surg 2008;139(6):805–10.
[20] Lee DY, Kang SH, Kim JH, Kim M-S, Oh KH, Woo J-S, et al. Survival and recurrence of resectable tongue cancer: resection margin cutoff value by T classification. Head Neck 2018;40(2):283–91.
[21] Brinkman D, Callanan D, O'Shea R, Jawad H, Feeley L, Sheahan P. Impact of 3 mm margin on risk of recurrence and survival in oral cancer. Oral Oncol 2020;110(April):104883. https://doi.org/10.1016/j.oraloncology.2020.104883.
[22] Daniell J, Udovicich C, Rowe D, McDowell L, Vital D, Bressel M, et al. Impact of histological oral tongue cancer margins on locoregional recurrence: a multi-centre retrospective analysis. Oral Oncol 2020;111:105004.
[23] Goel V, Parihar PS, Parihar A, et al. Accuracy of MRI in prediction of tumour thickness and nodal stage in oral tongue and gingivobuccal cancer with clinical correlation and staging. J Clin Diagnostic Res 2016;10(6). https://doi.org/10.7860/JCDR/2016/17411.7905.
[24] Singh A, Mishra A, Singhvi H, Sharin F, Bal M, Laskar SG, et al. Optimum surgical margins in squamous cell carcinoma of the oral tongue: Is the current definition adequate? Oral Oncol 2020;111:104938.
[25] Bajwa MS, Houghton D, Java K, Triantafyllou A, Khattak O, Bekiroglu F, et al. The relevance of surgical margins in clinically early oral squamous cell carcinoma. Oral Oncol 2020;110:104913.
[26] Varvares MA, Poti S, Kenyon B, Christopher K, Walker RJ. Surgical margins and primary site resection in achieving local control in oral cancer resections. Laryngoscope 2015;125(10):2298–307. https://doi.org/10.1002/lary.25397.
[27] Ettl T, El-Gindi A, Hautmann M, Gosau M, Weber F, Rohrmeier C, et al. Positive frozen section margins predict local recurrence in R0-resected squamous cell carcinoma of the head and neck. Oral Oncol 2016;55:17–23.
[28] Yang ZH, Chen WL, Huang HZ, Bin PC, Li JS. Quality of life of patients with tongue cancer 1 year after surgery. J Oral Maxillofac Surg 2010;68(9):2164–8. https://doi.org/10.1016/j.joms.2009.09.048.
[29] Lanschot CGF, Mast H, Hardillo JA, Monserez D, Hove I, Barroso EM, et al. Relocation of inadequate resection margins in the wound bed during oral cavity oncological surgery: a feasibility study. Head Neck 2019;41(7):2159–66.
[30] de Koning KJ, Varvares MA, van Es RJJ, Dankbaar JW, Breimer GE, de Bree R, et al. Response to: Should ultrasound-guided resection be the new norm for oral tongue resections? Oral Oncol 2022;124:105473.
[31] Black C, Marotti J, Zarovnaya E, Paydarfar J. Critical evaluation of frozen section margins in head and neck cancer resections. Cancer 2006;107(12):2792–800. https://doi.org/10.1002/cncr.22347.
[32] Umstattd LA, Mills JC, Critchlow WA, Renner GJ, Zitsch III RP. Shrinkage in oral squamous cell carcinoma: an analysis of tumor and margin measurements in vivo, post-resection, and post-formalin fixation. Am J Otolaryngol 2017;38(6):660–2.
[33] González-Ballester D. The tissue shrinkage phenomenon on surgical margins in oral and oropharyngeal squamous cell carcinoma. Plast Aesthetic Res 2016;3(5):150. https://doi.org/10.20517/2347-9264.2016.23.