

Ultrasound-guided oral cancer surgery

-An accessible technique for an impactful disease-

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Ultrasound-guided oral cancer surgery

Echogeleide mondkankerchirurgie

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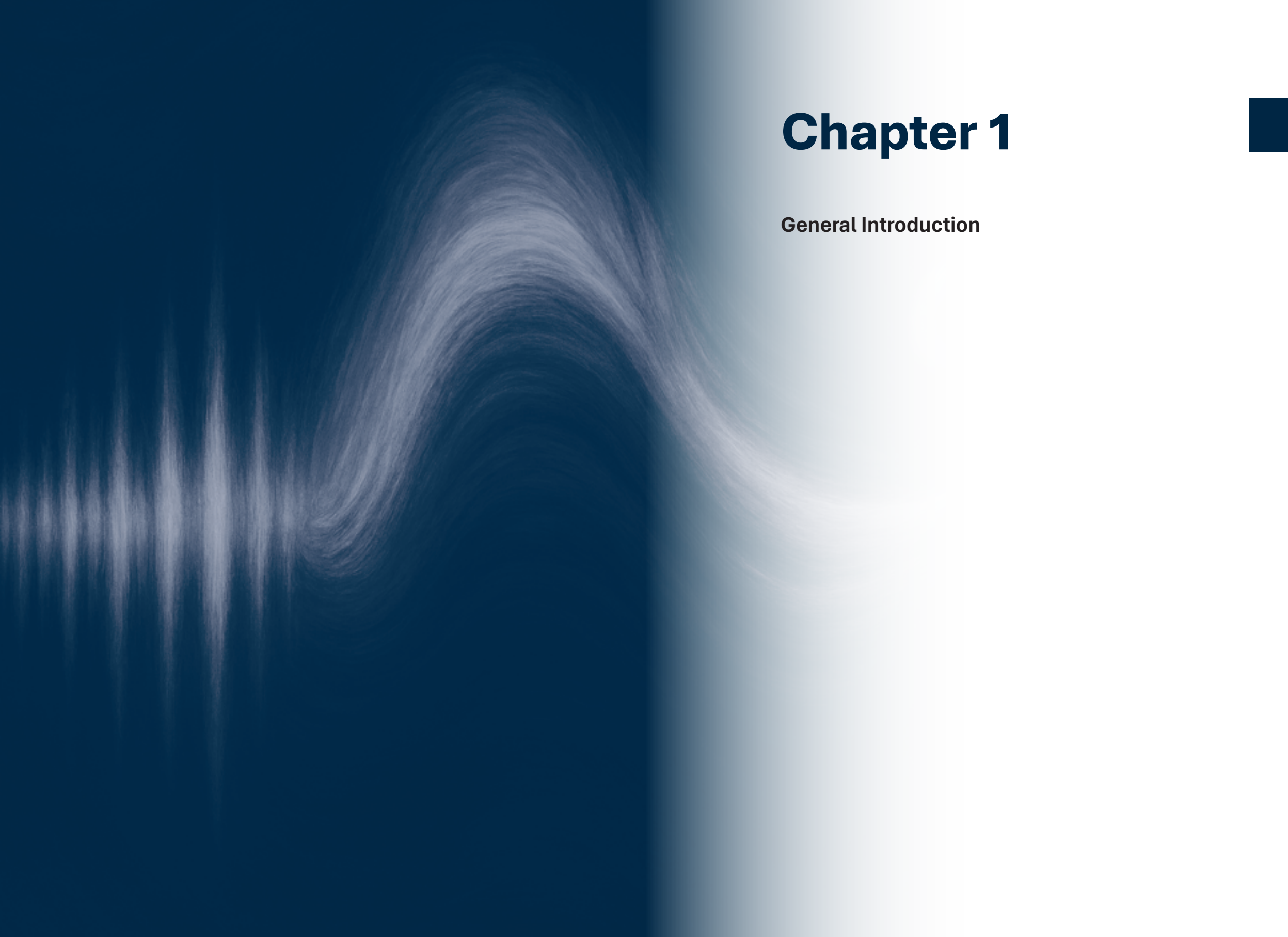
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Chapter 1

General Introduction

Oral cancer

‘Oral cancer’ encompasses several types of head and neck cancer that occur in the oral cavity. This thesis focuses on oral squamous cell carcinoma (SCC), which accounts for approximately 90% of all oral cancers (1).

Treating the primary tumour of oral SCC is also called ‘local treatment’. The most effective way to perform local treatment is adequate surgical removal of the SCC (2). This is crucial to prevent metastases or a recurrence. Once metastasized, oral cancer has a worse prognosis. If spread only regionally, it can be successfully cured with surgery and/or radiotherapy of the neck. If metastasized to distant sites, only palliative systemic treatment options are available in most cases, such as chemotherapy and/or immune therapy (3).

The definition of an ‘adequate resection’ remains a matter of debate and will be discussed later in this introduction. Nevertheless, incomplete surgical removal of a SCC is not uncommon. Although oral SCC originates from the superficial lining of the oral cavity, i.e. the mucosa, it often grows deeper into other structures, such as bone, fat, muscle, and salivary glands. The surgeon does not have a direct view of the tumour’s deeper extent and must rely completely on the preoperative imaging and palpatory feedback during surgery. It has been reported that up to 27% of the resections appear to be incomplete, meaning that cancerous cells are left in the patient (4).

This thesis primarily focuses on a novel image-guided surgery technique to aid surgeons in achieving adequate resections: ultrasound-guided surgery. To a lesser extent, this thesis also discusses other image-guided surgery techniques for oral cancer, including magnetic resonance-guided surgery.

Anatomy of the oral cavity

The oral cavity is the first part of the digestive tract. The oral vestibule is the space at the buccal or labial side of the teeth, bordered by the lips, gum, and alveolar process. The cavity propria is at the lingual side of the teeth, primarily filled with the tongue when closed. The oropharyngeal isthmus is the border between the oral cavity and oropharynx (5).

The anterior two-thirds of the tongue is part of the oral cavity and the posterior third of the tongue is part of the oropharynx. Both parts are separated by the terminal sulcus. The muscles of the tongue can be subdivided into intrinsic (Figure 1) and extrinsic (Figure 2A) muscles. The intrinsic muscles, which are responsible for the tongue’s deformation, are closest to the tongue’s mucosa. They can be subdivided into superior, inferior, transverse and vertical muscles. The extrin-

sic muscles of the tongue, which are responsible for movement of the tongue, are the genioglossus, the hyoglossus, the styloglossus and palatoglossus. The lingual nerve supplies sensation, the hypoglossal nerve supplies motor function, and the lingual artery supplies blood (5).

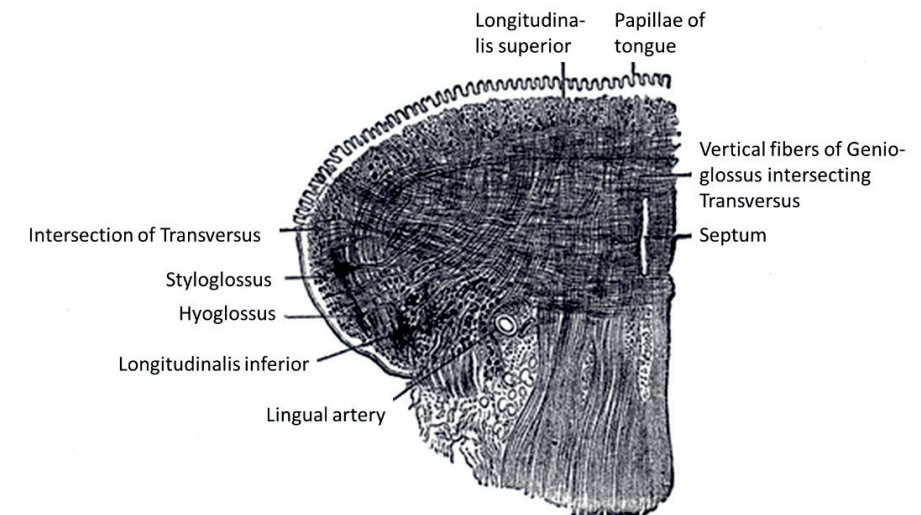


Figure 1: Coronal view of the tongue’s intrinsic muscles. Source: public domain.

The orbicularis oris underlies the labial mucosa. Just underneath the cheek mucosa, one can find the buccinator, a thin rectangular muscle, connecting the mandible and maxilla (Figure 2B). More posteriorly, the masseter muscle, a masticatory muscle, underlies the cheeks as well (5).

The oral mucosa is kept moist by saliva, which is secreted by the minor and major salivary glands and released through their ducts into the oral cavity. The major salivary glands are the sublingual gland, situated below the tongue, the submandibular gland, situated below the mandible, and the parotid gland, situated in front of the ears. The 800 to 1000 minor salivary glands are located submucosally throughout the oral cavity and have a size of approximately 1 to 2 mm (5).

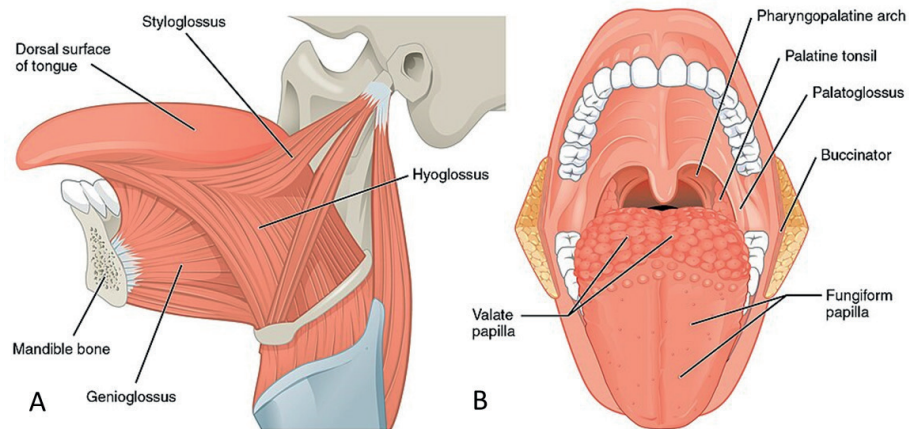


Figure 2: Anatomy of the oral cavity. A: extrinsic muscles of the tongue, B: frontal view of the oral cavity. OpenStax / CC BY (<https://creativecommons.org/licenses/by/3.0/deed.en>) original image modified.

Histopathology of oral cancer

The oral mucosa consists of several layers. It is lined with an epithelial lining of which the top cells are flat and resemble fish scales (in Latin: squama) (6). At the bottom of the epithelial line, one can find basal cells, from which epithelial cells originate. Once divided from a basal cell, an epithelial cell migrates to the most superficial layer of the mucosa (7). After every two to three weeks the most superficial layer of squamous cells is completely replaced (8). This process accelerates when the mucosa is damaged, as seen with alcohol consumption and smoking, which increases the risk of mutations during cell division. The accumulation of mutations may activate oncogenes and/or deactivate tumour suppressor genes (7).

Subsequently, cells within the mucosa may progress through a preneoplastic stage, eventually becoming dysplastic (Figure 3). Dysplasia is a condition characterized by disordered growth and differentiation, although the cells are not entirely autonomous. Dysplasia is often detected in leukoplakia (white plaques) or erythroplakia (red plaques). Both are lesions that cannot be scraped from the mucosal surface. Thus, if (severe) dysplasia is found in the oral cavity, usually by biopsy, there is often an indication to remove this premalignant lesion (9).

Once dysplasia progresses into SCC, the malignant cells gain the ability to invade deeper tissues by breaching the basement membrane. This allows the cancer to infiltrate structures such as bone, fat, muscle, and salivary glands. If left untreated, SCC is able to metastasize through the lymphatic vessels to cervical lymph nodes. In later stages, oral SCC is able to migrate through blood vessels to the lungs or to other vital organs, which is mostly fatal for the patient (10).

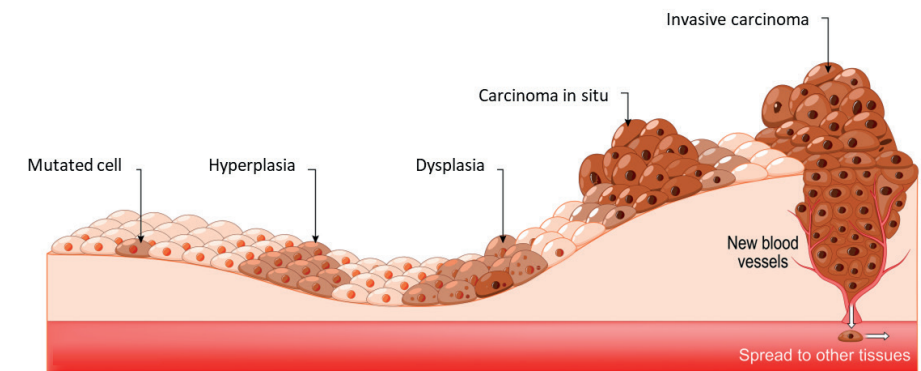


Figure 3: Schematic representation of tumour progression. A mutated cell may first undergo hyperplasia (increase in cell number), followed by dysplasia and cancer formation. A malignant tumour has the potential to metastasize through lymphatic or blood vessels to distant tissues. Image licensed from iStock.

Because there is a vast variety of possible sequential mutations in every unique human genome, every oral SCC is different. To identify the most suitable treatment, every oral SCC can be classified according to the 'TNM' coding method (11). 'T' refers to the tumour size, 'N' to the presence and severity of regional neck metastasis and 'M' to the presence of distant metastases. TNM can be used after clinical evaluation, referred to as 'cTNM' and after histopathological evaluation, referred to as 'pTNM' (11). 'T' is outlined in Table 1. Histopathological examination can only be done when an excised tumour (the resection specimen) is microscopically analysed by the pathologist.

The pathologist may analyse other tumour characteristics as well, such as:

- unfavourable histopathological growth factors, defined as:
 - perineural growth (i.e. tumour growth along nerves, also known as perineural invasion),
 - vaso-invasive growth (i.e. tumour growth into blood- and lymphatic vessels, also known as vascular invasion), and
 - non-cohesive growth (i.e. tumour growth in a disorderly, spidery, infiltrative manner; one could find small clusters of tumours on a distance from the tumour bulk, i.e. tumour budding) (12);
- tumour differentiation; and
- severity of the dysplasia (around the tumour).

Additionally, non-cohesive growth can be scored as the 'worst pattern of invasion,' introduced by Brandwein-Gensler et al. (13).

Table 1. Coding of the T-stage of the TNM classification

<i>T stage</i>	<i>Clinical (cT)</i>	<i>Histological (pT)</i>
Tx	Primary tumour cannot be assessed	Primary tumour cannot be assessed
T0	No evidence of primary tumour	No evidence of primary tumour
Tis	Carcinoma in situ	Carcinoma in situ
T1	Tumour ≤ 2 cm in greatest dimension or ≤ 5 mm DOI	Tumour ≤ 2 cm in greatest dimension and ≤ 5 mm DOI
T2	Tumour ≤ 2 cm in greatest dimension and > 5 and ≤ 10 mm DOI or tumour > 2 cm and ≤ 4 cm in greatest dimension and ≤ 10 mm DOI	Tumour ≤ 2 cm in greatest dimension and > 5 and ≤ 10 mm DOI or tumour > 2 cm and ≤ 4 cm in greatest dimension and ≤ 10 mm DOI
T3	Tumour > 2 cm and ≤ 4 cm in greatest dimension and > 10 mm DOI or tumour > 4 cm in greatest dimension and ≤ 10 mm DOI	Tumour > 2 cm and ≤ 4 cm in greatest dimension and > 10 mm DOI or tumour > 4 cm in greatest dimension and ≤ 10 mm DOI
T4a	Tumour > 4 cm in greatest dimension and > 10 mm DOI or tumour invades adjacent structures (e.g. through cortical bone of the mandible or maxillary sinus, or invades the skin of the face)	Tumour > 4 cm in greatest dimension and > 10 mm DOI or tumour invades adjacent structures (e.g. through cortical bone of the mandible or maxillary sinus, or invades the skin of the face).
T4b	Tumour invades masticator space, pterygoid plates, or skull base and/or encases the internal carotid artery	Tumour invades masticator space, pterygoid plates, or skull base and/or encases the internal carotid artery

Abbreviations: DOI: Depth of invasion

Epidemiology

Worldwide, head and neck cancer is the 6th most common cancer type. Within head and neck cancer, oral and laryngeal SCCs are the most reported subsites. Identifying incidence and prevalence of oral SCC is complicated, as multiple reports define oral cancer differently or report the statistics by combining oral cancer with other types, e.g. oropharyngeal cancer (14) or lip cancer (15).

The following categories of the “International Classification of Diseases for Oncology” (ICD-O) of the World Health Organization (WHO) (16) are used to define oral cancer (17):

- inner aspects of the upper (C00.3) and lower lip (C00.4);
- the dorsal (C02.0), lateral (C02.1), ventral (C02.2) and anterior two-third of the tongue (C02.3);
- upper (C03.0) and lower gum (C03.1);

- floor of mouth (C04);
- hard palate (C05.0);
- cheek mucosa (C06.0), vestibule of mouth (06.1) and retromolar areas (C06.2).

This definition is also used throughout this thesis.

The combined incidence of oral and lip cancer was worldwide 389,846, causing 188,438 deaths in 2022 (18). However, the distribution of oral and lip cancer incidence varies greatly geographically. The incidence is in general higher among men than women, with the highest rates observed in south-central Asia, where oral cancer ranks as the third most common cancer type. It is most likely caused by specific practices such as tobacco chewing, betel nut chewing and alcohol consumption (19).

For Europe, the lowest incidence of oral and lip cancer in 2022 was reported in Luxembourg, with an estimated 7.8 cases per 100,000 inhabitants, while Hungary had the highest incidence, at an estimated 24 cases per 100,000. The European mortality rate for oral and lip cancer was estimated at 5.9 per 100,000 inhabitants. However, the Netherlands reported a mortality rate well below this average, with only an estimated 3.4 deaths per 100,000 inhabitants (15). This could be attributed to the fact that most patients have good access to dental care. Routine dental checkups lead to early detection of lesions, followed by a referral to a maxillofacial surgeon or an otolaryngologist (20).

Regarding presence at subsites of the oral cavity, oral SCC is most present on the tongue (43%), followed by the floor of mouth (17%), and gum (14%) (21).

Diagnosis and work-up

Besides the patient’s medical history, physical examination is the first step in diagnosing oral cancer. Initial lesions are usually asymptomatic because of their small size. They may also present as leukoplakia and/or erythroplakia. When palpated, the area may feel rougher and less elastic than the healthy tissue. In due course, the patient may encounter some discomfort. When oral SCC is more advanced, the lesion often ulcerates and the patient may experience severe pain. Other symptoms may be bleeding of the lesion, difficulty while eating, swallowing (dysphagia) or speaking, weight loss and loosening of teeth (22). Although these features are very characteristic for oral SCC, an incisional biopsy for histopathological examination has to confirm the diagnosis (2). Differential diagnosis of oral SCC can be, for example, an infection with candidiasis, a traumatic lesion (23), or a syphilitic ulcer (24).

Intraoral examination is combined with palpation of the neck to detect metastasis in cervical lymph nodes. When palpated, neck metastases may feel as enlarged, rounded, non-tender lymph nodes. A cytological biopsy by fine needle aspiration, if desired guided by ultrasound (US), can confirm neck nodal metastasis (25).

Further work-up includes medical imaging. Computer tomography (CT) or magnetic resonance (MR) imaging of the head and neck can be used to evaluate the extension of the primary tumour and to detect lymph node metastases. With a chest X-ray or CT, the presence of lung metastases, mediastinal lymph node metastases and second primary lung cancer are evaluated. CT can be combined with [^{18}F]-Fluorodeoxyglucose positron emission tomography to evaluate for distant metastases if multiple neck metastases are suspected. MR imaging is used to evaluate the tumour's extent in soft tissue and presence of macroscopic perineural growth, simultaneously detection of neck metastases is performed (2). Intraoral US can be used to evaluate extension of the tumour in soft tissue (26).

In the Netherlands, the clinical TNM stage and treatment planning are determined during a multidisciplinary team meeting. This meeting includes head and neck surgeons, radiation oncologists, medical oncologists, radiologists, nuclear medicine physicians, pathologists, dentists, geriatricians, anaesthesiologists and other specialists.

Treatment

Local surgery and histopathological assessment

As already mentioned, the first treatment choice of primary oral SCC is surgical resection of the tumour (2). Adequacy of the resection is evaluated by the pathologist through histopathological examination of the resection specimen. If tumour cells in the cutting plane are found (also called a tumour cut-through), it is defined as a 'positive margin' (27) which indicates that there are still residual tumour cells in the patient. According to the guidelines of the Royal College of Pathologists, which are adopted at our centre, tumour cells located less than 1 mm (< 1 mm) from the resection plane are also considered a positive margin (28).

There is a general consensus that, besides preventing a cut-through, an extra safety margin of healthy tissue around the tumour is needed for better local control (29). Although there is discussion about the extent of this margin, current guidelines state that this safety margin needs to be at least 5 mm (≥ 5 mm) in all directions on histopathological examination (27,28). This is termed a '(tumour) free margin'. The rationale behind a 5 mm safety margin is multiplex:

- Statistical analyses. Many researchers have identified a 5 mm margin as the optimal cutoff for balancing overall survival and disease-free survival with quality of life (QoL) and oral function (29). However, this 5 mm margin is still a subject of debate. A substantial number of studies recommend a smaller (30) or larger cutoff value (31), or suggest that the ideal margin is dependent on other factors, such as tumour stage and unfavourable histopathological growth factors at the tumour front. Nevertheless, the 5 mm cutoff remains persistent in the current guidelines (27,28).

- Removal of tumour budding. In extreme non-cohesive growing tumours, one can find small clusters of less than five tumour cells outside the tumour bulk. These small, dissociated tumour cells are considered the primary step for invasive growth and metastasis (32). It is paramount to include these tumour nests in the resection, however the current method for histopathological examination has a limited sampling rate and might miss these tumour nests. A safety margin gives more assurance that the tumour nests are taken within the surgical resections. A recent study found that in head and neck cancer these tumour nest can be found up to 5 mm from the tumour bulk (33).
- Removal of preneoplastic cells in the epithelium. It is believed that the primary tumour can origin from an area with preneoplastic cells, however these cells are not always identifiable as such (34). This area is also able to generate new primary tumours (which may appear as recurrences) (35). This hypothesis is also known as the 'field cancerisation model', which is supported by molecular analysis of surgical margins (36). A ≥ 5 mm margin may increase the chance that preneoplastic cells are taken within the resection.

A histopathological margin larger than a < 1 mm positive margin but less than a ≥ 5 mm free margin is defined as a close margin. Close and positive margins are not uncommon. Literature reports that oral cancer resection specimens have up to close margins in 45% of the cases and up to positive margin in 43% of the cases (4).

In practice, to ensure surgery results in a ≥ 5 mm histological margin, the surgeon tries to attain a surgical tumour-free margin of approximately 1 cm (37). Recent guidelines even advise surgical margins of 10-15 mm (38). This accounts for the issues of post-resection tissue shrinkage, non-palpable tumour clusters or protrusions in case of extreme non-cohesive growing tumours. Both of these issues can lead to histopathological margins being smaller than resected. However, deviations from this preferred distance may be necessary when vital structures are at risk, particularly in large T3 or T4a tumours.

After the surgical resection of oral cancer, initial surgical margins are assessed primarily through visual inspection and palpation, as tumour tissue is often stiffer than healthy tissue. Several treatment centres use frozen section analysis (FSA), which involves taking a sample from the margin and sending it to the histopathological department for examination. Because the sample is frozen, it bypasses the need for formalin fixation, allowing results within less than an hour (39). FSA is the only intraoperative margin assessment technique currently accepted as a clinical standard (40).

Local adjuvant treatment

If inadequate margins on histopathological examination are obtained, alone or in combination with other unfavourable histologic growth factors, local adjuvant treatment of the oral cancer may be indicated. A positive margin is a hard indicator for adjuvant treatment (41). Local adjuvant treatment is employed to address possible residual cancer cells. It can involve either a re-resection in a second tempo or local adjuvant (chemo)radiotherapy. Both types of adjuvant treatment have their disadvantages.

A re-resection in a second tempo has disadvantages in that the precise location of the inadequate margin needs to be derived from the final histopathological report. Subsequently, the inadequate margin must be retraced into a closed wound bed or reconstructed area, resulting in a lack of confidence whether the inadequate margin has been fully addressed (42). Therefore, a re-resection is mostly employed when an inadequate margin is found at the (sub)mucosal portion of the resection specimen, which is less challenging to retrace (43).

Local adjuvant radiotherapy can have severe side effects, having large effects on the patient's QoL. Examples of possible side effects are mucositis, i.e. inflammation of the mucosa, i.e. xerostomia, extreme dry mouth due to salivary gland damage and osteoradionecrosis, i.e. radiotherapy-induced necrosis of the mandible or maxilla (44,45). A combination with chemotherapy may also induce nephrotoxicity, peripheral neuropathy, (46) and sensorineural hearing loss (47). Moreover, adjuvant radiotherapy, e.g. 30-35 daily fractions of radiation, burdens the patient and the health care system.

The disadvantages of these local adjuvant treatments are the main reasons the patient profits from an immediate free margin.

Ultrasound

Sound is mechanical vibration propagating through a medium (a gas, liquid or solid). The mechanical vibration creates regions of compression (high pressure) and rarefaction (low pressure) in the surrounding medium. The frequency of these vibrations, expressed in Hertz (Hz), determines how sound is perceived in terms of pitch, with higher frequencies corresponding to higher-pitched sounds. Humans can hear sound within a range between 16 and 16.000 Hz (16 kHz). A frequency below 16 Hz is considered infrasound and a frequency above 16 kHz is considered ultrasound (48).

When a vibration travels through a medium, the vibration is passed through with a certain velocity, i.e. the speed of sound. This velocity decreases with increasing density or compressibility of the medium. Another acoustic property of a medium is its acoustic impedance Z , which is depended on the speed of sound in that medium.

If sound meets another medium, a fraction of its intensity is reflected, while the remainder is transmitted through the boundary (Figure 4). The reflection coefficient R of the reflected intensity I_r and the incident intensity I_i , when sound travels through medium 1 with impedance Z_1 and meets medium 2 with impedance Z_2 is calculated as follows:

$$R = \frac{I_r}{I_i} = \left(\frac{Z_2 - Z_1}{Z_2 + Z_1} \right)^2$$

Additionally, the transmission coefficient T is calculated as follows:

$$T = 1 - R$$

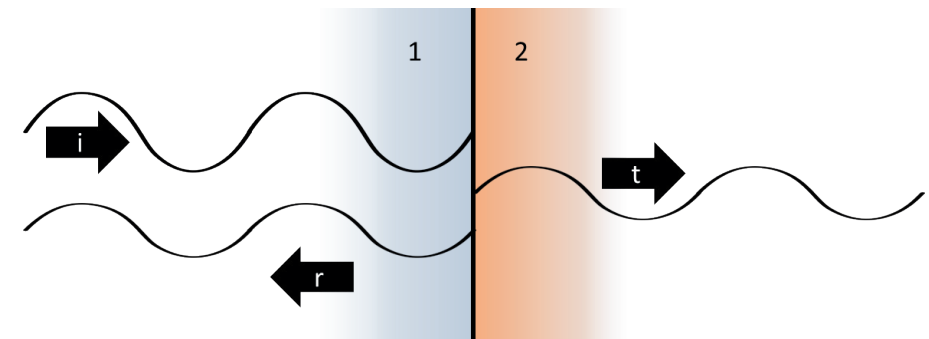


Figure 4: schematic depiction of the transmission and reflection of an incident sound wave when it encounters a transition between medium 1 and 2.

Ultrasonography or simply US, relies heavily on the differences of impedance between mediums. An US-probe contains piezoelectric material, which plays a dual role as both source and detector of ultrasound waves. This material has the ability to vibrate, thus making a sound, with the same frequency of an electric field with an oscillating voltage that runs through the material. Conversely, a vibration (thus an oscillating pressure) applied to a piezoelectric material creates an oscillating voltage.

A US image is based on a pulse-echo technique. Hereby, a US-pulse, which can range between 5,000,000 Hz (5 MHz) and 20 MHz, is applied to a tissue under investigation. At the interface of two tissues with different acoustic impedances, a reflected US-pulse travels back towards the transducer and is detected as a so-called echo. The time interval between emitting the pulse and receiving the echo indicates the boundary between tissue types; longer intervals correspond to greater distances.

An array of transducers can produce a B-mode image, while a single transducer produces an A-mode image. In a B-scan, the brightness of the screen

corresponds to the intensity of the reflection, plotted against the position of the body in a plane. This B-mode is interpreted as a cross-sectional image of a person's anatomy (49,50).

Advantages of US are that it is non-invasive, easy to use and relatively affordable. Additionally, US can acquire anatomical and functional information in real-time, making it a valuable tool in clinical practice. However, an important limitation is the attenuation of sound. While traveling through tissue, energy of the sound is absorbed and scattered, leading to an intensity that decreases exponentially per travelled distance. Attenuation is directly proportional to frequency. Higher frequencies (e.g. 15-20 MHz) provide high spatial resolution, but have a limited imaging depth, typically only several centimetres. In contrast, lower frequencies (e.g. 2-3 MHz) offer greater imaging depth, often exceeding 10 cm, but with lower spatial resolution. Consequently, US requires a trade-off between maximizing imaging depth and achieving optimal resolution (48,49).

Ultrasound for oral cancer surgery

US can be applied intraorally to assess tumour thickness of the oral SCC (26). This is done by placing the probe's transducer directly on the surface of the tumour. SCC is detectable as a hypoechoic lesion on US, meaning that a relatively low intensity of sound is reflected from this type of cancer (51). Thus, SCC appears darker than most other oral structures on US images. Intraoral US measurements of SCC thickness of the tongue has shown to have a good correlation with histopathology (52). It also seems to have a good correlation with depth of invasion, which is an important parameter in determining T-stage (51). This means that the tumour front can be visualized reliably by intraoral US, providing opportunities to use intraoral US to guide tumour resections.

Several small studies investigated US-guided resections of SCC of the tongue to obtain free margins. A challenging aspect is visualizing the cutting plane for which multiple methods have been investigated. For instance, sutures and needles were placed under the tumour, guided by US, until the intended distance from the tumour was reached (53–56). Tarabichi et al. (57) performed a more straightforward method by creating a small layer of air between the partially resected specimen and the wound bed. As the impedance difference of air is a factor 3800, most sound is reflected from the layer of air (48). Hence, the resection margin appears as bright hyperechoic line. Tarabichi et al. (57) achieved an adequate deep margin in 10 out of 12 patients treated by US-guided resections.

In this thesis, the impact of intraoral US-guided surgery on current clinical practice is further investigated.

Magnetic resonance imaging

Although US-guided surgery is the main scope of this thesis, MR imaging is another technique that could play a similar role evaluating resection margins in cancer treatment. Therefore, it is worthwhile to introduce this imaging technique.

MR imaging is based on the principle of nuclear magnetic resonance. When exposed to a static magnetic field, generated by the MR imaging machine (the 'B0 field'), nuclei with an odd number of protons and/or neutrons display a magnetic moment that precesses at a frequency depending on the strength of the B0 field. The precession of these magnetic moments can be perturbed by a second oscillating magnetic field, i.e. the B1 field, at the frequency of the precession, i.e. the resonance frequency. The rotating magnetic moment will induce a current that can be measured in a coil. MR imaging relies mostly on detecting these currents induced by hydrogen nuclei (^1H), which are abundant in the human body and highly sensitive to nuclear resonance (50).

Commonly, head and neck tumours can be distinguished from their environment in a T2 weighted MR image. T2 contrast is dependent on the transverse relaxation time: a time constant that describes the exponential decay of the magnetisation in the plane perpendicular to the B0 field. It is affected by the microenvironment of the water molecules. Muscle tissue has a shorter T2 relaxation time than oedema in or around a tumour. Therefore, muscle tissue commonly shows a lower signal intensity than tumours on a T2 weighted MR imaging.

The strength of the B0 field, expressed in Tesla (T) varies between different types of MR imaging machines. Clinical MR imaging scans typically have a field strength of 1.5 T or 3 T (for comparison, the earth's magnetic field strength is approximately $6 \cdot 10^{-5}$ T) (50). More experimental MR imaging machines possess a strength of 7 to 10 T or even more and are categorized as 'ultrahigh field MR'.

The higher the field strength of an MR machine, the more signal can be obtained from (hydrogen) nuclei. This effect can result in higher image quality, such as a higher signal to noise ratio that can be used to increase the spatial resolution. Consequently, ultrahigh field MR imaging may be particularly useful for examining SCC resection specimens to assess tumour-free margins. Since resection specimens require a smaller field of view, this can offset the typically longer image acquisition times associated with ultrahigh field MR imaging (58).

As safety precautions are needed because of the strong magnetic field, which is even present in low field MR machines (i.e. 0.1 T to 0.3 T), MR machines are generally unsuitable for use in operating room. This poses several logistical challenges for imaging during surgery and makes it necessary to adapt operating protocols (59). For imaging of the surgical specimens, it is not mandatory to perform the imaging in the operating theatre. One of the advantages of (ultrahigh field) MR imaging, is the possibility to generate a three-dimensional representation of the resection specimen, enabling the assessment of margins in multiple angles and perspectives.

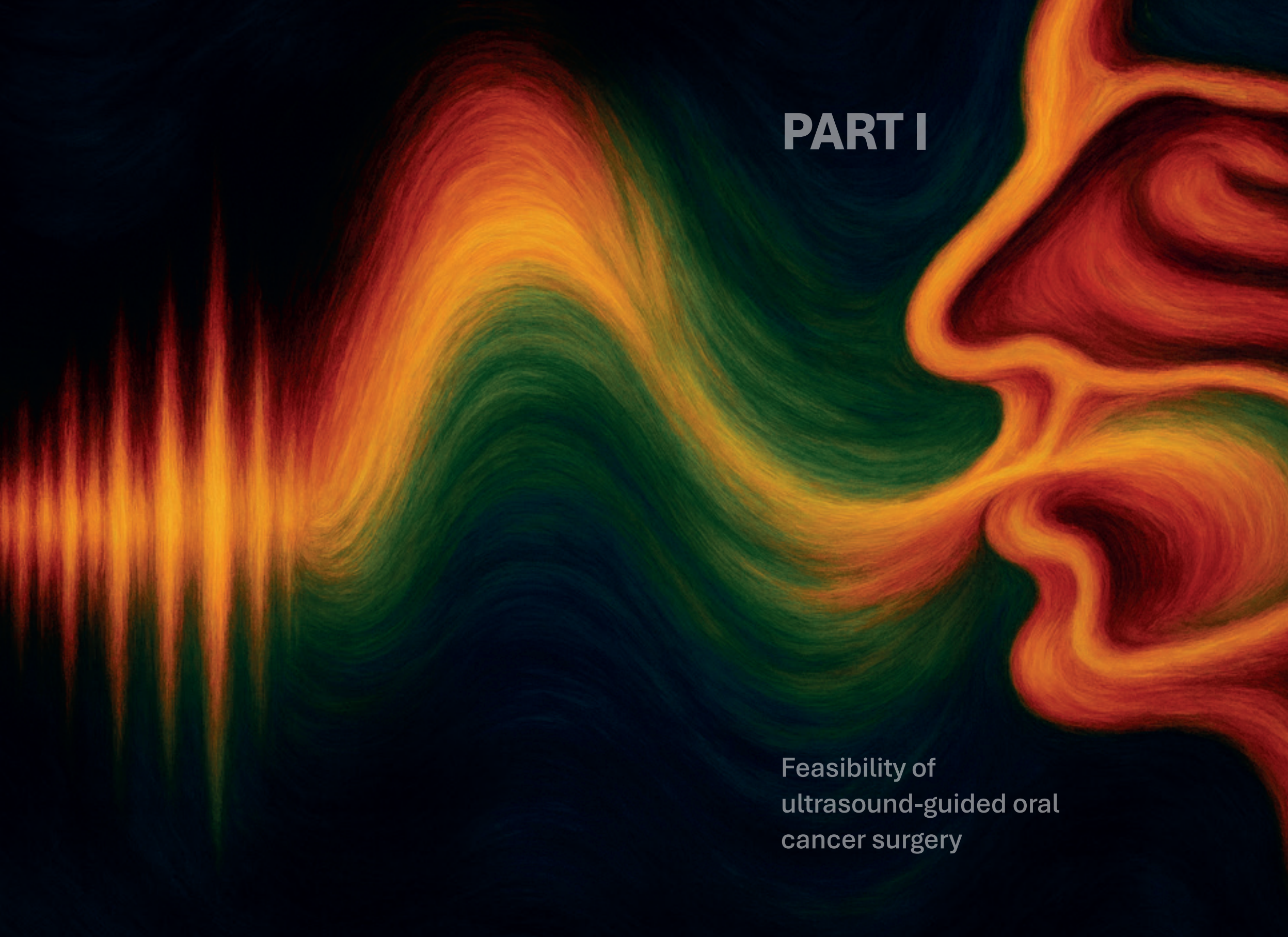
Aim and outline of this thesis

The aim of this thesis is to evaluate the use and impact of US-guided oral cancer surgery in clinical practice. The thesis is subdivided in three parts.

Part 1 introduces and tests the feasibility of an intraoperative surgical workflow for two oral cancer subsites most suitable for intraoral US: the tongue (chapter 2) and buccal mucosa (chapter 3). Both studies compare measurements made by US with histopathology in a small cohort of patients to assess the feasibility and accuracy of these techniques. Additionally, chapter 2 compares the US-guided cohort with a retrospectively analysed cohort of conventionally treated patients to investigate the impact of US guidance on histopathological margins and to ensure it does not lead to overtreatment due to underestimation of the tumour-free margins.

Part 2 continues with a detailed evaluation of the application, accuracy, and impact of US-guided surgery of SCC of the tongue. Chapter 4 provides a thorough assessment on accuracy of SCC of the tongue in a larger cohort and examines the effect of US-indicated intraoperative re-resections. The impact on margin status (i.e. the closest margin found the resection specimen) and the administration of adjuvant treatment is evaluated by comparing the US-guided cohort with a conventionally treated cohort. Chapter 5 compares local disease-free survival between the primary tumours of chapter 4, to evaluate how US-guided surgery affects local control. Chapter 6 describes the protocol of a Dutch multicentre randomized controlled trial involving eight centres. This study aims to evaluate the impact of US-guided surgery on margin status across different Dutch head and neck cancer centres, as well as its impact on adjuvant treatment, QoL, and oral function.

Part 3 explores alternative margin visualisation techniques for oral cancer, other than US. It discusses techniques that could be used in conjunction with or as supplementary to US to compensate for its limitations. Chapter 7 is a systematic review of other imaging techniques intended to obtain adequate deep resection margins, similar to US. Chapter 8 is a systematic review of other imaging techniques intended to define the mucosal margin. Chapter 9 explores the feasibility of an ultrahigh field MR-based three-dimensional digital specimen, which could potentially play a role in intraoperative ex-vivo assessment. We hypothesize that this digital specimen can map regions of inadequate margins on its surface, aiding the surgeon's orientation for optional intraoperative re-resections.



PART I

Feasibility of
ultrasound-guided oral
cancer surgery

Chapter 2

Feasibility study of ultrasound-guided resection of tongue cancer with immediate specimen examination to improve margin control – comparison with conventional treatment

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Abstract

Objectives: Squamous cell carcinoma of the tongue (SCCT) is preferably treated by surgery. Free resection margins (≥ 5 mm) provide local control and disease-free survival. However, close (1-5 mm) and positive margins (< 1 mm) are frequently encountered. We present our first experience of in-vivo ultrasound (US) guided SCCT resections followed by ex-vivo US control on the resection specimen to obtain free margins. We compare the results with those from a historical cohort of 91 conventionally treated SCCT patients.

Materials and Methods: Ten patients with SCCT were included in a consecutive US-cohort. We aimed for a 5-10 mm margin during surgery, while we visualized the resection plane on US. Ex-vivo US measurements on the resection specimen determined whether there was any need for an immediate re-resection. US measurements were then compared with histopathology. Histopathological margins were compared with a consecutive cohort of 91 patients who had undergone conventional surgery for a SCCT.

Results: In the US-cohort, 70% of the margins were free. In the conventional cohort, this figure was 17% ($p = 0.005$). US predicted minimal histopathological margin distance with a mean error of 1.9 (SD 1.8) mm. The mean of the histopathological overall submucosal/deep margin distance was 7.9 (SD 2.1) mm in the US-cohort and 7.0 (SD 2.2) mm in the conventional cohort ($p = 0.188$). Ex-vivo examination through use of US indicated an immediate re-resection, which prevented local adjuvant treatment.

Conclusion: Use of US-guided SCCT resection is feasible and improves margin control.

Introduction

Squamous cell carcinoma of the tongue (SCCT) is preferably treated by surgery. Free margin status, i.e. a minimal histopathological margin distance of ≥ 5 mm, is essential for local control and disease-free survival (2,43). A close (1-5 mm) or positive (< 1 mm) margin status is frequently encountered; analysis of SCCTs that have been surgically treated between 2004 and 2010 in our centre revealed that 64% of the patients had a close and 26% had a positive margin status. Submucosal and deep margins in particular are often inadequate (43). These results are in line with those that have been published in the literature, which report that oral cancer patients have up to 45% close-margin status and up to 43% positive-margin status after surgery (4).

Close or positive margin status frequently leads to a requirement for adjuvant therapy such as re-resection or (chemo)radiation (2,43). A previous study that was conducted in our centre revealed that adjuvant treatment at the primary tumour site was given to 35% of the patients with oral cancer. This could have been diminished by better surgical margin control (43). One major disadvantage of re-resection is that relocation of close or positive margins poses a challenge that could result in uncertainty about definitive margin status (42,60). (Chemo) radiation affects the patients' quality of life due to significant morbidity and (oral) discomfort due to, e.g., mucositis, fibrosis and possible osteoradionecrosis.

Conventional SCCT resections are guided by digital palpation and preoperative imaging. However, digital palpation does not provide accurate information about intraoperative margin distances. The only method that can provide supplementary intraoperative feedback on margin distances is analysis of frozen sections, but this method is not routinely available in every centre. Moreover, it offers low sensitivity in predictions of close/positive margin status due to its low sample rate (61,62). There is no reliable, standard method that reduces either the risk of close/positive margins or the risk of overtreatment, i.e. excessively large margin distances.

Intraoral ultrasound (US) is an accurate method that is used to predict histopathological tumour thickness (TT) in early SCCT (52,63). US-guided tumour resections are conducted in several surgical disciplines (64,65). In a large trial that involved 134 palpable breast-cancer patients, Krekel et al. (64) showed that US-guided breast-sparing surgery was superior to conventional, palpation-guided surgery when clinicians aimed for a free margin status. Several small projects have studied US-guided resections of SCCT (55–57). In the most recent study, Tarabichi et al. (57) used a US-guided surgery technique on 12 patients who had early SCCT. The researchers completed all procedures successfully without any complications that were related to the use of US. They aimed for a 10 mm margin distance; they achieved a deep histopathological margin distance of 9.7 (SD 1.2) mm. Their study confirmed the safety of this approach and suggested

that US-guided resection of SCCT could be used to acquire free margin status without excessive margin distances (57). Brouwer de Koning et al. (66) evaluated how ex-vivo US measurements on resection specimens could be used to predict the minimal margin distance on definitive histopathological results. They found a mean error of only 1.1 (SD 0.9) mm, which suggests that ex-vivo US measurements are reliable as well.

In line with these promising studies, we present our first experiences with US-guided SCCT resections. The presented method combines both intraoral in-vivo US measurements and immediate intraoperative ex-vivo US measurements of the resection specimen. With this method we aimed for a free margin status that did not incorporate overtreatment. In this study, the feasibility of this method was evaluated in a consecutive cohort of ten patients. US-measured TT and margin distances were compared with histopathological results. Histopathological margin distances were compared with those that were obtained in a retrospectively evaluated consecutive cohort of 91 patients who had been conventionally treated for SCCTs, to gain insight into undertreatment and overtreatment in conventionally resected SCCT.

Materials and methods

This study was performed in accordance with the 1964 Declaration of Helsinki and guidelines for good clinical practice. The local independent Medical Ethics Review Board of our institute approved the study protocol (trial ID: NL8336).

US-cohort

Patient inclusion

A consecutive cohort of ten patients who underwent treatment for SCCT between November 2019 and January 2020 was investigated (Tables 1 and 2). Patients were enrolled for the study during visits to our outpatient clinic. A patient was eligible for inclusion if: 1) a SCCT was diagnosed; 2) the tumour's mucosal surface was within reach of the US probe; 3) the tumour was detectable as a hypoechoic region on US; and 4) the surgical treatment was scheduled to be performed under general anaesthesia. A 16MHz hockey-stick shaped US probe (L16-4Hs, Mindray Bio-Medical Electronics, Shenzhen, China) was used for intraoral examination. This probe provides better accessibility in the oral cavity than a symmetrically shaped US probe (Figure 1). A technical physician (KJK) measured the TTs during these examinations.

Intraoperative technique

Under general anaesthesia, the TT was measured through use of the hockey-stick shaped US probe (Figure 1A-B). A mucosal margin distance of 10 mm was marked around the lesion. The surgeon then resected the tumour from the anterior by use of a monopolar diathermic surgical knife. When the resection plane reached under the anterior mucosal tumour border, US measurements were performed again. It was ensured that a thin layer of air was created between the specimen and the wound bed, by placing the specimen back in its original location (Figure 1C). This was visible as a hyperechoic border on US (Figure 1D). The closest distance from the tumour border to the resection plane was measured. The surgeon used this distance as feedback to aim at an echographic margin distance of between 5 mm and 10 mm. The same procedure was repeated when the resection plane reached the middle portion and posterior mucosal border of the tumour. The resected specimen was then marked with sutures for orientation. During the same session, a high-resolution, symmetrically shaped 20MHz US probe (L20-5s, Mindray) was used to measure ex-vivo the margins at five locations: anterior, posterior, craniomedial, caudolateral and central (Figures 1E-F and 2). If one or more of these margin distances was measured as less than 5 mm on US, an immediate re-resection was executed on the corresponding location of the tumour bed. A note was made of occasions when the tumour border was hard to distinguish ('unclear') and this problem provoked a discussion about its location during surgery. A technical physician (KJK) or a head and neck oncological surgeon (RJJE) performed the US measurements. An experienced radiologist (JWD) was consulted for image acquisition and understanding.

Conventional cohort

To analyse the conventional treatment of SCCT, we selected a consecutive cohort of 91 patients who had histological T1-3 SCCTs (*Tumour, nodes and metastases (TNM) Classification of Malignant Tumours*, 8th edition)(11) and who were conventionally treated between July 2014 and September 2018 in our centre. The exclusion criterion was the performance of excisional biopsies or surgery without curative intention. The results of frozen-section analysis were not analysed as a variable, since this analysis method was used in only 2% of the cases. Demographic and clinical data were extracted from the medical electronic database (Table 1).

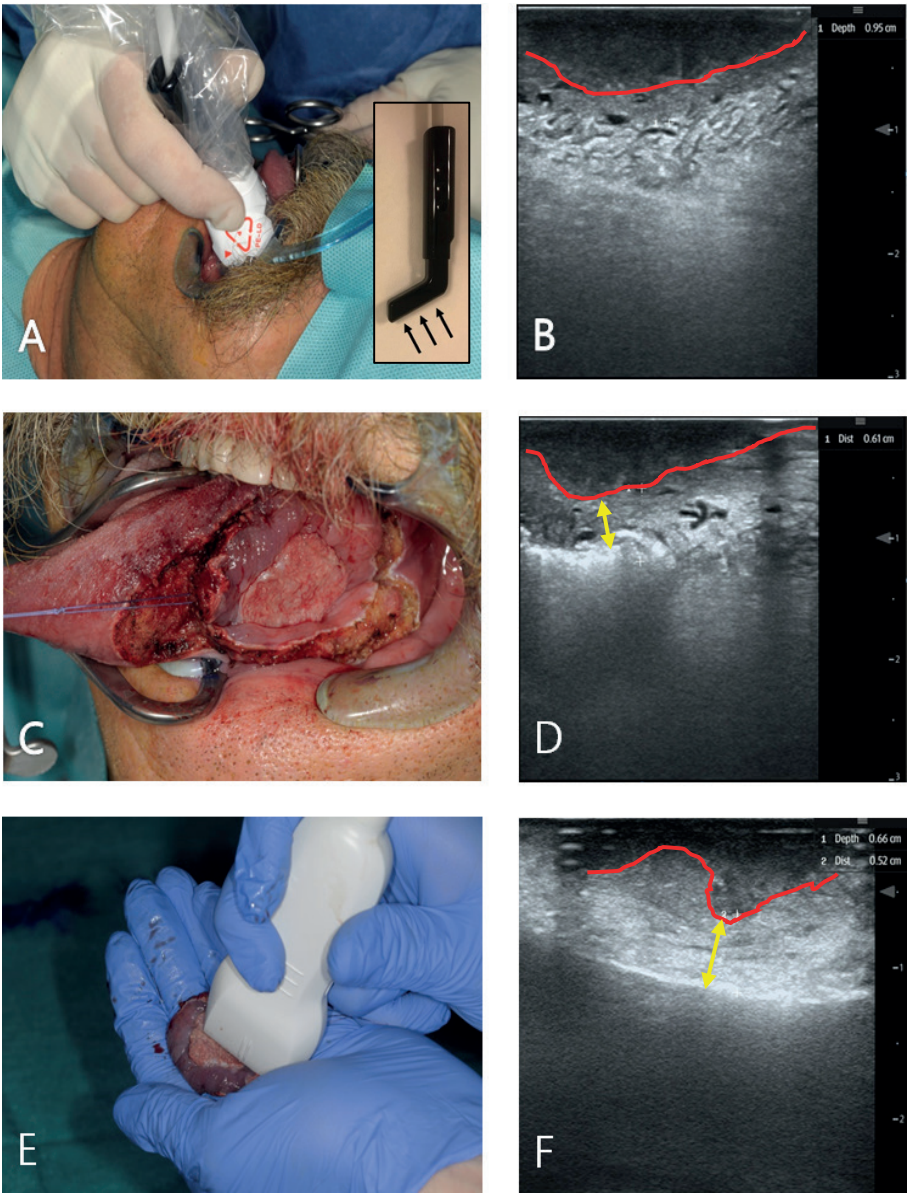


Figure 1: Photographs of the surgical workflow used in this study (patient 9). The tumour border is marked with a red line and the margin distance at the central location is depicted with yellow arrows. A-B: intraoral US for in-vivo determination of TT with the 16 MHz hockey-stick shaped probe (indent, black arrows point to transducer). C: resection of the lesion with a 10 mm mucosal margin. The resection plane has reached the middle of the tumour. D: the resection plane is visible as a white border during in-vivo examination. E-F: ex-vivo determination of TT and margin distances with the 20 MHz probe.

Table 1. Demographical data and tumour characteristics US and conventional cohorts

	US-cohort (n = 10)	conventional cohort (n = 91)	p-value
<i>Gender (n)</i>			
Male (%)	7 (70)	53 (58)	0.736
Female (%)	3 (30)	38 (42)	
<i>History (n)</i>			
Oral cancer (%)	1 (10)	7 (8)	0.579
<i>Age (years)</i>			
Mean (SD)	59.9 (12.2)	66.6 (12.7)	0.117
<i>Depth of invasion (mm)</i>			
Median (IQR)	6.2 (3.6-7.4)	6.1 (3.7-9.5)	0.446
<i>Pathological tumour stage (n)^b</i>			
pT1 (%)	3 (30)	33 (36)	0.527
pT2 (%)	9 (60)	37 (41)	
pT3 (%)	1 (10)	21 (23)	
<i>Growth pattern (n)</i>			
Non-cohesive (%)	7 (70)	44 (48)	0.318
Perineural (%)	4 (40)	25 (28)	0.467
Vaso-invasive (%)	2 (20)	5 (5)	0.142

Abbreviations: US: ultrasound

Histopathological examination

The resection specimens from both the US and conventional cohorts were paraffin-embedded and dyed for orientation. Specimens were cut into slices of ~3-5 mm. The mean thickness of the slices was determined retrospectively by dividing the reported length of the specimens by the reported number of slices. A 4 µm thin section of each slice was obtained, and each was stained with haematoxylin and eosin and digitalised. The TTs and tumour growth patterns (non-cohesive, perineural and vaso-invasive) were recorded. Margin distances at submucosal/deep level at the five specific locations (anterior, posterior, craniomedial, caudolateral and central, Figure 2) were determined by a dedicated senior pathologist in training (SAK) and a dedicated head and neck pathologist (SMW). Margin distances were determined by use of a digital ruler or were calculated by multiplying the number of tumour-free slices as determined through use of a microscope with the mean slice thickness.

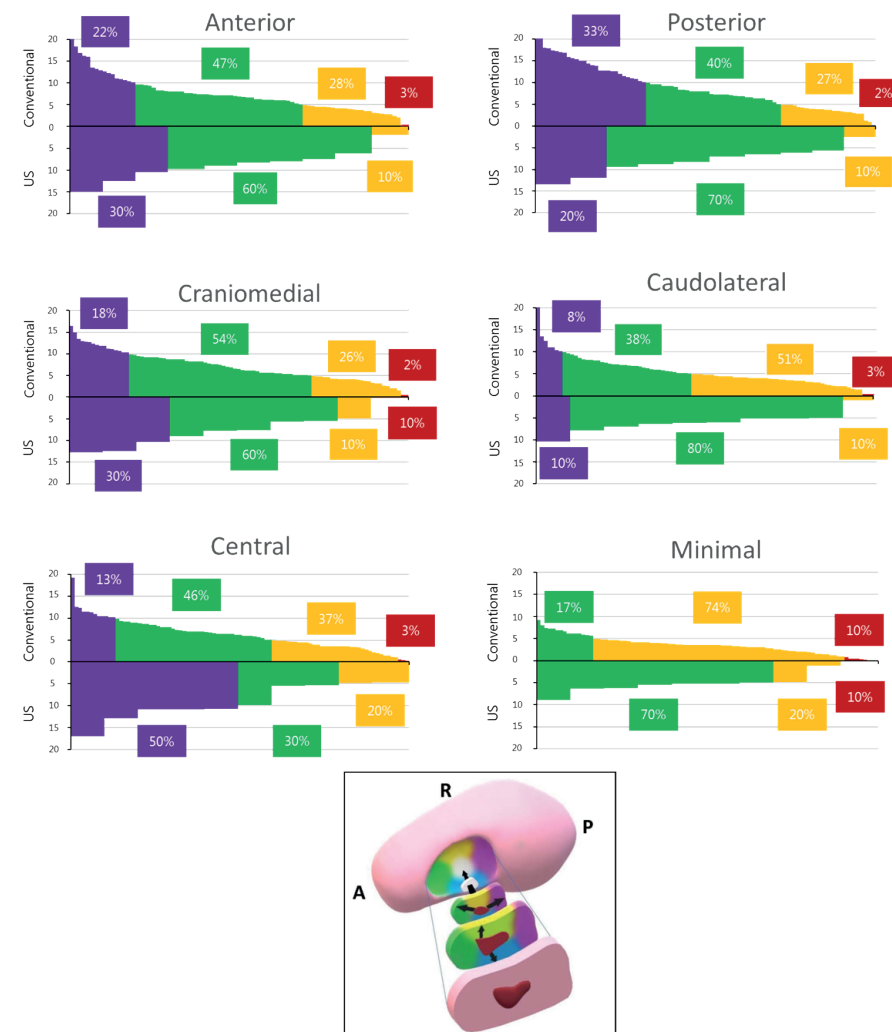


Figure 2: Depiction of margin distances in specific directions at submucosal/deep level, together with the minimal distances of both cohorts. Purple: overtreatment; green: free margins; orange: close margins; red: positive margins. Numbers in coloured boxes represent frequency. In the black box, the specific locations for margin assessment at submucosal/deep levels are visualised in a virtually sliced resection specimen. Note that mucosal levels are not evaluated. Green: anterior; purple: posterior; yellow: craniomedial; blue: caudolateral; white: central. The black arrows indicate examples of the measured margin distance in all directions. A: anterior; P: posterior; R: right side of tongue.

Analysis

In the US-cohort, we calculated the mean prediction errors in the histopathological results for TT (both for in-vivo and ex-vivo US) and for minimal margin distance (only for ex-vivo US). In both cohorts, the measured margin distances at submucosal/deep level were categorised as: ‘overtreatment’ (defined as a margin of > 10 mm), free (≥ 5 mm), close (1-5 mm) and positive (< 1 mm) margins. The frequency of occurrence of these categories was determined for each of the five submucosal/deep locations: anterior, posterior, craniomedial, caudolateral and central (Figure 2). In cases in which patients underwent re-resections that changed the margin distances at that location, the margins were re-defined for these analyses.

The minimal margin was taken to determine the definitive margin status in both cohorts. The mean minimal margin distances were determined in both cohorts and compared with each other. All location-specific margin distances (Figure 2) were averaged to determine the ‘overall margin distance’ of the resection specimen. For both cohorts, the mean location-specific margin distances and mean overall margin distances were compared with each other.

Fisher’s exact tests were performed to identify statistically significant differences in frequency of demographical data, clinical data and margin status between both cohorts. Independent sample t-tests were performed to identify statistically significant differences in mean values of demographical data, clinical data and histopathological margin distances. In cases in which data were not normally distributed (according to histograms, Q-Q plots and Shapiro-Wilk tests), Mann-Whitney U tests were performed instead, to identify differences between medians.

Calculations and tests were performed through use of IBM SPSS Statistics for Windows, Version 25.0 (Armonk, NY: IBM Corp., 2012).

Results

Demographical and clinical data

Table 1 shows the demographical and clinical data of both the US and conventional cohorts. No significant differences were found between groups regarding gender, age, history of oral cancer, T classification, depth of invasion or histopathological growth factors.

US-cohort

Table 2 shows patient-specific clinical data and compares the TTs that were measured echographically and margin distances with the histopathology of the US-cohort. One patient (no. 1) had experienced two previous primary SCCTs. Eight patients had received a sentinel node procedure (SNP), i.e. a peritumoural injection of a radioactive nanocolloid tracer. In one patient (no.

5), the nanocolloid had been injected on the same day as the surgery was performed (one-day protocol), while the seven other patients had received it one day prior to surgery (two-day protocol). All procedures were completed without any complications that were related to the use of US. The use of US in-vivo led to a predicted TT with a mean error of 1.9 (SD 1.4) mm, while its use ex-vivo led to a predicted TT with a mean error of 1.4 (SD 1.3) mm. The mean error of the minimal margin distance between ex-vivo US and histopathology was 1.9 (SD 1.8) mm.

Three patients (nos. 2, 3 and 8) initially were classified with close or positive margin status (Table 2). Patient no. 3 had an immediate intraoperative re-resection, because a 2.2 mm margin distance was found by use of US in the resection specimen. Since this re-resection induced a free margin of 5.3 mm according to histopathological examination (Table 2), local adjuvant treatment was prevented. Patient no. 8 received an immediate intraoperative re-resection at a location that showed a histopathological close distance, yet this appeared not to be found at the location of the minimal margin distance. It was determined that extensive microscopic non-cohesive growth had occurred. Small tumour nests (of diameter 0.5 mm) were found close to the resection plane (minimal margin distance 0.2 mm), and these nests caused this margin to be undetectable during ex-vivo US (Figure 3A).

Two patients, nos. 1 and 5, had tumour borders that were hard to distinguish on US during surgery (Table 2). These difficulties gave rise to discussion about the exact location of the borders. In patient no. 1, who had undergone two previous ipsilateral resections of SCCT, deeply included salivary tissue was mistaken for tumour on US because of a similar echodensity in both tissue types (Figure 3B). Patient no. 5 was the only one who underwent a one-day SNP protocol.

US vs. conventional cohort

In some cases that were part of the conventional cohort, location-specific margin distances were not measurable (in 9%, 5% and 2% of the anterior, posterior and caudal locations, respectively), due to lack of information in the histopathological reports and the absence of digital sections. The location-specific margin distances are depicted in Figure 2 for both cohorts. Considering the US-cohort, overtreatment was found most often at the central location (50%). Considering the conventional cohort, overtreatment was found most often at the posterior location (33%), while the most close and positive margins were found at the caudolateral location (41%).

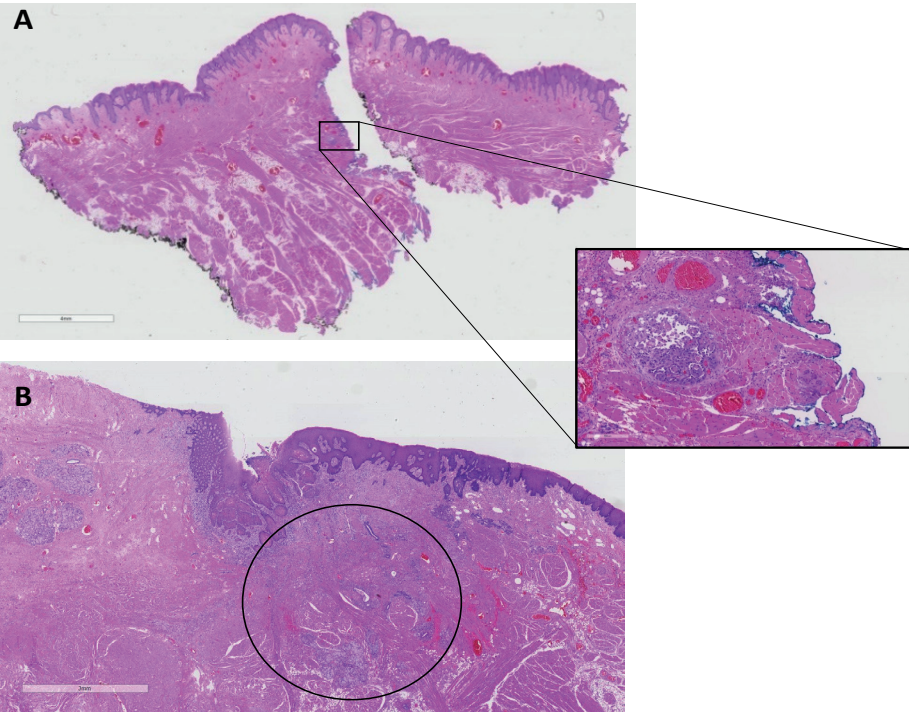


Figure 3: Microscopic views of HE stained histopathological sections of tumours taken from two patients. A: patient 8. In the black box, an infiltrative, microscopic tumour nest is detectable due to microscopic perineural growth. It caused a close margin from the resection plane (blue ink). The nest was not detectable on US. B: patient 1. Deep salivary gland tissue can be seen in the black circle. This caused a false-positive measurement during US examination. Its location is probably due to previous SCCT surgery.

Table 3 compares the histopathological margin results of both cohorts. Free margin status was significantly more frequent in the US-cohort (70%) than in the conventional cohort (17%); these figures are also depicted in Figure 2. There is a significant difference ($p = 0.007$) between the mean margins for the two cohorts at the central location: 9.2 (SD 4.1) mm in the US-cohort and 6.2 (SD 3.3) mm in the conventional cohort. The mean minimal margin distance was significantly different ($p = 0.046$) between the cohorts: 4.9 (SD 2.5) mm in the US-cohort and 3.5 (SD 2.0) mm in the conventional cohort. The mean overall margin distances were not significantly different.

#	Clinical data				Tumour thickness			Margin distance		Other									
	Head and neck cancer history	Pathological tumour stage	Location	SNP (protocol)	Measured		Error	Minimal	Error	Re-resection based on US	Growth pattern			US border					
					In-vivo US	Ex-vivo US	Histopathology	In-vivo US	Ex-vivo US		Histopathology	Non-cohesive	Perineural		Angio-invasive				
1	Yes	pT1	B	2-day	5.5	5.9	3.7	1.8	2.2	6.5	6.4	.1	No	-	Yes	No	No	U	Distinguishable
2	No	pT2	B	2-day	6.5	10.5	10.4	-3.9	0.1	7.2	1.1	6.1	No	-	Yes	No	Yes	C	
3	No	pT3	B	No	10.0	10.2	10.2	-0.2	0.0	3.9	2.2 ^a	1.7	Yes	Yes	No	No	No	C	
4	No	pT2	B	2-day	6.5	6.6	5.7	.8	0.9	6.8	5.2	1.6	No	-	Yes	Yes	Yes	C	
5	No	pT1	D	1-day	4.8	5.0	3.5	1.3	1.5	5.8	5.0	.8	No	-	Yes	No	No	U	
6	No	pT2	B	2-day	6.3	6.8	6.7	-0.4	0.1	7.1	9.0	-1.9	No	-	No	No	No	C	
7	No	pT2	B	2-day	5.1	5.1	7.0	-1.9	-1.9	5.2	6.2	-1.0	No	-	Yes	No	Yes	C	
8	No	pT2	B	2-day	3.4	3.2	6.7	-3.3	-3.5	3.8	.2	3.6	Yes	Yes	Yes	Yes	Yes	C	
9	No	pT2	B	No	9.5	6.7	5.7	3.8	1.0	5.2	5.5	-.3	No	-	Yes	No	No	C	
10	No	pT1	B	2-day	4.5	6.4	3.2	1.3	3.2	7.3	4.9	2.4	No	-	No	No	No	C	

Abbreviations: B: border of the tongue, D: dorsal surface of the tongue, C: clear, U: unclear

^a This patient received an intraoperative resection margin based on US, changing minimal margin distance from 2.2 to 5.3 mm. This distance is taken into account in all other analyses.

	US-cohort (n = 10)	Conventional cohort (n = 91)	P-value
Margin status (n)			
Free (%)	7 (70)	15 (17)	0.005^a
Close (%)	2 (20)	67 (74)	
Positive (%)	1 (10)	9 (10)	
Margin distances (mm) – mean (SD)			
Submucosal/deep			
- Anterior	9.3 (3.7)	7.4 (4.4)	0.200
- Posterior	7.6 (3.4)	8.9 (5.6)	0.462
- Craniomedial	7.6 (3.8)	7.1 (3.3)	0.616
- Caudolateral	6.1 (2.4)	5.5 (3.2)	0.613
- Central	9.2 (4.1)	6.2 (3.3)	0.007^b
Minimal	4.9 (2.5)	3.5 (2.0)	0.046^b
Overall	7.9 (2.1)	7.0 (2.2)	0.188

^a Statistical significance was determined with Fisher's exact test.

^b Statistical significance was determined with independent t-test.

Discussion

This feasibility study has evaluated the applicability of US-guided resection of SCCT combined with direct ex-vivo control of the resection specimen. Histopathological results were compared with those that were obtained through conventional treatment of SCCT. To our knowledge, this is the first study that has evaluated margins at five different submucosal/deep locations. As such, it has demonstrated both the locations at which it is difficult to achieve adequate resection margins and the locations where improvement can be made in terms of preventing overtreatment, i.e. margins of > 10 mm.

This study has exposed several advantages of the used methodology. First, because of its shape, the 16 MHz hockey-stick shaped probe proved to be useful to visualise intraoral in-vivo tumour borders (Figure 1A-B). It did not cause disproportionate discomfort to the patient during inclusion tests that were performed in our outpatient clinic. Second, the 20 MHz probe provided images that showed a higher resolution during ex-vivo US measurements. This improved resolution contributed to the achievement of a precise re-resection in the same session for one patient (Table 2). Third, the application of US did not extend surgical time more than the use of frozen sections would have done. Moreover, sample rate was higher, and costs were lower than if frozen sections had been used

(61,62). Lastly, the resection plane was clearly visible in all cases because an air layer was created between the resection specimen and the wound bed. Other methods have been applied that aim for an echographic free margin; these involve the placement of sutures (53), needles (55,56) or retractors (54) under the echographic tumour border. However, we prefer this more straightforward method, which shows less chance of tumour seeding as it images the resection plane itself, similar to the technique of Tarabichi et al. (57).

US-guided resection seems to increase the minimal resection margin and thereby improve free margin status (70%) when compared with conventional surgery (17%). This is in line with a comparable study by Beak et al. (56). These researchers increased the deep, free margin status from 55% in a conventional cohort to 95% of patients in the US-cohort. In our study, at all specific locations the frequency of free resection margin status was improved (see Figure 2). Most improvement was seen at the caudolateral location. This might be because the tongue must be torqued to display this location. The torque stretches the margin, which may generate a risk of overestimation when no US is applied. Thus, US guidance seems to provide better margin control than conventional treatment.

US guidance seems not to lead to resection of excessive amounts of healthy tissue, given the mean overall margin distance of 7.9mm (Table 3). Aiming for a 10 mm echographic margin, however, will lead to overtreatment at some specific locations. This can be seen in the mean margin distances at the central location, as shown in Figure 2 and Table 3. These findings are in line with those published in the literature; Tarabichi et al. (57) also had deep resection margins of more than 10 mm in 42% (5/12) of the patients studied. However, US guidance can also prevent excessively large resections of healthy tissue, as can be seen at the posterior location shown in Figure 2; the posterior course of the resection can be determined more precisely than without US guidance, since the anterior and deep resection planes are already visualised. US-guided resection has the potential to lead to resections of just the right amount of tissue, instead of insufficient or excessive resection margins. In breast cancer, Krekel et al. showed that US-guided resections generated significantly more free margins, but with a smaller volume of the resection specimen when compared with conventional surgery (64).

Several notable results were found when US measurements were compared with the histopathology results. The smaller mean error that was found in the prediction of histopathological TT with use of ex-vivo US (1.4 mm) suggested that the ex-vivo US method was more reliable than in-vivo US (1.9 mm). This might be in line with the results of Umstattd et al. (67), who found that an overall mucosal tumour shrinkage of 19.6% occurred immediately after the resection (from in-vivo to ex-vivo), but that there was no shrinkage after formalin fixation (from ex-vivo to histopathology). The fact that we found a

higher mean error in the prediction of minimal margin distance (1.9 mm) than Brouwer de Koning et al. (1.1 mm) (66) might be due to pressure on the resection specimen. Although Brouwer de Koning et al. (66) used a non-contact technique with US gel, we prefer a contact technique that offers more control over the resection specimen. A future study to assess reproducibility and reliability of different US-measurement techniques, e.g. hand-held, gel-based or water-based, may clarify the optimal method.

Although these results are promising, several limitations of US-guided resection of SCCT should be addressed. First, we encountered several false-positive measurements (i.e. overestimation of TT during use of US) in patient nos. 1, 5 and 10 (Table 2). In patient no. 1, this was due to previously relocated deep salivary gland tissue that was mistaken for tumour. Despite this patient having undergone two previous ipsilateral SCCT resections, we decided to include the results for this patient, since our conventional cohort also contained patients with a history of oral cancer. In patient no. 5, a peritumoural tracer injection that was performed during the one-day SNP may have made the tumour less distinct on US (Figure 3B). Patient no. 10 had a dense muscular layer directly under the tumour front that changed abruptly into fatty tissue, which was perhaps mistaken for tumour front. However, similar cases should be investigated to confirm this.

Second, we encountered several false-negative measurements (i.e. underestimation of TT during use of US) in patient nos. 7 and 8 (Table 2). This might be explained by the presence of microscopic, non-cohesive and perineural growth towards the central location, the small size of which lay outside the limits of the echographic spatial resolution (Figure 3A). Therefore, aiming for a 10 mm echographic margin rather than 5-10 mm might be more appropriate in such cases to control non-cohesive infiltrative growth in the resection margin.

Third, harmonisation of the in-vivo orientation with the ex-vivo orientation of the resection specimen should be improved. Although immediate ex-vivo US examination seems more efficient than frozen section analysis (61), it does not solve the problem of relocating close or positive margins on the tumour bed. This is difficult and not always accurate (42,61). Therefore, it is preferable to aim for a resection with a direct free margin status rather than even immediate re-resections (42).

Fourth, when US is used, experience and skills are required to differentiate between TT and the depth of invasion (an important determinant in tumour-staging (11), in case the tumour has an exophytic component. This is because the exophytic part of the tumour can only be visualised if there is a minimal amount of pressure from the probe. However, Klein Nulent et al. found that TT measured by US was a good predictor of depth of invasion (52).

Regarding these limitations, we advise that US should be used for guidance in the resection of SCCTs and that clinicians should be aware of its re-

strictions and pitfalls. Moreover, we advise that an experienced radiologist should be consulted for image acquisition and image interpretation during the first sessions.

In conclusion, this study has demonstrated that performance of resections of SCCT under US guidance, combined with immediate intraoperative ex-vivo measurements as a final check, is feasible. It was found that, when compared with a cohort of conventionally treated SCCT, it improved resection margin status without resection of excessive amounts of healthy tissue. Future studies should validate these findings in larger cohorts and investigate whether the use of US guidance during resections of SCCT indeed improves resection margin status, reduces the requirement for adjuvant treatments and ultimately improves local tumour control, thereby improving the quality of life of patients.

Chapter 3

Ultrasound-guided resection for squamous cell carcinoma of the buccal mucosa: a feasibility study

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Abstract

Background: Image-guided surgery could help obtain clear (≥ 5.0 mm) resection margins. This feasibility study investigated ultrasound-guided resection accuracy of buccal mucosa squamous cell carcinoma (BMSCC).

Methods: MRI and ultrasound measurements of tumour thickness were compared to histology in 13 BMSCC-patients. Ultrasound measured margins (at five locations) on the specimen were compared to the corresponding histological margins.

Results: Accuracy of in- and ex-vivo ultrasound (mean deviation from histology: 1.6 mm) for measuring tumour thickness was comparable to MRI (mean deviation from histology: 2.6 mm). The sensitivity to detect < 5 mm margins using ex-vivo ultrasound was low (48%). If an ex-vivo ultrasound cutoff of ≥ 7.5 mm would be used, the sensitivity would increase to 86%.

Conclusions: Ultrasound-guided resection of BMSCCs is feasible. In- and ex-vivo ultrasound measure tumour thickness in BMSCC accurately. We recommend ≥ 7.5 mm resection margins on ex-vivo ultrasound to obtain histological clear margins. Additional research is required to establish the effect of a 7.5 mm ultrasound cutoff.

Introduction

In the western world, approximately 10% of all oral cancers are squamous cell carcinomas of the buccal mucosa (BMSCC) (68,69). The treatment of choice is surgery, and tumour-free margins are the most important prognostic factor for recurrence-free survival (70,71). Various imaging modalities can be used to evaluate the extension of the tumour preoperatively. In BMSCC, magnetic resonance imaging (MRI) is preferred over computed tomography (CT) for determination of tumour thickness (TT) because of its better soft-tissue contrast (72–74). However, MRI can overestimate the tumour boundaries due to the visualisation of inflammation or peritumoural oedema (73). During surgery, digital palpation predominantly guides the BMSCC resections (70).

Obtaining clear resection margins in BMSCC is challenging. Multiple studies have shown that the buccal mucosa subsite is a risk factor for positive or close tumour-free margins compared to other oral subsites (70,71,75). Currently, 7–40% of the margins in BMSCC resections appear involved (76–79). Examples of factors associated with involved or close tumour-free margins in oral squamous cell carcinomas (SCC) are higher T-classification, increasing TT, perineural growth, and vascular invasion (80,81). When in doubt, a surgeon may use frozen sections for intraoperative feedback. Unfortunately, this method has low sensitivity in predicting close or positive margins due to sampling and interpretation errors (61,62,82).

An intraoperative imaging modality is needed to assess margin status and adjust the resection plane if necessary to obtain a higher percentage of clear margins (≥ 5 mm). A study by de Koning et al. showed a significant improvement of adequate resection margins in tongue cancer when using intraoperative ultrasound (US) (83). In this study, we investigate the feasibility of this technique in BMSCC and evaluate the accuracy of intraoperative US for the measurement of tumour-free margins in BMSCC resection. Furthermore, the accuracy of the US for the measurement of TT will be assessed by comparing the US measurements with the preoperative MRI and the histology of the resection specimen.

Methods

Patients

This study was performed in accordance with the 1964 Declaration of Helsinki and guidelines for good clinical practice. Our institute's local independent Medical Ethics Review Board approved the study protocol (trial ID: NL8336). All patients with a histologically proven BMSCC, primarily treated with surgery under general anaesthesia with curative intent between 04-2021 and 01-2022, were included in this retrospective study. Patients were excluded if: there was no re-

cent (< 1 month) pre-operative MRI available, or the tumour was out of reach of the intraoral US (ioUS) probe. The variables sex, age, tumour location, and pT/pN classification (as per TNM, 8th ed. (17)) were obtained from electronic medical records. Adjuvant treatment was reported to see the influence of intraoperative US on the need for adjuvant treatment.

Magnetic resonance imaging

Patients received a preoperative MRI-scan of the head and neck with a field strength of either 1.5 T or 3 T as standard care. One dedicated head and neck radiologist (JWD) analysed the MRI-scans. The MRI protocol included: axial proton density weighted with fat suppression (4 mm slice thickness at 4 mm distance), coronal T2 with and without fat suppression (3 mm slice thickness at 3.3 mm distance). An axial (4 mm slice thickness at 4 mm distance), and coronal (3 mm slice thickness at 3.3 mm distance) T1 with gadolinium with and without fat suppression (3 mm slice thickness at 3.3 mm distance). An axial diffusion weighted imaging / apparent diffusion coefficient (4 mm slice thickness at 5 mm distance). Three separate measurements of the maximal tumour thickness (TT) were acquired on either axial or coronal images depending on the orientation of the tumour on: proton density weighted T2 with fat suppression, T1 with gadolinium with fat suppression, and apparent diffusion coefficient. The mean measurement was calculated and used in the statistical calculations (Figure 1A).

Surgery and ultrasound measurements

The border of the tumour was identified by visual inspection (Figure 1B), and the mucosal resection margin was drawn at 10 mm, aiming to obtain histological tumour-free margins of ≥ 5.0 mm. Before starting the tumour excision, a 16 MHz hockey-stick shaped ioUS probe (L16-4Hs, Mindray Bio-Medical Electronics, Shenzhen, China) was placed intraorally, perpendicular (90°) on the tumour. The submucosal and deep extension of the tumour was visualized by moving the ioUS probe over the tumour. The tumour was visible as a hypoechoic (darker grey) mass, and the TT was measured where this hypoechoic mass was thickest (Figure 1C). Hereafter, the surgeon started the tumour-resection at the anterior border. During the resection, the tumour-free margin was measured when the surgical resection plane was located beneath the tumour's anterior, deep, and posterior border (Figure 1D). To measure this, the ioUS probe was moved over the tumour and the tumour-free margin was identified by measuring the minimal tumour-free margin distance between the hypoechoic mass, and a hyperechoic line created by a layer of air between the wound bed and the partly resected specimen (Figure 1E). The US images were interpreted and measured by one of two researchers (CA or KK). If the tumour-free margin was < 5.0 mm on ioUS, the resection plane could be immediately adjusted. After excision of the tumour, and still in the operation room, the TT and tumour-free margins on five

locations, anterior, deep, posterior, cranial, and caudal, (Figure 2) were measured on the resected specimen by ex-vivo US, in a plastic cup filled with 0.9% NaCl to minimize compression or in a gloved hand (Figure 1F). These measurements were performed by placing a 20 MHz symmetrically shaped US probe (L20-5s, Mindray) perpendicular on the anterior, posterior, cranial, and caudal transition from healthy mucosa to tumour, and central on the tumour for the deep tumour-free margin, as described by de Koning, et al. (83). The minimal tumour-free margin distance for every location was noted. In total, 10 US measurements were performed per tumour: one pre-excision intraoral for the TT, three intraoral during the resection for tumour-free margins, and six ex-vivo: one for TT and five for tumour-free margins. In the current study, the patient met the criteria for re-resection in case an echographic tumour-free margin of < 5.0 mm was found on ex-vivo US. The surgeon was informed and decided if a wider resection or immediate re-resection was possible and desired. A stitch marked the closest tumour-free margin measured on ex-vivo US at the cutting edge of the specimen (margin-mark) to evaluate the accuracy of US to find the closest tumour-free margin. Since re-resections were at suspected close tumour-free margins, and based on US measurements, they were also counted as margin-marks.

A hard indication for local adjuvant radiotherapy was an involved (< 1.0 mm) margin. Soft criteria for local adjuvant therapy were a close (1.0-4.9 mm) tumour-free margin, and adverse growth patterns of the tumour (i.e. perineural growth, vascular invasion, or non-cohesive growth) (84). The advice for adjuvant treatment was given by multidisciplinary consultation, and the decision was individualized for every patient.

Histology

All specimens were formalin fixed, paraffin embedded, and analysed by a dedicated head and neck pathologist (GB). The specimen's resection plane, the site of the margin-mark, and the location of the re-resection were stained in different colours for orientation. The specimens were cut into 3 to 5 mm slices, and a 4 µm thick section of each slice was stained with haematoxylin and eosin and analysed (Figure 1G). The TT, and adverse growth patterns of the tumour front (i.e., non-cohesive growth, perineural growth, or vascular invasion) were reported. We chose to measure TT because differentiation between the exophytic and infiltrating portion of the tumour is not reliable on US; hence the depth of invasion cannot be measured adequately. The TT was defined as the distance from the top to the bottom of the tumour, measured on the histological slice with the most extensive depth of invasion. The minimal tumour-free margin distance was measured for all five locations (i.e., cranial, caudal, anterior, posterior, and deep, Figure 2) from the tumour to the resection plane. The five minimal tumour-free margins on ex-vivo US were compared to the corresponding minimal tumour-free margins measured during histological evaluation. Margins at the cranial, caudal and deep parts of the specimen were measured digitally. The margins

at the anterior and posterior parts were measured digitally at the first or last slice containing tumour, respectively (Figure 2). In case of a re-resection, the macroscopic thickness of the re-resection was added to the tumour-free margin to calculate the definite tumour-free margin. Histology was considered the reference in this study.

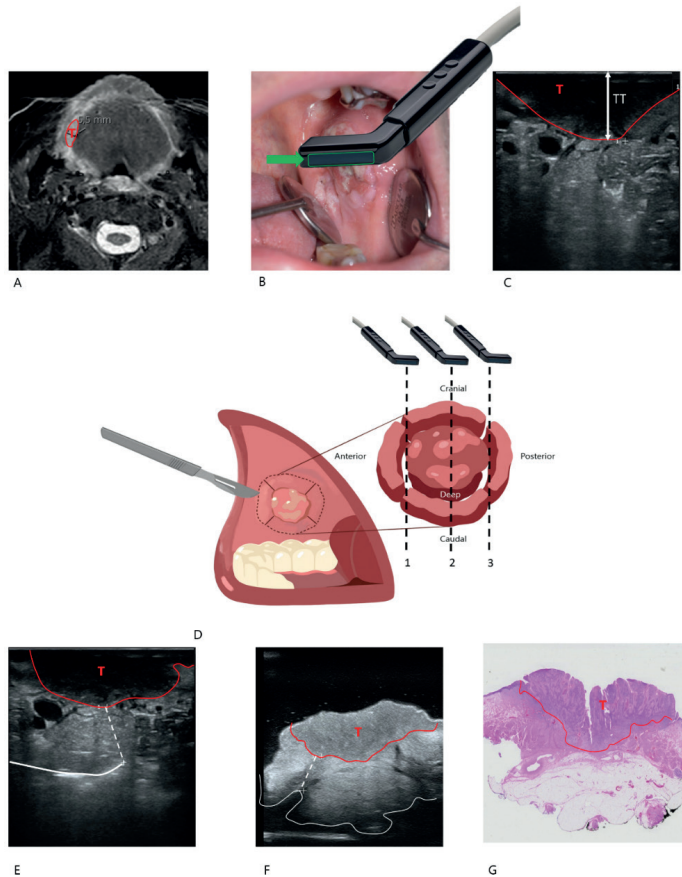


Figure 1: A: Image of BMSCC on axial MRI PD STIR. The red circle indicates the tumour (T). B: The transducer area of the ioUS probe, indicated by the green arrow and rectangle, was placed perpendicular on the BMSCC. C: The tumour thickness (TT, white arrow) was measured before the start of the resection on the place where the tumour was thickest. Red line is tumour border. D: Once the resection plane reached the anterior border (1), central beneath the tumour (2), and the posterior border (3) of the tumour, the tumour-free margin was measured. E: The resection plane was visible as a hyperechoic line (white line), due to the creation of a layer of air between the wound bed and the specimen. The tumour-free margin (white dotted line) was measured. F: The tumour-free margins were measured ex-vivo in saline, to prevent pressure on the specimen. The closest tumour-free margin was measured on 5 locations (anterior, deep, posterior, cranial, and caudal, Figure 2). G: The corresponding histological section of the BMSCC. Abbreviations: T: tumour, TT: tumour thickness, BMSCC: buccal mucosa squamous cell carcinoma, and MRI PD STIR: magnetic resonance imaging proton density short TI inversion recovery.

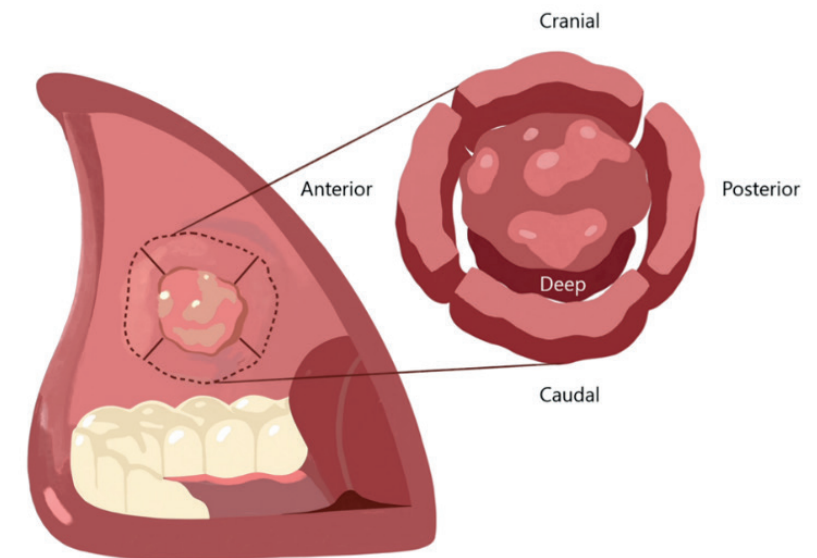


Figure 2: The location of the surgical tumour-free margins, shown in the mucosa of the right cheek.

Statistics

A repeated measures ANOVA was used to calculate significant differences between the TT on the three different MRI sequences and the histological TT, because the Mauchly's test (chi-square (5) = 7.582, $p = 0.18$) did not indicate any violation of sphericity. For the difference in TT between the different imaging techniques and histology, the variables were tested for normality. The Friedman's two-way analysis of variance by ranks was used since not all the variables were normally distributed. The Pearson correlation (2-tailed) was used to calculate the correlation between the TT and histology. The correlation was considered as follows: < 0.30: 'poor', 0.30-0.59: 'fair', 0.60-0.79: 'moderate', 0.80-0.99: 'very strong', and 1.0: 'perfect' (85). A scatter plot analysed the data of the five tumour-free margin measurements. The area under the curve (AUC) for receiver operating characteristic (ROC) curves was calculated for ioUS and ex-vivo US. The AUC was considered as follows: 0.50-0.69: 'poor', 0.70-0.79: 'fair', 0.80-0.89: 'good', and ≥ 0.90 : 'excellent' (86). The sensitivity of the ultrasound at a 5 mm tumour-free margin was calculated. The difference between the distance from the tumour front to the margin-mark or to the resection plane measured with ioUS or ex-vivo US, and histology was calculated with the related-samples Wilcoxon signed rank test. Results were considered significant if $p < 0.05$.

Results

The tumour was out of reach of the US probe in one patient. Thirteen patients were included in this feasibility study. None of the patients had a history of head and neck cancer. The mean time between the MRI and the surgical resection was 16 (SD 6.5) days. The mean age of the patients at the time of surgery was 73 (SD 11) years, and 61.5% were female (Table 1). Out of 13 tumours, 10 (76.9%) had a non-cohesive growth pattern, two (15.4%) showed perineural growth, and none showed vascular invasion.

Tumour thickness

There were no significant differences between TT on the three different MRI sequences and the histology ($F(3;33) = 1.031$, $p = 0.39$). In addition, there were no significant differences between the average TT of the three MRI sequences, ioUS measured TT, ex-vivo US measured TT, and histological TT, chi-square (3) = 4.477, ($p = 0.21$). The correlation between the TT on MRI and histology was ‘very strong’: 0.89 ($p < 0.001$). The correlation between the TT measured with ioUS and ex-vivo US was ‘very strong’: 0.95, and also the correlation between TT measured with ioUS or ex-vivo US, and on histology was ‘very strong’: 0.91 ($p < 0.001$), and 0.93 ($p < 0.001$), respectively. The mean deviation of the 13 TT measurements from histology was 2.6 (SD 1.1) mm for MRI, 1.7 (SD 1.2) mm for ioUS, and 1.6 (SD 1.0) mm for ex-vivo US, which did not differ significantly from each other (chi-square (2) = 3.231, $p = 0.20$).

Tumour-free margins

On histology, the closest tumour-free margin of the 13 patients was ‘involved’ (< 1 mm) in three (23%) patients and 3 of the 65 (5%) measurements, ‘close’ (1.0–4.9 mm) in nine (69%) patients and 23 of 65 (35%) measurements, and ‘clear’ (≥ 5 mm) in one (8%) patient and 39 of 65 (60%) measurements. The histological mean tumour-free margin of the 65 measurements was 4.7 (SD 2.2, median 5.1) mm. The assessment of resection margins by US was feasible in all 13 patients.

Intraoperatively, there were 30 tumour-free margin measurements by ioUS (anterior, deep, and posterior) because nine measurements were missing. The tumour-free margin was < 5.0 mm in 17 (57%) measurements. The AUC for the measurements of the tumour-free margins was 0.6 (95% CI: 0.40–0.81, $p = 0.32$), which is poor. There was no significant difference between the tumour-free margins measured with ioUS and on histology ($p = 0.21$).

Table 1. Patient- and tumour characteristics, TT measured with MRI (the average of the three sequences), US, histology, and number of re-resections

Patient	Sex	Age (years)	pT	TT MRI (mm)	TT ioUS (mm)	TT ex-vivo US (mm)	TT histology (mm)	Number of re-resections
1	F	76	1	7.9	2.4	4.1	3.3	2
2	M	54	1	5.1	3.8	4.6	1.9	0
3	F	65	3	12.4	14.4	16.1	16	0
4	F	77	4a	16.6	20.7	19.1	19.7	2
5	F	75	2	8.8	6.5	8.5	6.3	0
6	M	79	3	9.6	6.9	7.6	5.9	1
7	F	94	2	5.9	6.4	6.5	5.7	1
8	M	72	2	9.8	8.3	8	7.3	1
9	F	81	1	7.6	6.6	6.4	4.7	0
10	F	59	2	7.8	7.5	7.8	9.8	0
11	F	72	3	9.3	7.8	10.7	11.9	2
12	M	59	4a	12.9	9.9	7.7	11.3	2
13	M	82	3	9.7	12.1	10.8	8.1	0

Abbreviations: pT: pathological tumour-classification, TT: tumour thickness, MRI: magnetic resonance imaging, (io)US: (intraoral) ultrasound, M: male, F: female

The ex-vivo US measured the tumour-free margins in the five different locations (Figure 2). Three ex-vivo US measurements were missing. Therefore, 62 tumour-free margin distances were plotted against histology (Figure 3A). The tumour-free margin was overestimated in 45/62 cases (73%; mean overestimation: 3.5 (SD 3.0) mm). The mean deviation between the ex-vivo US measurement and histology was 3.1 (SD 2.8) mm. Of the 29 < 5.0 mm tumour-free margins on histology, only 14 (48%) were correctly identified as < 5.0 mm by the ex-vivo US. Three of four tumour-free margins measured as > 10.0 mm by ex-vivo US but < 5.0 mm during histological evaluation were from one patient with extremely non-cohesive growth, where the tumour nest was located 5.0 mm from the tumour bulk. Ex-vivo US indicated a re-resection in 20 of the 62 tumour-free margins (32%), which also was an inadequate margin on histology in 14 (70%) tumour-free margins. The surgeon refused a re-resection in 11 of these 20 on US inadequate tumour-free margins (55%); see Table 2. Although, in six of these 11 (55%) tumour-free margins, there was a true indication on histology; one tumour-free margin was < 1.0 mm, four were 1.0-2.9 mm, and one was 3.0-4.9 mm. In combination with the re-resections, this resulted in correct treatment in 58%, overtreatment in 3%, and undertreatment in 39% of the tumour-free margins.

The tumour-free margins measured on ex-vivo US were plotted in a ROC curve (Figure 4). The area under the curve was 0.70 (95% CI: 0.56-0.84, $p = 0.007$) for < 5.0 mm histological tumour-free margin, which was fair. The sensitivity of the ex-vivo US at 5.0 mm tumour-free margin was 48%, which means that in 48% of < 5.0 mm histological margins, the ultrasound measured < 5.0 mm.

Table 2. The re-resection indications by ultrasound and the execution by surgeon

	No re-resection by surgeon	Re-resection by surgeon	Total
US correctly identified ≥ 5.0 mm tumour-free margin	23	4	42
US incorrectly identified ≥ 5.0 mm tumour-free margin	13	2	
US correctly identified < 5.0 mm tumour-free margin	6	8	20
US incorrectly identified < 5.0 mm tumour-free margin	5	1	
Total	47	15	62

Note: Crosstab for the indication for re-resection by US and execution by the surgeon.
Abbreviations: US: ultrasound

Margin-marks and re-resections

In one patient, the stain at the margin-mark was not visible after histological processing, and in one patient, the margin-mark was not placed; therefore, 11 margin-marks were analysed. The distance from the resection plane to the tumour front at the location of the margin-mark and the 11 re-resections were measured on ex-vivo US and during histological analysis. The mean deviation of the measurement on ex-vivo US from histology was 2.5 (SD 2.8) mm. The ex-vivo US measurement did not differ significantly from histology ($p = 0.69$).

Four out of 12 (33%) margin-marks were placed at the exact location of the closest tumour-free margin. In four out of 12 (33%), it was placed near the closest tumour-free margin, and in two out of 12 (17%), it indicated a close tumour-free margin; however, not the closest tumour-free margin. Two out of 12 (17%) margin-marks were not placed at or near the closest tumour-free margin.

The 11 re-resections covered 15 locations because some of the re-resections covered more than one location (Figure 2, Table 1). Therefore, a re-resection could cover a location where the US did not indicate a re-resection. Five of the 11 (46%) re-resections were executed at the exact location, of adequate size, and prevented an involved or close tumour-free margin, three (27%) were in the right place but too small, and three (27%) were unnecessary.

Table 3. the new cutoff point for re-resection and the histology

Hypothetical	Histology < 5.0 mm tumour-free margin	Histology ≥ 5.0 mm tumour-free margin	Total
US tumour-free margin < 7.5 mm	25	17	42
US tumour-free margin ≥ 7.5 mm and therefore re-resection	4	16	20
Total	29	33	62

Note: Here the hypothetical results are shown if a tumour-free margin of < 7.5 mm on the ex-vivo US would lead to a re-resection and the consequence for the histological tumour-free margin. Abbreviations: US = ultrasound

Adjuvant therapy

Six out of 13 patients (46%) qualified for adjuvant treatment of the primary tumour. Of these six patients, four (31%) received adjuvant radiotherapy, one declined adjuvant radiotherapy, and one received a re-resection.

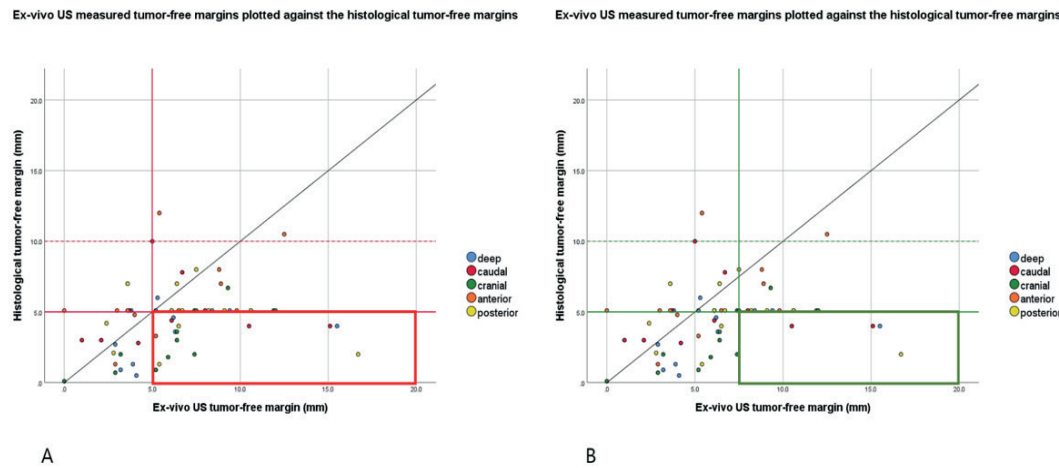


Figure 3: A: The tumour-free margins at the five different locations (Figure 2), measured by ex-vivo US. The red lines indicate the 5.0 mm tumour-free margin on US and histology. The red rectangle indicates the margins that are ≥ 5.0 mm on the ex-vivo US, but < 5.0 mm at histological evaluation. The dotted line indicates the histological 10.0 mm tumour-free margin. **B:** The green lines indicate the hypothetical ex-vivo US tumour-free margin measurement with cutoff value of 7.5 mm to obtain a 5.0 mm histological tumour-free margin. The dotted line indicates the 10.0 mm histological tumour-free margin. Abbreviations: US: ultrasound.

Discussion

Complete removal of the tumour with ≥ 5.0 mm tumour-free margin is a challenge in BMSCC surgery (70,71,75). Especially at the deep resection plane, where up to 100% of the involved margins are reported (87). The application of ioUS appears of value for complete tumour removal and optimal resection volumes in tongue- and breast cancers (83,88). This study shows the potential of this technique in BMSCC surgery. To our knowledge, this is the first study that uses intraoperative US to measure TT and tumour-free margins in BMSCCs objectively. The results show that ioUS, and ex-vivo US are reliable for estimating TT. Furthermore, a 5.0 mm tumour-free margin on ex-vivo US was the cutoff point to qualify for re-resection. When aimed for a ≥ 5.0 mm ex-vivo US and histological tumour-free margin, only 48% of the involved or close tumour-free margins were correctly identified by the ex-vivo US.

ROC curve: ex-vivo US for < 5 mm histopathological tumour-free margins

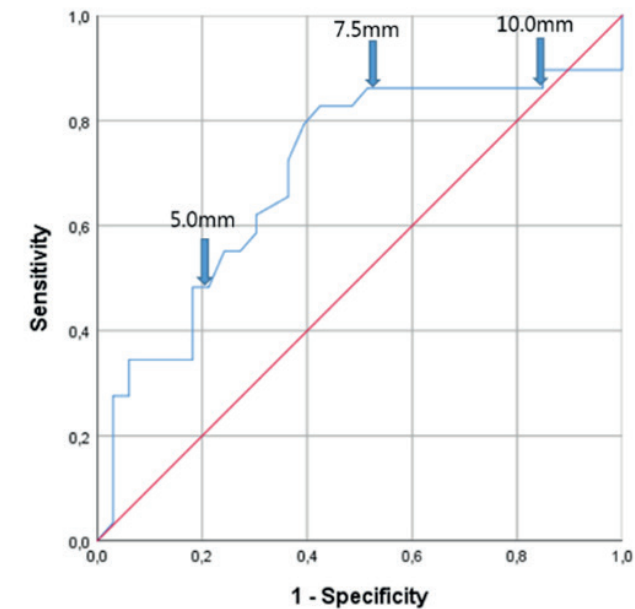


Figure 4: The ROC curve: margins measured on ex-vivo US for a < 5.0 mm histological tumour-free margin. The arrows indicate the sensitivity of the US at a certain tumour-free margin measured with the ex-vivo US. Abbreviations: US, ultrasound; ROC, receiver operator characteristics.

Tumour thickness

In this study, TT was measured to validate the accuracy of intraoperative US by comparing it to MRI and histology. MRI, ioUS, and ex-vivo US measured TT accurately since there were no significant differences compared to histology, and their correlations were very strong. Therefore, US is a reliable imaging technique for measuring TT. Literature shows correlations of 0.80-0.96 for measuring TT with US in oral cancer, which is comparable to our results for BMSCC (51,52,89,90). Moreover, ex-vivo US had the highest correlation and therefore measured TT most accurately, with the least deviation from histology, followed by ioUS. There are multiple advantages of US over MRI, such as the costs, which are lower, and the ability to visualize the tumour real time during resection.

Tumour-free margins

An adequate tumour-free margin is pivotal for recurrence-free survival. Unfortunately, BMSCC itself is a risk factor for involved or close tumour-free margins, possibly due to overestimation of the tumour-free margins, which is undesirable (70,71,75). We aim to excise the tumour with a 5.0 mm histological tumour-free margin. Unfortunately, the ex-vivo US overestimated the tumour-free margins in 45 of the 62 tumour-free margins (73%). This could be due to non-cohesive growth, which was present in 77% of the tumours, as microscopic tumour-nests cannot be identified with US (83). Furthermore, the direction of specimen slicing could be slightly different from the angle the US-probe, resulting in a different cross section and a shorter distance from the tumour to the outside of the specimen (26). Probably most important: in BMSCC, it is known that tumour-free margins can shrink up to 72% prior to and during fixation (71,76,91,92). The specimen release from the surrounding supporting structures and contractility of the underlying muscles are held responsible for this shrinkage (93). When a tumour-free margin of 7.5 mm on ex-vivo US would be the cutoff point for re-resection, this problem could be intercepted. Such an approach is probably more precise than the use of only visual inspection and palpation, which is the current clinical practice. The sensitivity of the ex-vivo US would then increase from 48% to 86% (Figure 3B, Table 3), and the undertreatment of the tumour-free margins would decrease from 39% to 6%. The negative predictive value of the US would be 80%, and 58 of the 62 (94%) tumour-free margins would be ≥ 5.0 mm on histology or identified by the US as being < 5.0 mm on histology (Figure 3B). The three out of four tumour-free margins that would have been missed even with ≥ 7.5 mm on ex-vivo US were still from only one patient with extreme non-cohesive growth. In this patient, the invasive tumour nests were localized 5.0 mm from the tumour bulk. We recommend a ≥ 7.5 mm ex-vivo US tumour-free margin for a ≥ 5.0 mm histological tumour-free margin, with an immediate re-resection in case of a closer ex-vivo US tumour-free margin. We aim to validate this recommended tumour-free margin in a new study.

BMSCC is an aggressive cancer with different biological and clinical behaviour than other oral cavity carcinomas (94). Until now, no studies have been published on BMSCC-specific intraoperative tumour-free margin imaging. There are imaging modalities that try to improve tumour-free margins in oral cavity carcinomas, such as fluorescence, which is a promising technique. However, it is invasive because of intravenous injection of conjugated antibodies. Moreover, the sampling depth is less than 5.0 mm (95). Other techniques are Raman spectroscopy, diffuse reflectance spectroscopy, hyperspectral imaging, and optical coherence tomography. All these techniques struggle with superficial sampling depth, which is insufficient to rule out close tumour-free margins (95). Frozen section analysis is a routinely used technique in daily clinical practice. However, this technique is prone to false negatives because only a tiny fraction of the specimen is evaluated (82,95). In addition, the evidence to support this commonly used technique is lacking (96).

Margin-marks and re-resections

There is room for improvement regarding the application of margin-marks and re-resections. The involved or close tumour-free margins were visible and measured correctly in most cases. Eighteen of the 23 (78%) margin-marks or re-resections were placed at or near an involved or close tumour-free margin. There were multiple steps in the process susceptible to interpretation error. The biggest error proved to be the misinterpretation between the ex-vivo US and the correct re-resection site.

Paired tagging could decrease this interpretation error. Van Lanschot et al. placed numbered tags on both sides of the resection line, enabling accurate re-resections (97). Another problem of BMSCC surgery is the limited amount of resectable healthy tissue. The indication for re-resection, based on the ex-vivo US, was sometimes ignored by the surgeon because this would have caused an orocutaneous defect, a mandibular or maxillary resection, or damage to the facial nerves and muscles (Table 2) (71). Nevertheless, at least five of the 11 re-resections (46%) prevented an involved or close tumour-free margin by correct re-resections based on the ex-vivo US. This reflects the consideration between morbidity and possible recurrence. However, patients with an involved or close tumour-free margin have a higher risk of recurrence and qualify for adjuvant treatment, which also carries a high risk of morbidity (70,71,98,99). Furthermore, the chance of correctly re-resecting an involved or close tumour-free margin in a second operation is low (100).

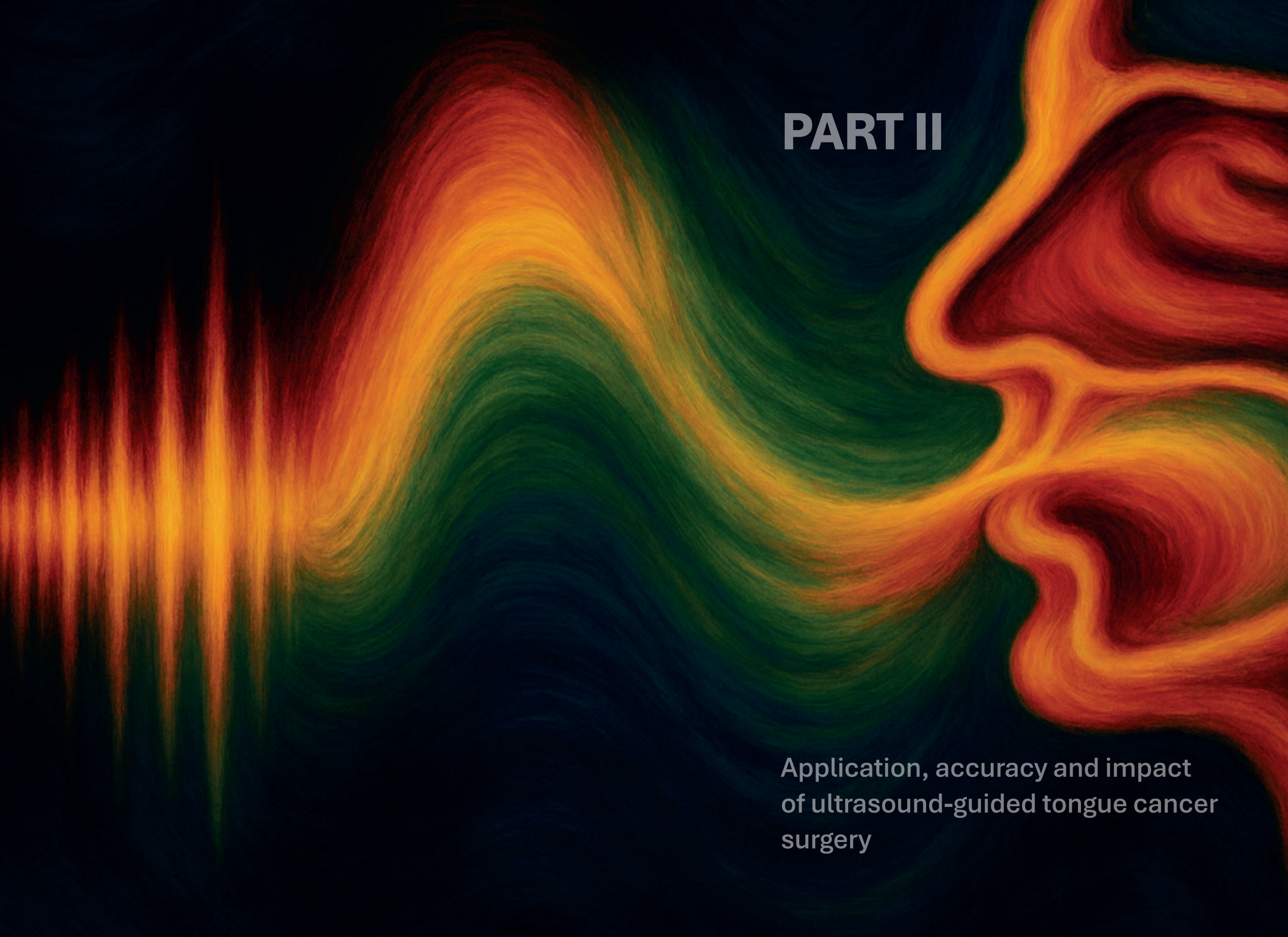
Because US is non-invasive, affordable, widely available, and easy to insert in the surgical workflow, it is a suitable tool for every head and neck surgeon to guide their resection. This study gives surgeons a guideline for using intraoperative US and a US tumour-free margin to strive for during surgery.

Limitations

This study had a limited number of included participants. Nevertheless, ioUS and ex-vivo US were able to measure TT correctly. In addition, the long-term advantages of more correct resections and immediate re-resection on local recurrences and quality of life were not analysed because of the short follow-up. Another limitation is the limited number of ioUS measurements. Therefore, it is impossible to calculate a reliable indication for a wider resection for ioUS. Further research is needed to evaluate the effect of obtaining an US tumour-free margin of 7.5 mm instead of 5.0 mm, so that the overestimation can be evaluated. This was not possible in the current study because we do not know what the tumour-free margins would be if re-resection was done at an US tumour-free margin of 7.5 mm. We intend to continue this line of research and analyse the effect of the adjustments based on this study.

Conclusions

This feasibility study aimed to validate ioUS and ex-vivo US in the resection of BMSCC by comparing it to MRI, and to find an appropriate US tumour-free margin cutoff point for re-resection. MRI, ioUS, ex-vivo US were able to accurately estimate histological TT, making them reliable imaging modalities to measure TT in BMSCC. The ioUS and ex-vivo US show potential for measuring tumour-free margins. However, US tended to overestimate the tumour-free margins. Therefore, we recommend a 7.5 mm tumour-free margin on ex-vivo US to obtain a histological tumour-free margin of ≥ 5.0 mm for future studies. Additional research is required to establish the effect of 7.5 mm ultrasound cutoff.



PART II

Application, accuracy and impact
of ultrasound-guided tongue cancer
surgery

Chapter 4

Application and accuracy of ultrasound-guided resections of tongue cancer

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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 with those of 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 margin 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 cutoff 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) (2). 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 (83). These numbers are in line with those of other studies in which up to 45% close margins and 43% positive margins in oral cancer (4) 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, which is non-cohesive growth, perineural growth, 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 RT on the oral cavity has several side effects (e.g. mucositis, xerostomia and osteoradiationecrosis (44)), 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 (42). 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 (43). 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 (52,101) and is a better predictor than manual palpation (63). 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. (57). 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%) (83).

In this study, we evaluate the application and accuracy of the US-guided SCCT resection technique as described previously in a larger cohort (83). 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 (17)*) 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 Figure 1A-C. A mucosal margin of 10 mm was marked around the lesion (Figure 1D). Next, the surgeon started the 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 un-

der the resection plane, comparable with the methods of Songra et al. (54) (Figure 1E). 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 (Figure 1F). 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 echographic 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 (L20-5s, Mindray) over all five areas and measuring the smallest margin for those areas (Figure 3B). 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 Figure 1G-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 factors 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.

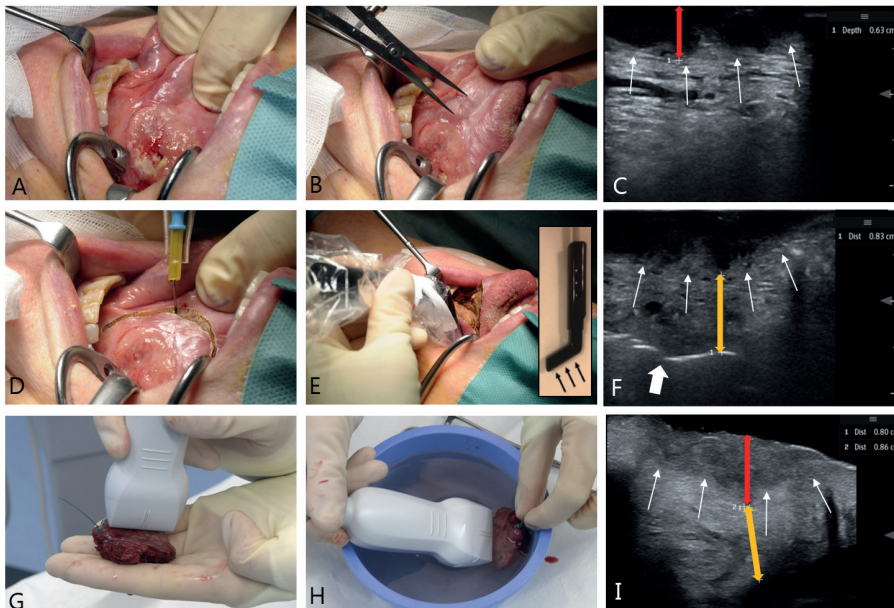


Figure 1: US-guided resection of SCCT. Red double arrows define echographical TT, yellow double arrows define the margin, and white arrows define the tumour border. A: SCCT of the tongue. B: Demarcation of a mucosal margin of 10 mm. C: Intraoral US for in-vivo determination of TT. D: Resection of the 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.

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 (Figure 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 haematoxylin-eosin (HE) and digitalised according to the methods described by Stathonikos et al. (102).

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, Figure 3) were determined by a dedicated head and neck pathologist. Margins at the craniomedial, caudolateral and central parts of the specimen were measured digitally (102) (Figure 2). Margins at the anterior and posterior parts were calculated by multiplying the number of tumour-free slices, as determined by microscopic images, by the mean slice thickness (Figure 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 3B), 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 ≥ 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' (≥ 5 mm), 'close' (1-5 mm) or 'positive' (< 1 mm). The smallest margin of all these areas determined 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 Figure 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.

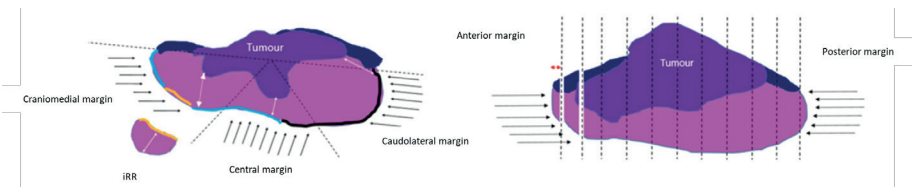


Figure 2: Schematic representation of histopathological assessment of the resection specimen. Pink: normal HE stained tissue. Purple: HE stained tumour. Dark blue: HE stained mucosa. A: HE section with light-blue ink demarcating the craniomedial side and black ink demarcating the caudolateral side of 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 number of tumour-free slices.

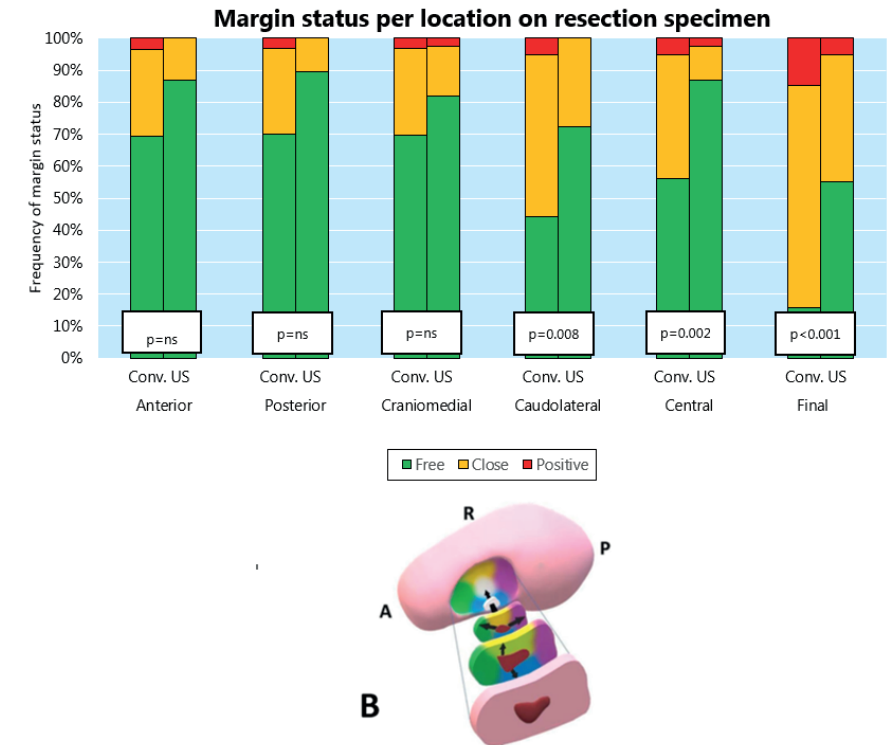


Figure 3: Margin status of all analysed locations of the resection specimen and final margin status of both cohorts. P-values are representative of all comparisons (found using a Fisher's exact test) in the black indents. B: The analysed locations are schematically depicted in a virtually sliced resection 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. Accuracy of US-guided resections

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 different from 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.

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

Abbreviations: ns: not significant, US: ultrasound.

^a determined with independent t-test.

^b determined with chi-square test.

Results

US vs. conventional cohort

Forty-four patients were initially included for this study in our outpatient clinic. Four dropouts 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 Figure 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 Figure 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.

Table 2. Results of US- and conventional cohort

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

Abbreviations: ns: not significant, US: ultrasound

^a Statistical significance was determined with Fisher's exact test.

^b Statistical significance was determined with independent t-test.

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)

Abbreviations: iRR: intraoperative re-resections, sUS: smallest US-measured tumour-free margin

Accuracy of US-guided resections

Modified Bland-Altman plots are shown in Figure 4. There was a mean difference in TT of 0.4 (SD 2.4) mm between in-vivo measurements on US and histopathologic examination, indicating a small overestimation of TT by US. For ex-vivo US, the measurement was 0.9 (SD 2.6) mm, indicating an overestimation. Neither of the means are statistically significantly different from 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.

Figure 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 echographic cutoff 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 (53–57,66,83,90,103), 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.

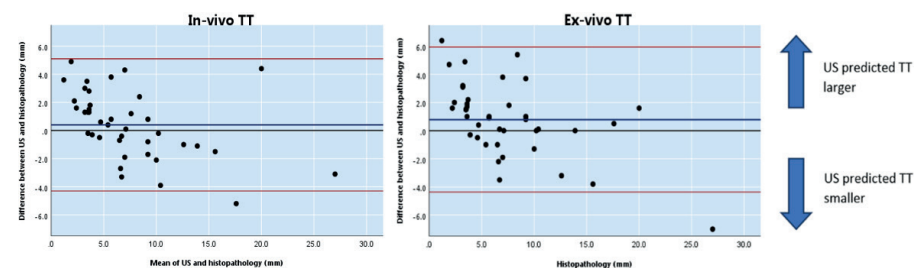


Figure 4: Modified Bland-Altman plots depicting the differences between in- and ex-vivo TT measurements on US and upon histopathology. The blue line depicts the mean difference between US and histopathology. For in-vivo US, it is 0.4 mm; for ex-vivo US, it is 0.9 mm. Red lines and blue arrows depict 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.

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 cutoff margin distance that significantly reduces the chance of recurrence without excessive removal of healthy tongue tissue (30,38,43,78,104–106). 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 (30,61,106,107). 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 (108) and tongue function (e.g. mobility and sensory function (44,108)). In a multiple regression analysis, Yang et al. (108) found that adjuvant RT has the most negative effect on QoL in tongue cancer patients ($B = -9.595$). In addition, Jehn et al. (44) described specific physical impairments (e.g. xerostomia and pain) that were significantly associated with mucositis as a result of local adjuvant RT after surgery.

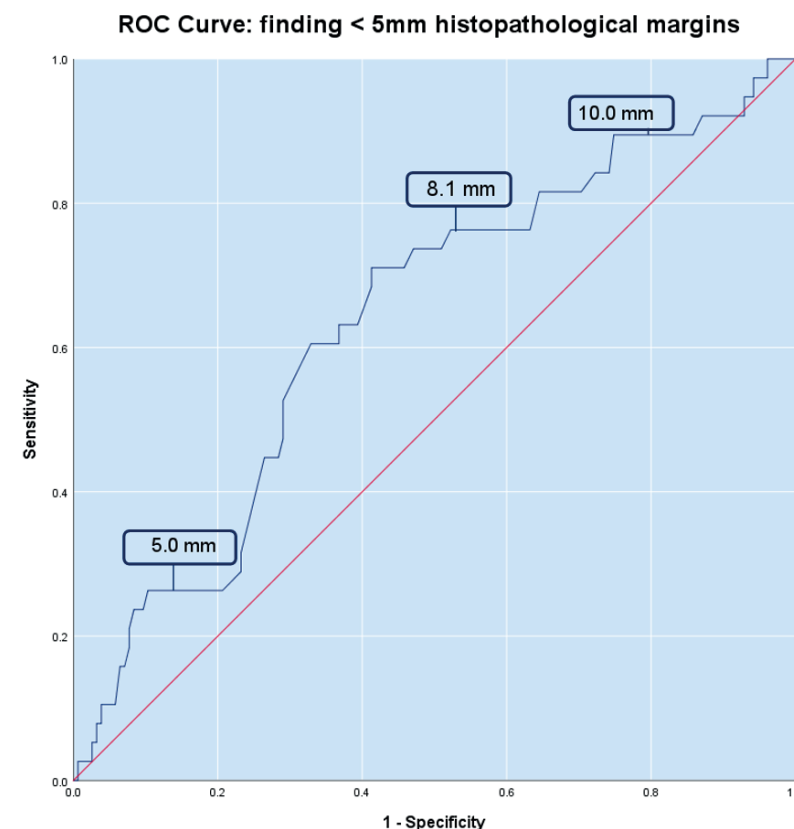


Figure 5: ROC curve (blue) depicting the diagnostic ability of US to identify < 5 mm margins (area under the curve: 0.633). The red line is a reference line for a method with no diagnostic value (area under the curve: 0.5). All margins measured by ex-vivo US and histopathology (anterior, posterior, craniomedial, caudolateral and central) were 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.

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 intraoperative echographic 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 (Figure 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 (Figure 3). Regarding the caudolateral area, this might be due to intraoperative margin overestimation because during conventional treatment the muscular tongue tissue might be overstretched to reveal the caudolateral portion of the tumour. 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 (Figure 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 (Figure 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 different from 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. (52) 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) (Figure 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. 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 (66,83), ex-vivo US control of the resection specimen prolongs surgical time by only 5-10 minutes 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 (4). Second, if an iRR was performed with 8 mm as an ex-vivo echographic cutoff 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 (Figure 5). Although this alternative cutoff 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. (97), 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 (109). As iRRs are not anatomically oriented (110), it is challenging to translate an echographically inadequate margin's location on the resection specimen to its corresponding location on the tumour bed (42). Several studies have paradoxically reported that iRRs, indicated by frozen sections, are predictors for local recurrence (61,107). We expect that 'parallel tagging' (97), 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 into their reliability.

Finally, we did not correct for post-excision or post-fixation specimen shrinkage. Although this phenomenon is described in the literature for the surface and mucosal margins of oral tumours (67,111), 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. vaso-invasive 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.

Chapter 5

Impact of US-guided surgery on local disease-free survival

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Submitted

Abstract

Objectives: Obtaining tumour-free margins to achieve local disease-free survival (DFS) during resection of squamous cell carcinoma of the tongue (SCCT) is challenging. US-guided SCCT surgery is suggested to increase tumour-free margins, particularly in the deep margin of the resection. This study aims to evaluate the impact of US-guided surgery on achieving a better local DFS.

Methods: A retrospective analysis was conducted on patients with primary T1-T3 SCCT, treated with US-guided or conventional surgery. Local DFS was compared using log-rank tests over a 30-month follow-up. Recurrences were analysed to identify their origin (deep or mucosal). Stratified analyses for margin status and adjuvant treatment effects were performed, along with a uni- and multivariate analyses, over a 60-month follow-up to assess risk factors for local DFS.

Results: US-guided surgery reduced frequency of positive margins (< 1 mm) threefold and increased free margins (≥ 5 mm) threefold compared to conventional surgery ($p < 0.001$). Local DFS did not differ statistically ($p = 0.945$). No deep recurrences were observed in the US-cohort, whereas at least 3/5 recurrences were deep in the conventional cohort. Patients who had positive margins in combination with adjuvant treatment had a better local DFS than those without adjuvant treatment ($p = 0.014$), but this was not the case for those with close margins. Multivariate analysis identified positive margin status and neck metastasis as independent predictors for local recurrence.

Conclusion: Because of the relatively low incidence of local recurrence in SCCT, larger cohorts are needed to elucidate the effects of US-guided surgery on local DFS. However, it shows potential in improving local DFS by reducing positive margins. It also seems to prevent deep recurrences and the need for adjuvant treatment, which significantly impacts quality of life and oral function.

Introduction

One of the major challenges of surgical removal of squamous cell carcinoma of the tongue (SCCT) is to obtain adequate tumour-free margins (2). Many studies have reported on SCCT surgery and the ideal definition of an adequate margin. This definition may be a trade-off between local control and maintenance of oral function. However, no consensus has been reached thus far (30,43,78,104,106,112). The most accepted guideline is a histopathological tumour-free margin of more than 5 mm, according to the guidelines of Royal College of Pathologists and the National Comprehensive Cancer Network (27,28).

In case the tumour-free margins are reported as 'inadequate' (i.e. < 5 mm), adjuvant treatment, i.e. local postoperative radiotherapy (PORT) or a re-resection, may be considered to prevent local recurrence. Both have disadvantages; radiotherapy has several side effects (44) and re-resection requires a second surgical procedure, wherein localizing the inadequate margin could be challenging (42). Moreover, the need for additional procedures, such as hospital admission, second surgeries, radiotherapy, or side-effect management, increases the burden on patients and healthcare costs.

Ultrasound (US)-guided SCCT surgery was introduced in our centre to prevent 'positive' (< 1 mm) or close (1-5 mm) margins and to obtain more tumour-free margins (≥ 5 mm) (83,113). US-guided SCCT surgery is suggested to increase tumour-free margins, particularly in the deep margin of the resection, i.e. the muscular tongue tissue and submucosal tissue. This may prevent deep local recurrences that originate from tumour residue in these tissues. Deep local recurrences deteriorate survival and typically require more invasive treatments, i.e. aggressive surgery and chemoradiotherapy. In contrast, mucosal recurrences are earlier recognized and subsequently treated (43).

In this study, we retrospectively analysed the local disease-free survival (DFS) of a cohort treated with US-guided surgery and compared it with a cohort treated with conventional surgery. Additionally, we determined which other factors may play a role in local DFS. Finally, we analysed whether US-guided surgery leads to less deep recurrences.

Methods

Patients

This study was performed in accordance with the 1964 Declaration of Helsinki and Good Clinical Practice. The US-guided cohort consisted of patients who consecutively agreed to participate in a prospective study approved by our institute's Medical Ethics Review Board (number: 19-414), which was conducted between November 2019 and June 2021. For the conventional cohort, retro-

spective analysis was approved by our Medical Ethics Review Board (number: 20-258) and consisted of patients who received SCCT surgery between July 2014 and September 2018. Both cohorts consisted of patients who were diagnosed with a primary T1-T3 SCCT, according to the 8th edition of TNM classification of malignant tumours (17). All patients were treated under general anaesthesia. Patients were excluded in case no residual tumour cells were found because a previous (excisional) biopsy resected all visible tumour cells already.

Table 1. Demographical data and tumour characteristics US and conventional cohorts

	Overall (n = 132)	US-cohort (n = 37)	Conventional cohort (n = 95)	p-value
<i>Age (years)</i>				
Median	69	63	71	0.001
Range (min -max)	23 - 95	23 - 83	33 - 95	
<i>Gender (n)</i>				
Male (%)	76 (58)	21 (57)	55 (58)	0.905
Female (%)	56 (42)	16 (43)	40 (42)	
<i>Ever smoked (n)</i>				
No (%)	46 (35)	17 (46)	29 (31)	0.095
Yes (%)	86 (65)	20 (54)	66 (70)	
<i>Ever alcohol (n)^a</i>				
No (%)	46 (35)	12 (32)	34 (37)	0.657
Yes (%)	84 (64)	25 (68)	59 (63)	
<i>Pathological T stage (n)</i>				
T1 (%)	45 (34)	12 (32)	33 (35)	0.537
T2 (%)	55 (42)	18 (49)	37 (39)	
T3 (%)	32 (24)	7 (19)	25 (26)	
<i>Maximal dimension (mm)</i>				
Median	20	19.5	20	0.985
Range (min - max)	3 - 64	3 - 57	4 - 64	
<i>Depth of invasion (mm)</i>				
Median	6.6	6.6	6.3	0.818
Range (min - max)	0.2 - 27.0	0.8 - 27.0	0.2 - 27.0	
<i>Unfavourable histopathologic growth factor (n)</i>				
Non-cohesive growth (%)	75 (57)	28 (76)	47 (50)	0.006
Perineural growth (%)	41 (31)	13 (35)	28 (30)	
Vaso-invasive growth (%)	9 (7)	3 (8)	6 (6)	

<i>Neck metastasis (n)</i>				
No (%)	74 (56)	18 (49)	56 (59)	0.284
Yes (%)	58 (43)	19 (51)	39 (41)	
<i>Margin status (n)</i>				
Free (%)	34 (26)	19 (51)	15 (16)	< 0.001
Close (%)	83 (63)	16 (43)	67 (71)	
Positive (%)	15 (12)	2 (5)	13 (14)	
<i>Adjuvant treatment (n)</i>				
Re-resection (%)	13 (10)	4 (11)	9 (10)	0.817
Local radiotherapy (%)	24 (18)	5 (14)	19 (20)	0.385
Chemoradiotherapy (%)	10 (8)	1 (3)	9 (10)	0.282

^aof two patients, no data regarding the use of alcohol was available.

Treatment and follow-up

In both cohorts, the tumour's extent was estimated by the surgeon with visual inspection under white light, palpation and diagnostic images (i.e. magnetic resonance imaging, preoperative US). Under general anaesthesia, the tumour's mucosal border was delineated with a 10 mm margin. During resection, the surgeon aimed for surgical margins of about 10 mm. In the US-cohort, additional to the conventional procedures, a hockey-stick shaped probe was placed onto the tumour to image its extent (L16-4Hs, Mindray Bio-medical Electronics, Shenzhen, China). During the resection, US images from the tumour border and resection plane were taken simultaneously to measure the surgical tumour-free margin. This functioned as additional feedback for the surgeon to aim for 10 mm margins. After in-vivo imaging with the hockey-stick shaped probe, the resection specimen was examined ex-vivo with a high-resolution symmetrical probe (Mindray L20-5s) to find unnoticed inadequate resection margins. If so, an additional re-resection was executed during the same session. The position of the immediate re-resection relative to the main resection specimen was either directly sutured onto the main specimen or indicated by placing a suture on the main specimen to mark its relative position. More detailed descriptions about this method were reported earlier (113). The neck was managed with sentinel node biopsy and/or neck dissection, which depended on preoperative diagnostic work-up.

For all included patients, the resection specimen and optional re-resections were cut in 3-5 mm thick blocks, paraffin embedded and dyed for orientation. Four µm-thick haematoxylin and eosin slices were obtained and digitalized according to the methods of Stathonikos et al. (102). Depth of invasion, unfavourable histopathological growth factors (i.e., non-cohesive, perineural and vaso-invasive growth) and the tumour-free margin distance at five specific locations (i.e. anterior, posterior, craniomedial, caudolateral and central) per specimen were

determined. The smallest margin distance at one of those five locations determined the margin status.

Depending on the histopathology report, the policy was either routine follow-up, a re-resection, or (loco)regional postoperative (chemo)radiotherapy. Routine follow-up was generally chosen for patients who had free margins or a close margin status (1-5 mm) and < 2 unfavourable histopathological growth factors. A re-resection during a second surgery was generally executed when a retrievable positive margin was found or a close (sub)mucosal margin together with ≥ 2 unfavourable histological growth factors. Local PORT was generally administered when a positive margin was found or a close deep margin in combination with ≥ 2 unfavourable histopathological growth factors (43). Adjuvant chemoradiotherapy was generally administered locally when a positive margin was found in patients fit for chemotherapy and aged < 70 years.

All patients were discussed in a multidisciplinary team consisting of the surgeon, a pathologist, radiotherapists and oncologist, taking also the patient's fitness, comorbidities and preferences into account. Out-patient follow-up was generally scheduled every 2 months in the first year, every 3-4 months in the second year, every 4-6 months in the third year.

Statistical analysis

Various clinicopathologic variables were evaluated for the conventional and US-cohort. Among these variables, continuous variables were presented as mean (SD) when normally distributed as confirmed by analysis of histograms, Q-Q plots and the Shapiro-Wilk tests and as median (range) if not. Statistical significance was set at $p < 0.05$ and tested with an independent t-test or Fisher's exact test if normally distributed. If not normally distributed, a Mann-Whitney U-test was performed to identify differences between medians. Categorical variables were reported as frequency (percentage) and statistical significance was calculated with the chi-square test.

In general, a 60-month follow-up period was chosen for the statistical analyses regarding survival. Only when the analysis' independent variable was the cohort (i.e. conventional or US-guided treatment), a 30-month follow-up period was chosen, since only 30 months passed between the last inclusion of the US-cohort and the follow-up status update.

Of both cohorts, local DFS was visualized with Kaplan-Meier curves over a 30-month follow-up period. Statistically significant differences in local DFS probability over time were analysed using log-rank tests. Cox regression analyses were performed to assess risk factors associated with local DFS over a 60-month follow-up period. Firstly, this was done with a univariate analysis. Subsequently, a multivariate analysis was performed with the statistically significant variables (defined as $p < 0.05$) from the univariate analysis, to eliminate their reciprocal influence. Categorical variables, such as margin status and TNM were split up in dichotomic variables.

As an additional analysis, local recurrences within a 30-month follow-up

period were differentiated between superficial recurrences originating from the mucosa or deep recurrences originating from the submucosal muscular or connective tissues. This was determined from clinical and histological (i.e. biopsies) records in accordance with a dedicated head and neck pathologist (GB). The location of the recurrence was compared with the location of narrowest margin of the original resection specimen.

Kaplan-Meier curves were made to visualize the effect of margin status on local DFS (i.e. positive, close and free margin status) and margin status in combination with adjuvant treatment. Subsequently, log-rank tests were used to determine the statistical significance of the differences between the curves.

Table 2. Results from univariate and multivariate analysis for local disease-free survival

Variable	Univariate			Multi-variate		
	HR	95% CI	p-value	HR	95% CI	p-value
US-guided	0.890	0.176-4.485	0.887	...	...	...
Age, years	1.003	0.956-1.052	0.898	...	...	...
Sex, female	1.569	0.421-5.847	0.502	...	...	...
Smoking	1.089	0.272-4.355	0.904	...	...	...
Ever alcohol	1.007	0.251-4.041	0.993	...	...	...
pT1	0.866	0.216-3.466	0.838	...	...	...
pT2	0.393	0.081-1.897	0.245	...	...	...
pT3	2.951	0.790-11.020	0.107	...	...	...
Maximal dimension	1.027	0.971-1.086	0.350	...	...	...
Depth of invasion	1.091	0.958-1.243	0.188	...	...	...
Non-cohesive growth	1.014	0.272-3.783	0.983	...	...	...
Perineural growth	2.935	0.788-10.937	0.109	...	...	...
Vaso-invasive growth	1.847	0.231-14.789	0.563	...	...	...
Neck metastasis	6.050	1.252-29.237	0.025	6.093	1.264-29.380	0.024
Positive margin status	7.797	1.907-31.880	0.004	7.943	1.943-32.628	0.004
Close margin status	0.382	0.102-1.430	0.153	...	...	...
Free margin status	0.804	0.167-3.879	0.786	...	...	...
Neck dissection	1.853	0.497-6.908	0.358	...	...	...
Re-resection	1.013	0.126-8.142	0.991	...	...	...
Local PORT	2.842	0.708-11.413	0.141	...	...	...
Regional PORT	2.646	0.708-9.887	0.148	...	...	...

Abbreviations HR: hazard ratio, CI: confidence interval, PORT: postoperative radiotherapy

Table 3. Clinical characteristics of the local recurrences within the 30-month follow-up period									
Case	Cohort	Indication mucosal or deep	Minimal margin distance and location	Death due to disease, months after surgery	Local PORT	No. of UHGF	Recurrence treatment	Pathological description of biopsy	Clinical description of appearance
1	US	Mucosal	6.9 mm, mucosal	No	No	1	Mucosal re-resection	"Severe dysplasia of the squamous epithelium with focal invasive growth, to be classified as SCC."	"Area of erythroplakia."
2	US	Mucosal	3.9 mm, deep	No	No	2	Mucosal re-resection	"Focal superficial well-differentiated SCC (DOI of 0.5 mm) against a background of severe dysplasia."	"Dysplastic-looking lesion in the floor of mouth."
3	C	Unclear Presumably deep	0 mm, submucosal	Yes, 16	Yes	1	Palliative	Unknown – biopsy in other hospital	"Swelling of the jaw"
4	C	Deep	0.3 mm, submucosal 3.5, deep	Yes, 7	Yes	3	Palliative	"In the lingual muscles a poorly differentiated SCC"	"Crater shaped ulcer on the floor of mouth, with necrosis extending deep into the tongue's base."
5	C	Deep	0 mm, deep	Yes, 15	Yes	0	Chemotherapy	Unknown – no biopsy of lesion	"Granulation tissue in the right floor of mouth, feels supple. Tongue is mobile." – PET/CT images show mass with necrotic centre in floor of mouth.
6	C	Mucosal	3.0 mm, mucosal	Yes, 24	Yes	2	Chemotherapy	At least severe dysplasia, suspicious for squamous cell carcinoma	"Tongue tender to palpation but appears calm."
7	C	Deep	7.1 mm	Yes, 22	No	0	Palliative	Deep biopsy shows SCC	PET-CT shows indications of recurrent tumour on the right side of the tongue.

Abbreviations: US: ultrasound, C: conventional, PORT: postoperative radiotherapy, UHGF: unfavourable histopathological growth factors SCC: squamous cell carcinoma; PET-CT: positron emission tomography – computed tomography

Results

US-guided vs. conventional surgery

Ninety-five patients had conventional surgery and 37 patients had US-guided surgery. Regarding patient-characteristics, the only statistically significant difference between both cohorts was found in age, i.e. 71 years (range: 33-95 years) in the conventional cohort vs. 63 years with (range: 23-83) in the US-cohort. Clinicopathological differences between the conventional and US-cohort were statistically significant for non-cohesive growth (50% vs. 76%, respectively) and margin status. In the US-cohort, the frequency of free margin status was more than three times higher than the frequency in the conventional cohort (51% vs. 16%). For positive margins, the frequency was almost three times as low as compared with the frequency of the conventional cohort (5% vs. 14%) (Table 1). Log-rank test on local DFS showed no statistically significant differences ($p = 0.945$) between the US-guided and conventional cohorts. Kaplan-Meier curves of both groups (Figure 1) show that local DFS probability is equal in the whole follow-up period.

Uni- and multivariate analysis

Considering local DFS, the variables positive margin status and neck metastasis were statistically significant in the univariate analysis ($p = 0.025$ and $p = 0.004$, respectively) and therefore included in the multivariate analysis. According to the multivariate analysis, both variables were also independently associated with local recurrence, with a hazard ratio (HR) of 6.093 ($p = 0.024$) for neck metastasis and an HR of 7.943 ($p = 0.004$) positive margin status (Table 2).

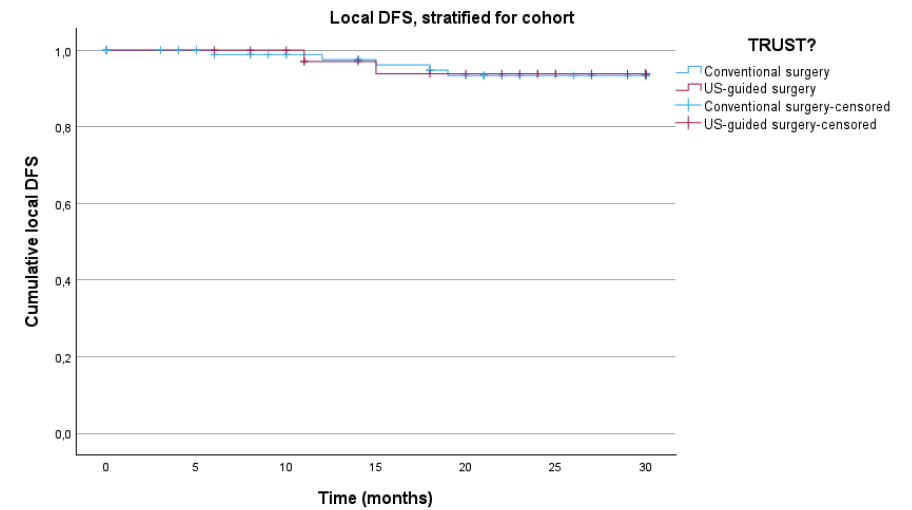


Figure 1: Kaplan-Meier showing differences in local DFS the US- and conventional cohort ($p = 0.945$).

Additional analyses

The only two local recurrences in the US-guided cohort originated from the mucosa and seemed to originate from a dysplastic area. One of these patients had a free margin (6.9 mm) but with an indication for severe dysplasia in the resection plane after surgery for the primary tumour. The other had only a close deep margin (3.9 mm). Regarding the conventional cohort, three of the five local recurrences originated from the deep muscular tissue, one from the mucosa, and one was of unknown origin. For the latter, it was presumed to be a deep recurrence, because the patient had a positive margin in the submucosal connective tissue after primary tumour surgery. Of the five local recurrences, three patients had a positive margin, one had a close margin, and one had a free margin.

Log-rank tests show a statistically significant difference in local DFS between patients who had free margins vs. positive margins ($p = 0.031$) and between patients who had close margin vs. positive margins ($p = 0.002$). No statistically significant difference was found between patients who had free vs. close margins ($p = 0.942$) (Figure 2).

When further stratified for local adjuvant treatment (either local PORT or a re-resection) log-rank tests showed the following statistically significant results (Figure 2):

- Better local DFS for patients with positive margins and adjuvant treatment than those with positive margins without adjuvant treatment ($p = 0.014$). There was however no significant difference between patients with close margins and adjuvant treatment than those with close margins without adjuvant treatment.
- Better local DFS for patients with free margins than those with positive margins, both for those with adjuvant treatment ($p = 0.006$) and without adjuvant treatment ($p < 0.001$). There was also a near-significant better local DFS for patients with free margins over those with close margins and adjuvant treatment ($p = 0.083$).
- Better local DFS for patients with close margins without adjuvant treatment than those with positive margins with ($p = 0.013$) and without ($p < 0.001$) adjuvant treatment.
- Better local DFS for patients with close margins with adjuvant treatment than those with positive margins without adjuvant treatment ($p < 0.001$).

Of all the studied patients who had a positive margin status, 9/15 (60%) received local adjuvant (C)RT, while 3/15 (20%) received a re-resection and 3/15 (20%) refused adjuvant treatment. Of all the studied patients who had a close margin, 15/83 (18%) received local adjuvant (C)RT, while 10/83 (12%) received a re-resection. The remaining 57/83 (69%) received a watchful waiting policy or refused adjuvant treatment.

Discussion

US-guided surgery has been introduced in SCCT surgery since manufacturers have been able to shape US-probes for intraoral use (53). As US reliably visualizes the tumour's deeper extent (52), it can provide the surgeon with intraoperative feedback. Different clinical studies have been conducted ever since, starting from small feasibility studies (53,55,56,83,101) to larger cohorts that compared larger US-guided cohorts with conventional cohorts (90,113,114). In these studies, US-guidance always enabled to obtain more adequate margins during surgery. However, to our knowledge this study is the first to investigate US-guidance's potential impact on local DFS.

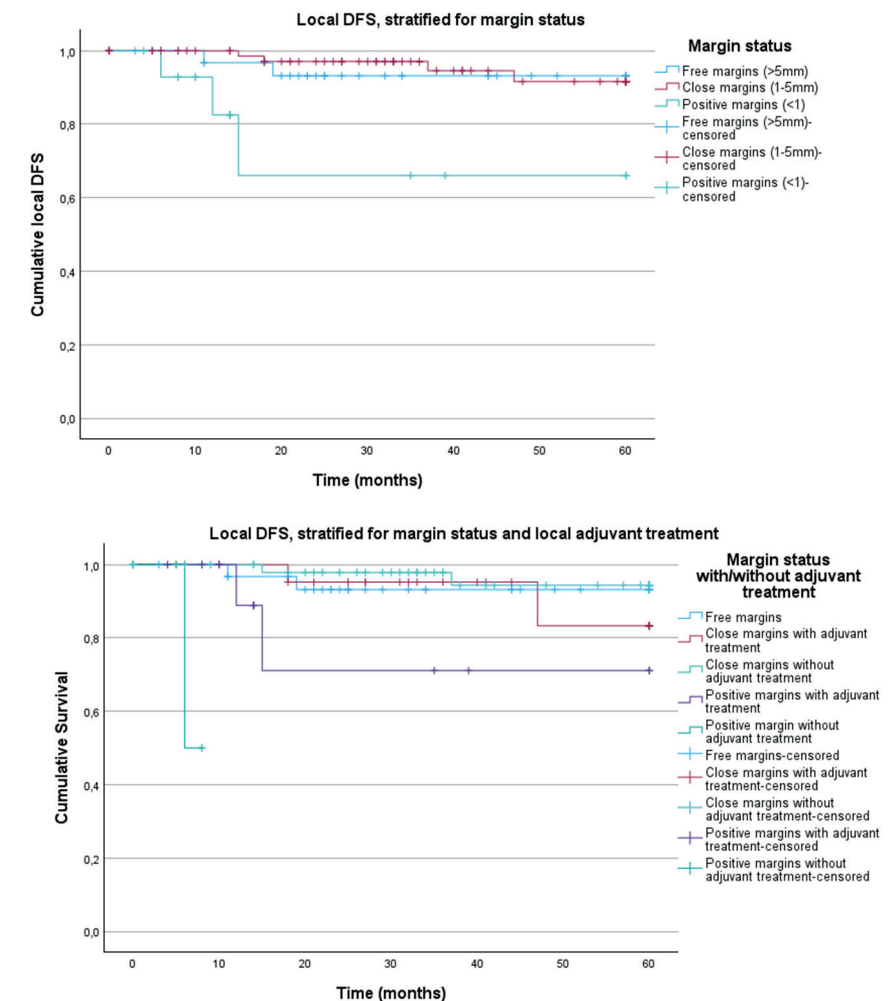


Figure 2: Effect of margin status on local DFS is visualized using Kaplan-Meier curves. Top: Kaplan-Meier curves stratified for margin status. Bottom: Kaplan-Meier curve stratified for margin status and local adjuvant treatment.

The US-cohort showed a statistically significant lower proportion of positive margins (1 mm) and a higher proportion of tumour-free margins compared to the conventional cohort, despite a higher prevalence of non-cohesive growing tumours in the US-cohort (it must be noted however, that low inter-observer agreement for grading non-cohesive growth has been reported (115)). The multivariate Cox regression analysis showed a statistically significant difference in HR for positive margins (HR: 7.797). This finding suggests that US-guided surgery could contribute to improved local DFS.

Despite the statistically significant difference in tumour-free resection margins in favour of the US-cohort, our analyses revealed no statistically significant differences in local DFS between the US-guided cohort and conventional cohort within a 30-month follow-up period. This is probably caused by the low incidence of local recurrence in both cohorts and the (partly) compensating effect of local adjuvant treatment among patients with positive margin status. Therefore, our study may be underpowered to show survival benefit.

An unexpected finding from the multivariate analysis was that neck metastasis is an independent predictor for local recurrence. Also, Sutton et al. (81) found in an analysis of two hundred oral cancer patients that, together with close and positive margin status, neck metastases were a predictor for local recurrence. Patel et al. (116) found that that local recurrence did not occur in oral cancer patients that had intraoperative margin revisions, provided that there was no neck metastasis. These findings suggest that neck metastasis may also be an indicator of tumour aggressiveness at the primary site. It might also explain to some extent why local DFS was similar in both cohorts, as the incidence of neck metastasis was slightly higher in the US-cohort (51% vs. 41%).

It is notable that patients in the US-cohort received less local PORT than in the conventional cohort, (14% vs. 20% respectively). Besides the fact that local PORT is associated with increased healthcare costs, it is also well known that it can be a significant burden for patients. Indeed, a multivariate analysis by Yang et al. showed that local PORT has the most negative effect on quality of life (QoL) among oral cancer patients (108). Those who received adjuvant radiotherapy often have a high probability in developing swallowing and speech impairment (117). This study suggests that US-guided SCCT surgery may also lead to a better QoL and oral function, since less patients have indication for local PORT while local DFS remains constant. A large randomized controlled trial, currently conducted across eight Dutch centres, is comparing margins, QoL, and oral function between US-guided SCCT and conventional SCCT surgery.

The efficiency of local PORT remains a topic under debate. A systematic review by Brown et al. (118) reports no improvement in local recurrence in patients who had local PORT regardless which stage of disease. Moreover, there is uncertainty about the impact local PORT in patients presenting with close margins and one or more unfavourable histopathological growth factors (43,119,120). In

our log-rank test, stratified on margin status in combination with adjuvant treatment (although both for RR and local PORT) a statistically significant better local DFS was observed for patients with positive margins who received adjuvant treatment vs. those who did not. However, among patients with close margins, no statistically significant difference was observed between those who received adjuvant treatment and those who did not.

From the log-rank tests it can also be concluded that close margin status, with or without local adjuvant radiotherapy, does not lead to worse local control than free margin status. The definition of a close margin, as stated by the NCCN (27) and Royal College of Pathologists (28) remains a subject of debate. Many studies compare histopathological margins with survival to find the optimal cutoff distinguishing between close and free margins (and thus whether to administer adjuvant radiotherapy or a re-resection). However, outcomes differ between 1 mm and > 7.5 mm. A possible explanation might be the variation in methods between those studies (30,43,78,104,106,112). Firstly, the endpoints that are used to determine the cutoff value (e.g. local DFS, locoregional DFS, disease-specific survival and overall survival) differ among studies. Secondly, the presence of (severe) dysplasia in the resection margin being classified as 'positive margin', varies. Thirdly, since all studies analyse retrospective cohorts, it is often unknown how consistently pathological examination was conducted and reported, such as complete microscopical examination and precision in margin reporting. For our study we reviewed all digitalized haematoxylin and eosin slices to be certain that margins addressed with 0.1 mm precision. Lastly, the statistical methods vary between studies. For instance, Kaplan-Meier curves and Cox regression analyses are used to portray survival statistics for groups with a range of margins, while receiver operating characteristic curves are used to display the predictive value (i.e. sensitivity and specificity) of different margins for survival outcomes.

It is notable that the only two local recurrences in the US-cohort most likely originated from the mucosal margins. US helps to visualize the deeper extent of the tumour, thereby enabling the surgeon to achieve an adequate the deep margin. Histologically, both recurrences originated from a dysplastic area, even though the margins at the mucosa were free: one patient had a close deep margin, i.e. 3.9 mm, but it was at the submucosal margin, not the mucosal. These findings suggest that the local recurrences in the US-cohort originated from preneoplastic areas that were already present. This is also known as field cancerisation (35,36) and therefore these recurrences might be considered second primaries. Due to the working mechanisms of US, it is impossible to use US for the detection of in-situ carcinoma or preneoplastic areas. Other techniques, ideally combined with US-guided surgery, may be useful to assess intraoperatively mucosal margins (121).

Of the local recurrences in the conventional cohort, one of five originated from the mucosal tissue. This recurrence is also most likely caused by field can-

cerisation, as the patient had a close margin (i.e. 3.0 mm) and the recurrence originated from a field of severe dysplasia (35). At least three patients had deep recurrences, of which three are most likely caused by positive margins in the submucosal connective or muscular tissue. Only one of these four patients had a deep recurrence despite a free margin status (minimal margin distance 7.1 mm). Three of these 4 recurrences could potentially have been prevented by the use of US guidance.

The impact of other intraoperative margin assessment techniques on local DFS has been investigated as well. This is primarily conducted with frozen section analysis, which is widely acknowledged worldwide as the clinical standard for margin assessment. However, its impact is under debate. In a cohort of 156 head and neck patients, Ettl et al. (61) found that revising positive margins intraoperatively to negative ones, guided by positive frozen section analysis, emerged as the strongest predictor for local recurrence. In a cohort of 108 oral cancer patients, Varvares et al. (107) found that positive to negative revised margins had better outcomes regarding local DFS when compared with close (< 5 mm) margins, but far worse when compared with initially negative margins. The limited impact of frozen sections is most likely caused by the sampling errors, as only a small part of resection specimen and/or wound-bed is sampled (0.1% - 1%) (4). This is underscored by DiNardo et al. (62), who found that frozen sections had a sensitivity of 60% in predicting a positive margin status (i.e. at least one positive margin in the entire specimen) and 34% in predicting a close margin (i.e. at least one close margin in the entire specimen). Upon sampling errors, it is challenging to appoint the location of a positive and close margin towards its corresponding location on the tumour bed, as frozen section samples are mostly small and not anatomically oriented (42). Intraoperative US is able to provide the surgeon real-time feedback, allowing for a direct and more precise removal of additional tissue by keeping the specimen's orientation.

Another survival study on intraoperative margin assessment technique was conducted by Durham et al. (122) which was a multicentre randomized controlled trial where oral cancer patients in the treatment group received surgical guidance by autofluorescence and patients in the control group received conventional surgery. Local DFS and other survival parameters were all similar in both groups. One explanation might be that autofluorescence does not use any contrast agents, requiring subjective interpretation of the fluorescence signal (123). This is also underscored by Durham et al. (122) themselves, as they suggested that the relative inexperience of the participating centres with autofluorescence led to no effect in survival rates. Moreover, since autofluorescence is only able to visualize the mucosal margins, it might add little effect to the surgery, as identifying the deeper extent of the tumour is way more challenging than the superficial extent (123). In contrast, intraoperative US is a margin assessment technique which is able to visualize the deep extent of the entire tumour (52).

Some limitations must be addressed. Firstly, the follow-up period of our US-guided vs. conventional surgery evaluation is limited to 30 months, which is in contrast to other co-variables in our model that could be evaluated until a follow-up period until 60 months. Therefore, the possible effect of US on survival endpoints could have been more pronounced if follow-up period was longer. However, the risk of local recurrence in oral squamous cell carcinoma after 2 years is extremely low (124). Secondly, the low incidence of local recurrences may have led to overfitting in the multivariate Cox regression model. This suggests that parameters such as neck metastases may not be independently correlated with local recurrence. Nevertheless, more studies do address the adverse effect of neck metastases on local DFS (81,116).

In conclusion, US-guided SCCT surgery shows potential in improving local DFS, likely due to a threefold reduction in positive margin status, which is strongly associated with local recurrence. While the reduction in close margin status may be less impactful in preventing recurrence, it still lowers the need for local adjuvant treatment, which potentially improves QoL and oral function. The role of local PORT for patients with close margins and unfavourable histopathological parameters remains unclear. Probably, within this group of patients, subgroups of patients have to be (re)defined: who may benefit (most) of adjuvant treatment and who do not. Since US-guided surgery provides real-time feedback on the tumour's extent, it helps to prevent deep recurrences originating from the muscular tissue, which tend to have a greater impact on survival, QoL and oral function. Because of the relative low incidence of local recurrence in oral cancer, larger cohorts are needed to elucidate the effects of US-guided surgery on local DFS and QoL. These cohorts will possibly be provided by a multicentre randomized controlled trial comparing US-guided and conventional resections of SCCT, currently conducted among eight Dutch cancer centres.

Chapter 6

Impact of ultrasound-guided tongue cancer surgery on margins, quality of life and oral function – methods of a multicentre randomized clinical trial (study design)

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Submitted

Abstract

Objectives: Surgery is the preferred treatment of squamous cell carcinoma of the tongue (SCCT). However, close (i.e. 1-5 mm) or positive (< 1 mm) margins often necessitate local adjuvant treatment, such as re-resection (RR) or postoperative radiotherapy (PORT), which negatively impacts quality of life (QoL). Recent studies suggest that ultrasound- (US) guided surgery increases frequency of free margins (≥ 5 mm) and reduces administration of PORT. The presented protocol is of a Dutch multicentre study that aims to evaluate the impact of US-guided surgery on margin status, administration of local adjuvant treatment, Quality of life (QoL) and survival in SCCT patients.

Methods: This randomized controlled trial (RCT) involves eight centres affiliated with the Dutch Head and Neck Society. Patients with primary cT1–T3 SCCT accessible via a hockey-stick shaped US probe are randomized to either conventional surgery (control-arm) or US-guided surgery. Primary outcome measurements include margin status, local adjuvant treatment rates (RR or PORT), and QoL measured by questionnaires during a 12-month follow-up period. Secondary outcome measurements include oral function test scores and survival parameters that are evaluated after 24-month follow-up period.

Discussion: The RCT that results from this presented protocol may elucidate the impact of US-guided SCCT surgery on the surgical management of SCCT. It is anticipated that an increase of free margin status and reduction of the need for local adjuvant treatment (both adjuvant PORT and RR), along with improvements in oral function and DFS, will be observed using US-guided resections. These outcomes are crucial for assessing the potential benefits of US-guided surgery in the management of SCCT and may dictate future treatment protocols.

Introduction

Squamous cell carcinoma (SCC) of the tongue (SCCT) is the most common type of oral cancer in the Netherlands (21). Its preferred treatment is surgical removal (2). Free margin status, i.e. a minimal histopathological margin distance of ≥ 5 mm is relevant for local control and disease-free survival (DFS) (79). However, resection margins are frequently close (i.e. 1-5 mm) or even positive (< 1 mm). A recent analysis of cT1-cT3 SCCT patients, who underwent surgery between 2014 and 2018 in our centre, revealed 70% close margins and 15% positive margins (113). These results are in line with literature, reporting close margin status up to 45% and positive margin status up to 43%, respectively (4).

Positive margin status in SCCT is a strong indication for local adjuvant treatment, while close margins in combination with certain unfavourable histopathologic growth factors are relative indications (27,43,125). Local adjuvant treatment could either be a re-resection (RR), or postoperative radiotherapy (PORT), which both have disadvantages. RRs often result in uncertainty whether it corresponds exactly with the location of the inadequate margin of the resection specimen (42). Local PORT has several side effects that deteriorate oral functions, i.e. xerostomia, fibrosis, mucositis and possible osteoradionecrosis (44,108). In some cases, adjuvant treatment is not administered because of the patient's wishes or condition. After conventional resection of SCCT in our centre, 30% of the patients received adjuvant treatment, of which 9% RR and 21% local PORT (113). This may be reduced with better resection margin control.

A recent study on image-guided resection of SCCT revealed that the application of ultrasound (US) guidance results in 55% free margin status, 40% close margin status and only 5% positive margins (113). The more than threefold increase in free margin status and the threefold decrease in positive margin status, compared to a cohort of conventionally surgically treated SCCT patients, led to a reduction of the administration of local PORT from 21% to 10%. Since free margins are associated with improved local disease-free survival (DFS), locoregional DFS, and overall survival, US-guided surgery has the potential to enhance patient outcomes. In addition, US-guided surgery could lead to better quality of life (QoL) and oral function due to the reduction of local PORT.

This chapter describes the protocol of a Dutch multicentre randomized controlled trial (RCT) on US-guided surgery in SCCT patients. Its goal is to evaluate the impact of US-guided SCCT surgery on margin status, administration of local adjuvant treatment, QoL and survival.

Methods

Objectives

Primary objectives

The primary objectives of this study are to evaluate whether the findings from a monocentre study on US-guided SCCT surgery — specifically, an increased frequency of free margin status, a decreased frequency of positive margin status, and a reduced need for local adjuvant treatment compared to conventional SCCT surgery — are reproducible in other Dutch Head and Neck centres, and whether these outcomes lead to an improvement in QoL.

Secondary objectives

The secondary objectives of this study are to evaluate if US-guided SCCT surgery leads to an improvement of oral function and survival parameters, i.e. local DFS, disease-specific survival (DSS) and overall survival (OS), when compared with conventional SCCT surgery.

Study design and population

This ‘multicentre tongue resection ultrasound-guided technique’ (multi-TRUST)-study is funded by grant 11906 of the Dutch Cancer Society (KWF) and is approved by the local independent Medical Ethics Review Board (study number NL76681.041.21). It is designed as a non-blinded RCT. The following centres, affiliated with the Dutch Head and Neck Society (NWHHT), participate:

- University Medical Center Utrecht, Utrecht (organising center),
- Antoni van Leeuwenhoek / Netherlands Cancer Institute, Amsterdam,
- Erasmus University Medical Center, Rotterdam,
- Haaglanden Medical Center, The Hague,
- Medisch Spectrum Twente, Enschede,
- Radboud University Medical Center, Nijmegen,
- Rijnstate Hospital, Arnhem,
- University Medical Center, Groningen.

This RCT contains two arms. Patients in the control-arm are conventionally treated according to standard clinical protocol of the participating centres, i.e. resections based on preoperative images, white light visualisation and intraoperative palpation, with optionally intraoperative margin assessment (100). Patients in the US-guidance-arm are treated additionally with US-guided surgery. Patient inclusion is conducted consecutively and independent of the inclusion frequency of the participating centres.

Patients are eligible for inclusion if diagnosed with a primary cT1-T3 (TNM 8th) SCCT (17). Tumours must be reachable with a hockey-stick shaped 16 MHz US-

probe (L16-4Hs, Mindray Medical, Shenzhen, China) and must be completely detectable as a hypoechoic area, i.e. a dark appearance on US images (51). Routine preoperative imaging of the centre in both arms is allowed. Patients must undergo surgery with curative intent under general anaesthesia.

Patients are excluded if 1) diagnosed with a cT4a tumour, 2) the tumour exceeds significantly to the floor of mouth, 3) treated earlier for ipsilateral tongue cancer (surgery with or without local PORT).

After informed consent, the patient is stratified for each participating centre and randomized according to a permuted block design, using block sizes of two and four. Blinding is impossible, since the surgeon is aware whether US-guidance is applied.

Surgical procedure

The surgical procedure in the US-guidance-arm consists of an in-vivo and an ex-vivo part. During the in-vivo part, the tumour is visualized by the abovementioned hockey-stick shaped probe as a hypoechoic area. Hence, the surgeon is able to determine the morphology and deep extension of the tumour. While performing the resection, the cutting plane is made visible by creating a small layer of air or by using a flat instrument between the partially resected specimen and wound bed. This produces a hyperechoic line (i.e. bright appearance on US images). By capturing the tumour border and resection plane in one image it is possible to measure tumour-free margin distance, providing the surgeon with real-time feedback. According to standard clinical care, the surgeon aims for a 10 mm surgical margin distance to achieve a histopathological margin distance of at least 5 mm (38). US-measured margin distances are recorded for the anterior, deep central and posterior portion of the resection. The ex-vivo part is applied directly after the resection for a final check on inadequate margins. The margin distances of the anterior, posterior, craniomedial, caudolateral and deep central part of the resection are recorded. This can either be done by imaging the resection specimen on a gloved hand or imaging it in a container of saline, to prevent deformation. Surgeons are advised to adjust the resection plane or perform an immediate intraoperative re-resection (iRR) in case they find a US-determined margin < 7 mm in-or ex-vivo. This recommendation is based on a previous study that demonstrates that less close and more free margins can be achieved if the US-determined tumour-free margin is increased by several millimetres. The locations for iRRs are oriented by a variant of ‘parallel tagging’ introduced by van Lanschot et al. (97). This involves the placement of paired sutures in at least four quadrants on both sides of the (sub)mucosal cutting plane. The sutures serve as landmarks to help the surgeon in relocating the exact spot of a US-determined inadequate margin within the wound bed.

Surgeons are allowed to perform iRRs in the conventional group as well, either indicated by digital palpation of the resection specimen, examination under white light or frozen sections.

Pathological examination

Histopathological examination is carried out by cutting formalin-fixed resection specimens in ~3-5 mm thick slices, in an anterior to posterior direction. Slice thickness is estimated by dividing the reported length of the specimens by the reported number of slices. Macroscopical margins are determined for five regions by 1) multiplying the number of tumour-free slices with the mean slice thickness for the anterior and posterior region and by 2) subdividing each slice in craniomedial, deep central and caudolateral region and measuring the margin with a ruler. In case an inadequate margin is suspected in one of these locations, a 4 µm section of the corresponding slice is obtained and stained with haematoxylin-eosin (HE) and reviewed microscopically to evaluate adequateness. In case microscopical examination redefined the macroscopical margin at that location, the microscopical margin is used for further analysis. Centres are free in deciding whether to microscopically analyse all slices taken from the resection specimen or to analyse at least the slices that seemed to have a macroscopical < 5 mm margin. Special attention is given to the iRRs, in order to verify whether it changes the margin status.

Follow-up

The following questionnaires, measuring general and head and neck related QoL, are administered digitally before treatment and three, six, and twelve months after treatment.

- EORTC QLQ-C30: Evaluates five functional scales (physical, role, emotional, cognitive, and social), three symptom scales (fatigue, pain and nausea/vomiting), a global health status scale, six single items (dyspnoea, appetite loss, insomnia, constipation, diarrhoea, and financial difficulties), with a maximum score of 100 (126).
- EORTC QLQ-H&N35: Assesses pain, swallowing, cognitive issues, speech, eating in public, social contact, sexuality, dental problems, open mouth, dry mouth, sticky saliva, cough, malaise, analgesic use, nutritional supplements, tube feeding, and weight changes, with a maximum score of 100 (126).
- EQ-5D-5L: Assesses five questions about mobility, self-care, usual activities, pain/discomfort and anxiety/depression and uses a visual analogue scale, ranging from 0 to 100 (127).
- Swallowing Quality of Life Questionnaire (SWAL-QOL): A 44-item questionnaire covering 10 domains including food selection, eating duration, eating desire, fear, burden, mental health, social functioning, communication, sleep, and fatigue (128).
- Speech Handicap Index (SHI): A 30-item questionnaire addressing speech problems (129).

Groningen Radiation-induced Xerostomia (GRIX): A 14-item questionnaire focusing on patient-rated xerostomia and sticky saliva, particularly in relation to radiation techniques aiming to reduce xerostomia (130).

In the organising centre, oral function tests are performed simultaneously with the questionnaires, as assessed by the function rehabilitation outcome grade-(FROG-) scale. This is a health care provider-rated measure assessing the function of the shoulder, mandible, teeth, lip, tongue, oropharynx and saliva (128).

Outcome measurements

Primary outcomes

Margin status of both study arms is drawn from the standard histopathological reports to evaluate whether US-guided SCCT surgery leads to more free margin status. Data regarding local adjuvant treatment via a RR or local PORT is extracted from records of the patient's electronic database to evaluate whether US-guided SCCT surgery leads to less local adjuvant treatment. To evaluate whether US-guided SCCT surgery leads to higher QoL, scores of each aforementioned QoL questionnaire are assessed before surgery and at three months, six months, and twelve months after surgery. Relative change in the results of QoL questionnaires between different follow-up periods is compared between both study arms. As the standard clinical care of different centres may differ (i.e. whether intraoperative margin assessment is applied (100)) the primary outcomes are assessed for the complete study cohort and for each participating centre.

Secondary outcomes

Scores of the FROG-scales are assessed before treatment and three, six, and twelve months after treatment, in order to compare oral function between a US-guided surgery strategy and a conventional surgery strategy over time. The relative change in oral function between different follow-up periods is compared between both arms within the organizing centre. Survival parameters (i.e. DSS, DFS and OS) are evaluated after follow-up periods of 24 months and derived from the patient's electronic database to evaluate whether US-guided surgery leads to better survival parameters.

Power analysis

A power analysis was conducted for the primary outcome of margin status and administration of local adjuvant treatment. All analyses were calculated with the following parameters, common in medical literature; a two-sided alpha of 0.05, beta of 0.20 and power of 0.80. Calculations were performed with "clinical sample size calculator" (131).

Smits et al. (4) reported 29% of their cohort received local PORT. Our previous monocentre study reported 10% local PORT (113). To demonstrate statistical significance for the expected difference in local PORT administration between the two study arms, 67 patients are required in each arm. An estimated drop-out of ~10% yields a need for 150 patients in total.

Interim analysis

To determine when the interim analysis needed to be conducted, another power analysis was conducted with a two-sided alpha of 0.05, beta of 0.20 and power of 0.80. Calculations were performed with “clinical sample size calculator” (131).

The interim analysis is a non-inferiority test to the impact of US-guided surgery on margin status. If there is a true difference of 40% in favour of US-guided surgery (reflecting the difference in free margin status from the previous studies used for the power analysis (4,113) then 24 patients are required to be 80% sure that the upper limit of a two-sided 90% confidence interval will exclude a difference in favour of the conventional cohort of more than 5%. Since all surgeons of the participating centres need to go through a learning curve, an additional three patients per centre are added. Hence the analysis will be performed after 48 patients. If US-guided surgery appears to be inferior, the study protocol is re-evaluated to decide whether the study must be terminated.

Statistical analysis

It is hypothesized that US-guided SCCT surgery results in a better free margin status with less positive or close margins and less local adjuvant treatments. A chi-squared test must confirm whether frequencies are significantly different. If needed, a Fisher’s exact test will be performed if $n < 5$. QoL and oral function will be compared between both study arms with a Wilcoxon signed-rank test, for different follow-up times. Changes in scores over the follow-up period will be analysed with Kruskal-Wallis tests. Possible confounders that could influence QoL will be analysed by stratifying the data by a possible confounder and performing Kruskal-Wallis test between the stratified groups. Multivariate linear regression will correct for possible confounders, if necessary.

Discussion

The most challenging aspect of SCCT surgery is achieving a free margin status. Most agree with the fact that a < 1 mm margin is classified as positive (28), because it is associated with worse DFS (30,61,106,107). However, the 5 mm cutoff for a free margin status has been a subject of debate (31,78,104,106); several studies consider a 3 mm tumour margin as adequate as 5 mm for early

oral cancers (43). Nevertheless, the 5 mm adequate margin is still established in the current clinical guidelines (27,28). These guidelines strongly advise adjuvant treatment in case of positive margins. In case of a close margin status, adjuvant treatment may be considered if several unfavourable histopathological growth factors are present in the tumour front. Consequently, less adjuvant treatment is indicated if more margins are free.

If US-guided surgery leads to less adjuvant treatment, it could also lead to better QoL and oral function among tongue cancer patients. Especially local PORT has an adverse effect on QoL. As the target tissue of local PORT is nearby or at the location of the salivary glands and jaw bones, there is a risk of side effects such as xerostomia, mucositis and osteoradionecrosis (44,108). These comorbidities can severely impair oral functions such as speech and swallowing. As a result, together with the burden of multiple fractions, patients who undergo local PORT may experience a lower QoL than those who do not receive local PORT.

One of the objectives of this RCT is to measure the effect of US-guided SCCT surgery on oral function and (oral function related) QoL. It is expected that the US-guidance-arm experiences less deterioration of QoL in general (measured with the EORTC QLQ-C30 and EQ-5D-5L) and head and neck function related QoL (measured with the EORTC QLQ-HN35), oral function related QoL (measured with SWAL-QOL, SHI and GRIX) and objectively measured oral function (measured by FROG-scale) when compared with the conventional cohort. As these questionnaires and objective tests have a focus on a broad range of parameters, a multiple regression analysis will allow to explore a wide range of QoL and oral function related outcomes that could be associated with local adjuvant treatment (i.e. both PORT and RR). Moreover, the broad range may also allow to correct for many confounding variables, offering a comprehensive understanding of the effect of US-guided surgery.

Another objective that is investigated is local DFS. As positive margins are the strongest predictors for local recurrence, US-guided surgery might have the ability to influence local DFS, as our previous study suggests that US-guided surgery reduces positive margin status threefold (43). However, the difference in local DFS, when compared with conventional surgery might be subtle or even non-significant. One reason is that local adjuvant treatment, which is administered in standard clinical practice when inadequate margins are found, could compensate for a deterioration in DFS. Another reason might be that positive margin status in SCCT has a relatively low prevalence and the number of events of local recurrence might be too low and require a larger number of patients.

A previous monocentre study in the organizing centre assessed the accuracy of ex-vivo US to identify inadequate (< 5 mm) margins on the resection specimen. Receiver operator characteristic (ROC) analysis demonstrated

that US guidance can substantially reduce the occurrence of histopathological margins < 5 mm when the US-determined tumour-free margin is increased by several millimetres. For this reason, we advise to perform an iRR if the US-determined free margin appears to be < 7 mm (61,107).

As this study registers measurements both performed by US and histopathology (i.e. tumour thickness and margins at the anterior, posterior, craniomedial, caudolateral and deep central locations), it enables additional analyses regarding the accuracy of US to assess tumour thickness and margin adequateness. For example, a new ROC analysis performed on the US measurements in the US-guidance-arm can elucidate the most appropriate US-determined cutoff value for obtaining histopathological ≥ 5 mm margins. Also, the correlation between the accuracy of US in measuring tumour thickness and measuring margin distance can be established.

Moreover, the frequency of correctly placed iRR can be analysed. In our previous study, only 23% of the iRRs were executed correctly which may be explained by the difficult relocation of inadequate margins to the wound bed. Several studies found that positive margins that were thought to be revised to free by RR, still had worse local DFS when compared with close and free margins (61,107). Our protocol adopted a simplified version of the ‘parallel tagging’ technique, which may decrease this relocation problem (97). However, there is still a risk iRRs might not be extensive enough. One possible conclusion might be that a first-time adequate SCCT resection, without the need for additional surgery, always leads to better DFS, even when (ex-vivo) US-indicated iRRs are highly accurate. As such, in-vivo US also has the potential to establish this ‘first-time right’ principle (42).

Several smaller studies have been conducted to assess the feasibility of US-guided surgery (53–57). These initial studies laid the groundwork for three larger, comparative studies that evaluated the outcomes of US-guided surgery cohorts against conventionally treated cohorts. One study was conducted in our own centre and showed a more than threefold increase of free margin status and a threefold decrease in positive margin status (4). Bulbul et al. (90) and Nilsson et al. (114) found an increase in free margin status, i.e. 70% vs. 48% and 59% vs. 41%, respectively. However these studies showed no statistically significant differences when comparing US-guided surgery and conventional surgery. This difference is possibly not significant due to lack of power and by the fact that the authors already established high frequency of free margins with their conventional technique. Larger cohorts could result in statistically significant results.

The aforementioned comparative studies (90,114) compared cohorts from different time periods, which could lead to data being confounded by the surgeon’s experience and variations in clinical (both surgical and histopathological) protocols over time. This is the first fully prospective RCT to investigate the value of US-guided surgery, which should eliminate these confounding factors.

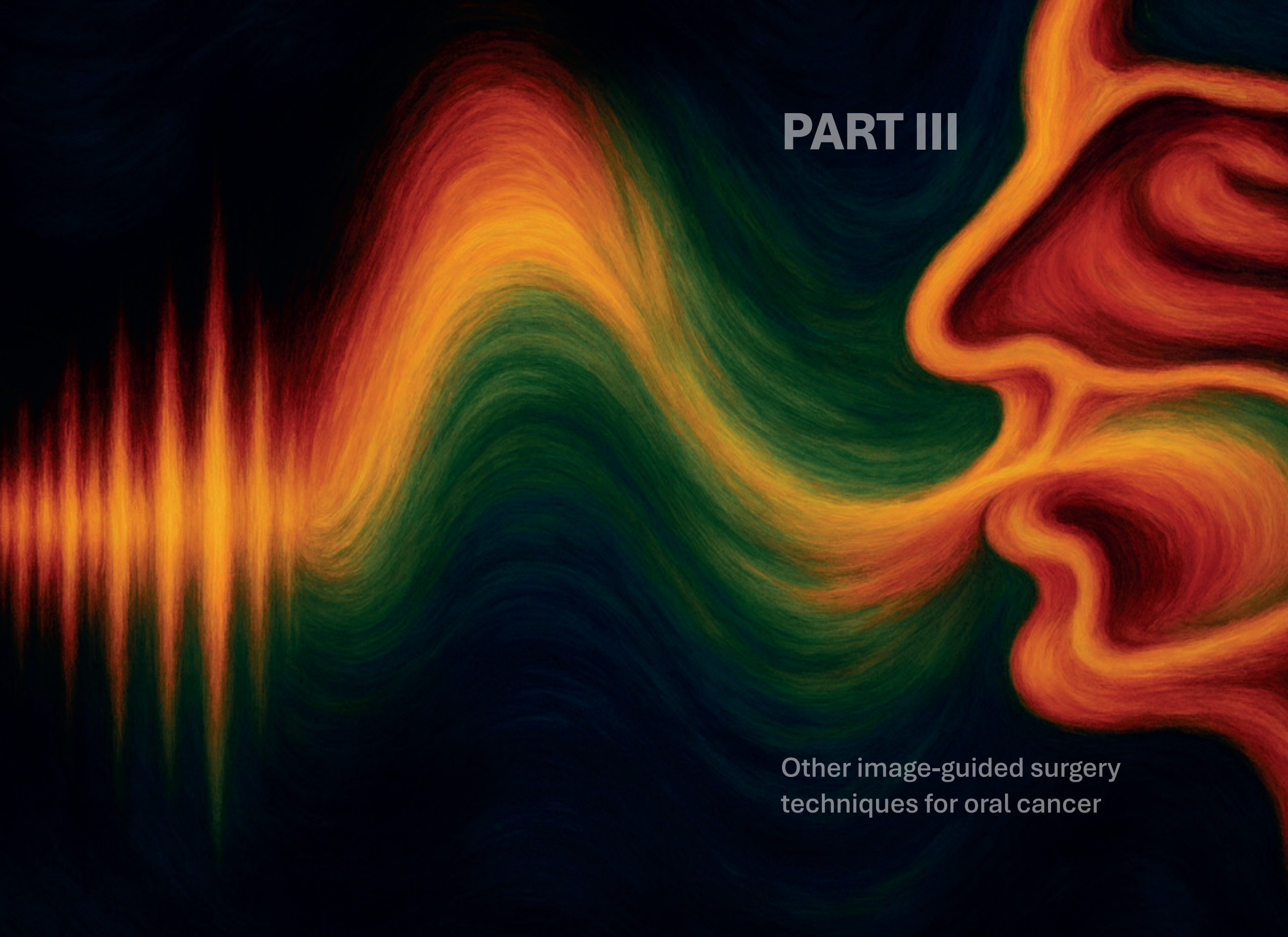
This RCT also differs from previous studies to US-guided SCCT surgery in its effort to acquire a homogeneous cohort. We exclude patients with large cT4a tumours extending beyond the mobile tongue and invading the floor of mouth, as well as those with lower T-stage tumours not clearly originating from the oral tongue’s mucosa. Additionally, patients previously treated for oral cancer are excluded. These exclusions are essential because scar tissue from prior treatments or the presence as non-muscle tissues such as salivary glands or bone, may obscure the tumour borders on US images. This effect was identified in one of our previous single-arm studies on US-guided surgery (83).

Final results in favour of US-guided surgery must be interpreted with caution, as RCTs are highly protocolized and might not always reflect real-world data (132). Moreover, the patient group is relatively homogeneous when compared to the broader SCCT population. However, performing a study in collaboration with the vast majority of the Dutch head and neck cancer centres will hopefully narrow the difference from real-world outcomes. Additionally, the prospective randomized nature of this study carries a risk that the control-arm may be influenced by the US-guidance-arm. Intraoral US constantly confronts the surgeon with the cutting plane’s position relative to the tumour border, creating awareness of the surgical technique during conventional surgery if the patient is not allotted to US-guidance. This can also result in a better margin status in the conventional cohort, masking the effect of US-guided SCCT surgery.

A possible limitation of this study might be the relatively large number of participating centres with a relatively small patient population. As both arms are filled consecutively, centres that treat lower volumes of SCCT patients might be less presented in the cohorts. Surgeons might not go fully through the learning curve of US-guided surgery. This was also suggested by an RCT on the effect autofluorescence-guided surgery on margins of the oral mucosa; margin status of both groups did not differ, possibly due to inexperience of the participating centres (122). On the other hand, multiple feasibility studies with a small population, ranging between 4 and 12 patients, already concluded that US-guided surgery has a positive effect on free margin status, indicating that not much experience is needed to complete the learning curve (53–57).

Another possible limitation is the fact that the participating centres have differences in their standard clinical care, which might affect outcomes in the conventional group. For instance, two participating centres use a specimen-driven intraoperative margin assessment method in their standard clinical protocol (100). This involves cutting the fresh resection specimen by the pathologist during surgery at margins that seem inadequate, followed by macroscopical examination. In case the margin is confirmed to be inadequate, an iRR is performed, oriented by parallel tags. Therefore, stratifying outcomes per participating centre is relevant to understand the effects of US-guided surgery on different clinical protocols and thus the whole US-guidance-arm.

In conclusion, the RCT that results from this presented protocol may elucidate the impact of US-guided SCCT surgery on the surgical management of SCCT. It is anticipated that increase of free margin status and reduction of the need for local adjuvant treatment (both local PORT and RR), along with improvements in oral function and DFS, will be observed. These outcomes are crucial for assessing the potential benefits of US-guided surgery in the management of SCCT and may dictate future treatment protocols.



PART III

Other image-guided surgery
techniques for oral cancer

Chapter 7

**Beneath the surface: a systematic review
on intraoperative imaging techniques for
deep margin assessment in oral squamous
cell carcinoma**

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Abstract

Resection margins of oral squamous cell carcinoma (SCC) are often inadequate. A systematic review on clinical intraoperative whole specimen imaging techniques to obtain adequate deep resection margins in oral SCC is lacking. Such a review may render better alternatives for the current insufficient intraoperative techniques: palpation and frozen section analyses (FSA). This review resulted in ten publications investigating ultrasound (US), four investigating fluorescence, and three investigating MRI. Both US and fluorescence were able to image the tumour intraorally and perform ex-vivo imaging of the resection specimen. Fluorescence was also able to image residual tumour tissue in the wound bed. MRI could only be used on the ex-vivo specimen. The 95% confidence intervals for sensitivity and specificity were large, due to the small sample sizes for all three techniques. The sensitivity and specificity of US for identifying < 5 mm margins ranged from 0 to 100% and 60 to 100%, respectively. For fluorescence, this ranged from 0 to 100% and 76 to 100%, respectively. For MRI, this ranged from 7 to 100% and 81 to 100%, respectively. US, MRI and fluorescence are the currently available imaging techniques that can potentially be used intraoperatively, and which can image the entire tumour-free margin, although they have insufficient sensitivity for identifying < 5 mm margins. Further research on larger cohorts is needed to improve the sensitivity by determining cutoff points on imaging for inadequate margins. This improves the number of adequate resections of oral SCCs and paves the way for routine clinical implementation of these techniques.

Introduction

The management of oral cavity cancer is complex due to the potential functional and aesthetic consequences of treatment in this area. Surgery is the preferred treatment of choice in most cases. Its goal is complete removal of the tumour with adequate tumour-free margins (2,27). Inadequate resection margins are associated with poorer clinical outcomes (133,134). In cases of inadequate deep resection margins, re-resection or local adjuvant (chemo)radiotherapy might be indicated (27,135). Unfortunately, a re-resection in a second tempo comes with relocation problems, especially since the wound bed has usually healed (100). Furthermore, it requires a second scheduled surgery under general anaesthesia. Local adjuvant radiotherapy could result in morbidities such as mucositis, fibrosis, osteoradionecrosis and xerostomia (98,99).

The definition of an adequate margin remains a topic of discussion and ranges from > 0 mm to > 7 mm in literature (136). Most commonly, histological margins are divided into positive (< 1 mm), close (1-5 mm), and free (≥ 5 mm) (28,137). Especially, obtaining free deep resection margins is challenging. Literature reports that resection margins are < 5 mm in 30-85% of the procedures, possibly because detailed intraoperative feedback is lacking (4). In a conventional setting, the deep margin can be estimated intraoperatively by usage of preoperative imaging, visual inspection, and palpation. Frozen section analysis (FSA), utilized by many surgeons, allows intraoperative analysis of resection margins for residual tumour tissue. FSA can be performed on the tumour bed or on the specimen. With FSA however, one samples only a small fraction of the entire margin. Therefore, this technique is prone to false-negative results (4,95). Furthermore, in the case of a positive FSA, relocating the original spot for a re-resection is difficult (138). Therefore, initial positive margins, regardless of re-resections based on FSA, lead to worse outcomes than initially free margins (60,61,139). The ideal intraoperative imaging technique for oral squamous cell carcinoma (SCC) is able to guide the resection real time and is applicable for both mucosal, submucosal, deep as well as bony margins.

There are multiple (systematic) reviews that give an overview of available intraoperative techniques for deep-margin assessment in patients with oral SCC. However, some reviews discuss techniques that are based on random sampling, which still may lead to sampling errors and location problems, as witnessed in FSA. Other reviews focus on techniques able to differentiate SCC from healthy tissue but do not measure the extent of the tumour-free margin. Furthermore, some reviews investigate either other sites than the oral cavity, only compare two techniques, or focus on the superficial (sub)mucosal margins. (95,96,140,141)

This systematic review aims to outline and compare the clinical intraoperative imaging techniques that are currently being investigated to obtain free deep surgical resection margins in patients with oral SCC. It focusses on the ability

of the technique to identify, localize, and estimate the extent of the tumour-free margin by imaging the margins of the whole specimen.

Materials and methods

This systematic review was carried out according to the Preferred Reporting Items for Systematic Reviews (PRISMA) guidelines (142).

Eligibility Criteria

A publication was considered eligible in case 1) the study population consisted of patients with a SCC of the head and neck area containing a sub-group of oral cavity SCC; and 2) it analysed a technique that aimed to assess the extent of the entire resection margin; and 3) it was used or will be used intraoperatively (i.e. in theatre directly before the incision, during the resection or immediately after the resection) on the fresh specimen; and 4) the margins were compared to the histological margins; and 4) the number of true positives (TP), false positives (FP), false negatives (FN), true negatives (TN), the number of free margins, or the number of positive margins were mentioned or could be extracted from the publication; and 5) the publication focused on deep margin assessment.

Exclusion criteria were: 1) publications before 2010; 2) non-clinical studies; 3) publications that described techniques that only used white light for tumour/margin visualisation e.g. trans-oral robotic surgery (TORS) without visual enhancement; 4) publications describing head and neck cancers with < 50% oral cancers or without subgroup analysis of oral cancer in the intervention group; 5) techniques that only investigate bony margins; 6) publications in languages other than English, Dutch, or German; 7) techniques that require the specimen to be cleaved; 8) dose-finding or dose-escalating studies.

Search strategy

A systematic search for publications was conducted in PubMed and Embase on August 31st, 2023 (KK). In PubMed, the search term focused on Title, Abstract, and MeSH-terms and included carcinoma, all subsites of the oral cavity and margins of excision. Moreover, the terms “thickness” or “depth of invasion” were also included. The same search terms were used in Embase, but instead of the aforementioned MeSH-terms, the “explode function” was used. The search syntax is shown in supplementary materials.

EndNote (Version 19.3.3, Clarivate Analytics, Philadelphia, PA, USA) was used to de-duplicate using the method described by Bramer et al. (143). Subsequently, data was exported to Rayyan QCRI (Hamad Bin Khalifa University, Ar Rayyan University, Qatar). At least two of the three screening authors (CA,

KK, and RN) independently screened all titles and abstracts for relevance using predetermined inclusion and exclusion criteria and achieved consensus by discussion. The remaining publications were included or excluded by reading full texts by two screening authors (CA, KK). A reference and citation check were performed on the selected publications to ensure the whole field of interest was covered.

Critical appraisal

The two screening authors (CA, KK) critically appraised the included publications separately using the Quality Assessment of Diagnostic Accuracy Studies (QUADAS-2) tool (Whiting, 2011). The following signalling questions were utilized:

Risk of bias: 1) Patient selection: did the study consist of a consecutively or randomly selected patient cohort? Was a relevant control group utilized? Were inappropriate exclusions avoided? 2) Index test: was the index test interpreted without knowledge of the reference standard? 3) Reference test: was the reference standard interpreted without knowledge of the index test? 4) Flow and timing: were the reference standard and index test conducted equally for all patients? Were all patients included in the statistical analysis?

Applicability was evaluated based on the first three items and their criteria: 1) Patient selection: did the study include both small (T1-T2) and large (T3-T4) oral SCCs? 2) Index test: did the publication give a clear definition of a positive index test? Could a positive index test be evaluated objectively? If applicable, was a clear description of the used dosage or devices given? 3) Reference test: was the definition of a free margin ≥ 5 mm? If frozen sections were applied, were they guided by the imaging technique?

All items were scored with 2 points when they complied, 1 point when there was unclarity, and 0 points when they did not comply. Each category was scored by summing the points and dividing them by the number of items. The scores were considered low if 0-0.5, intermediate if 0.6-1.4, and good if 1.5-2.0 (Figure 2).

Data extraction

The following information was extracted from the included publications: year of publication, imaging technique, subsite of the oral cavity, study methodology (prospective or retrospective), assessed margin, in- or ex-vivo measured, number of included tumours, number of measured margins, surgical margin aim, immediate revision, definition histological positive margin, definition histological free margin, TP, FP, FN, TN, and number of tumours with positive or free margins (whole specimen or margin-based). The definition of a TP was an inadequate margin based on the imaging technique and confirmed by histology. The definition of a TN was an adequate margin based on the imaging technique and confirmed by histology. An adequate margin was defined as ≥ 5 mm, unless

otherwise stated. If possible, the number of TP, TN, FP, FN, and positive and free margins were re-calculated for ≥ 5 mm. Some authors were contacted and requested to elaborate on their results. The sensitivity, specificity, and forest plots were calculated by using MetaDTA: Diagnostic Test Accuracy v2.0.5 (144,145).

Results and technique discussion

Search strategy

The search yielded 19,656 records (Figure 1). After removing duplicates, records in a language other than English, Dutch, or German, and non-original publications, 9,284 records remained. These were screened on title and abstract according to the predetermined inclusion and exclusion criteria. The full texts of the remaining 164 publications were screened, and 17 publications were included in this review. No additional publications were found after checking the references of the included studies.

Critical Appraisal

The 17 included studies were scored on risk of bias and applicability to our review question according to QUADAS-2 (Figure 2). There were no studies with a high risk of bias in the categories 'patient selection', and 'flow and timing'. In the study of Steens et al., risk of bias was suspected because the index test results were interpreted in a non-blinded fashion (146). In four studies, the reference standard was not blinded for the index test results, potentially resulting in bias for the category 'reference standard' (57,83,113,147). Only three studies mentioned that the reference standard was blinded for the index test results (59,148,149).

The applicability of the category 'patient selection' was intermediate or good for all the studies. The applicability of the category 'index test' was intermediate in all the studies because most index tests were subjective and therefore operator dependent. Only the index tests of studies investigating fluorescence could potentially be objective; however, none of the studies predefined the cutoff value for a positive test beforehand (149,150). The applicability of the category 'reference standard' was low for Wu et al. because their definition of a free margin was not described and because FSA was not guided by the imaging technique, which made it unclear whether these free margins were achieved due to the index test or the FSA (151).

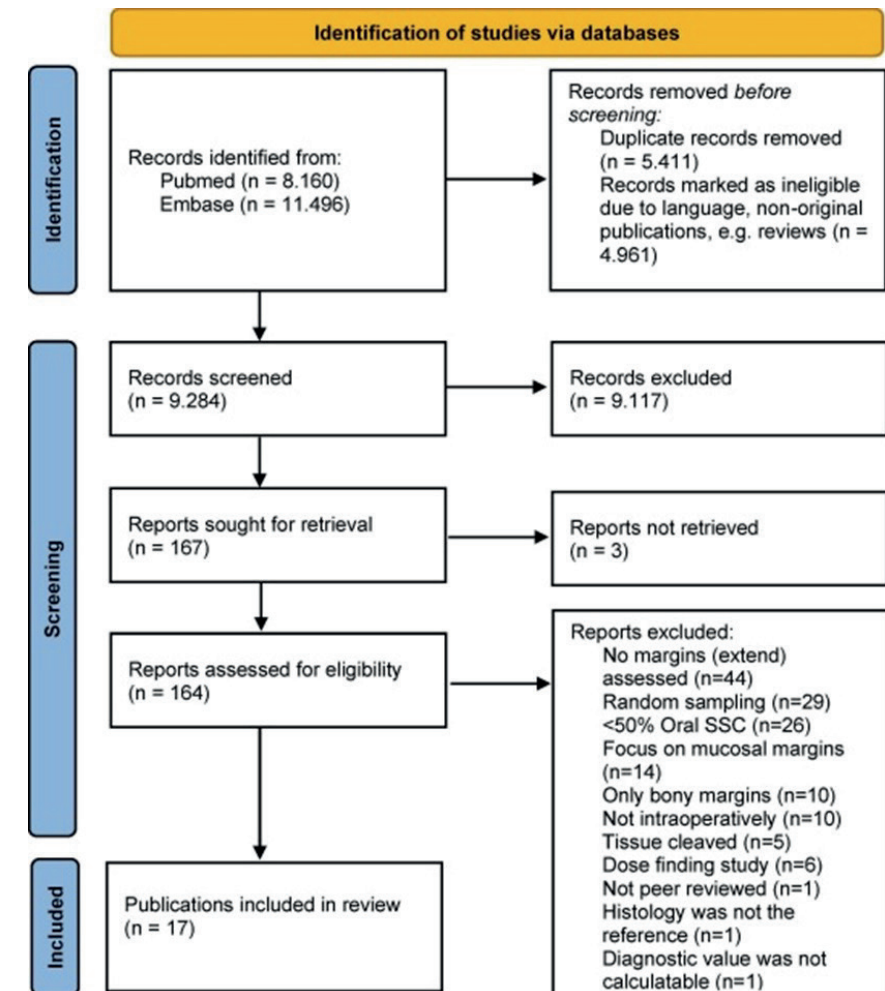


Figure 1: Prisma chart for inclusion and exclusion of publications. From, Page, 2021 (142).

Imaging techniques

Three different imaging techniques emerged from our search and are described separately in this systematic review: ultrasound (US), fluorescence, and magnetic resonance imaging (MRI).

Ultrasound

US is an imaging technique that can determine the tumour thickness and the deep border of the tumour accurately (52). There are two methods for intraoperative US imaging: visualisation of the surgical resection margin in-vivo to adjust the resection plane if needed, and ex-vivo imaging of the specimen to determine the margins and the possible need for an immediate re-resection.

	Risk of Bias			Applicability		
	Patient Selection	Index Test	Reference Standard	Flow and Timing	Patient Selection	Index Test
Ultrasound	(Au, 2023) (Adriaansens, 2023) (Nilsson, 2022) (de Koning, 2022) (Bekedam, 2021) (Bulbul, 2021) (de Koning, 2021) (Brouwer de Koning, 2020) (Tarabichi, 2018) (Kodama, 2010)	(Au, 2023) (Adriaansens, 2023) (Nilsson, 2022) (de Koning, 2022) (Bekedam, 2021) (Bulbul, 2021) (de Koning, 2021) (Brouwer de Koning, 2020) (Tarabichi, 2018) (Kodama, 2010)	(Au, 2023) (Adriaansens, 2023) (Nilsson, 2022) (de Koning, 2022) (Bekedam, 2021) (Bulbul, 2021) (de Koning, 2021) (Brouwer de Koning, 2020) (Tarabichi, 2018) (Kodama, 2010)	(Au, 2023) (Adriaansens, 2023) (Nilsson, 2022) (de Koning, 2022) (Bekedam, 2021) (Bulbul, 2021) (de Koning, 2021) (Brouwer de Koning, 2020) (Tarabichi, 2018) (Kodama, 2010)	(Au, 2023) (Adriaansens, 2023) (Nilsson, 2022) (de Koning, 2022) (Bekedam, 2021) (Bulbul, 2021) (de Koning, 2021) (Brouwer de Koning, 2020) (Tarabichi, 2018) (Kodama, 2010)	(Au, 2023) (Adriaansens, 2023) (Nilsson, 2022) (de Koning, 2022) (Bekedam, 2021) (Bulbul, 2021) (de Koning, 2021) (Brouwer de Koning, 2020) (Tarabichi, 2018) (Kodama, 2010)
Fluorescence	(Wu, 2022) (Pan, 2020) (de Wit, 2023) (Filip, 2023)	(Wu, 2022) (Pan, 2020) (de Wit, 2023) (Filip, 2023)	(Wu, 2022) (Pan, 2020) (de Wit, 2023) (Filip, 2023)	(Wu, 2022) (Pan, 2020) (de Wit, 2023) (Filip, 2023)	(Wu, 2022) (Pan, 2020) (de Wit, 2023) (Filip, 2023)	(Wu, 2022) (Pan, 2020) (de Wit, 2023) (Filip, 2023)
MRI	(Giannitto, 2021) (Heidkamp, 2020) (Steens, 2017)	(Giannitto, 2021) (Heidkamp, 2020) (Steens, 2017)	(Giannitto, 2021) (Heidkamp, 2020) (Steens, 2017)	(Giannitto, 2021) (Heidkamp, 2020) (Steens, 2017)	(Giannitto, 2021) (Heidkamp, 2020) (Steens, 2017)	(Giannitto, 2021) (Heidkamp, 2020) (Steens, 2017)

Figure 2: Critical appraisal according to QUADAS-2. The green checkmark indicates low risk of bias or good applicability, the yellow exclamation mark indicates intermediate risk of bias or intermediate applicability, and the red cross indicates high risk of bias or low applicability.

Ten studies that investigated the use of intraoperative US were included in this review (Table 1). Nine out of 10 investigated 2D US and one study investigated 3D US (103). Eight out of 10 studies used in-vivo and ex-vivo imaging and two studies only used ex-vivo imaging on the specimen (66,103). All studies used 5 mm as a cutoff value for free margins, which made it possible to compare these studies. Nevertheless, some studies strived for a certain minimal US measured margin and considered a re-resection in case of a smaller measured margin (55,57,90,114,148). Therefore, we assumed this minimal margin was achieved by doing an immediate re-resection in case of a smaller measured margin, unless otherwise stated. Five studies focused on the ability of US to image the deep margin (55,57,66,90,114). Bulbul et al. and Nilsson et al. used US to image the deep resection plane and also described the overall margin outcome. The other five studies imaged both deep and superficial margins (83,103,113,147,148). Five studies aimed for a ≥ 5 mm margin from the tumour during the surgery (83,113,114,147,148) and 3 studies aimed for > 10 mm (55,90,101). The acquisition time varied between 5 and 10 minutes for the studies that used US in the operating theatre (66,83,90,113,114). For one study, this was 33 minutes, because the imaging technique consisted of manual segmentation of a volumetric dataset to create a US-based 3D margin model (103). The 10 patients from the pilot study of de Koning et al., 2021, had a 100% overlap with the patients in the study of de Koning et al., 2022. There was also a 23 patient overlap between Au et al. and Bulbul et al. (90,148). These 10 and 23 patients were only included once when calculating the total number of tumours and margins, as well as when determining the medians. In total, 169 tumours and 371 margins were analysed by US and compared to histology. For the included US studies, the median percentage tumours with a ≥ 5 mm overall margin was 59% (range: 8% - 63%) for the US group, while it was 41% (range: 16% - 48%) in the control group (90,103,113,114,147,148). The median percentage of tumours with a ≥ 5 mm deep margin was 76% (range: 38% - 100%) for the US group, while this was 59% (range: 56% - 67%) in the control group (55,57,66,90,113,114). All studies investigated SCC of the tongue, except for Adriaansens et al., who focused on SCC of the buccal mucosa, a probably more difficult to visualize tumour location (147). They found that one of the 13 patients with buccal mucosal cancer had a tumour with free margins and that 60% of the margins (five margins per specimen) were free. Without this study, the median for overall free margins in tongue tumours in the US group was 60% (range: 55% - 63%) and this was 82% (range: 66% - 100%) for deep free margins.

For all the studies it was possible to calculate the sensitivity and specificity for inadequate margins (< 5 mm), except for the study of Kodama et al. (Table 2, Figure 3). Kodama et al. placed a needle 10 mm from the deepest

portion of the invasive front of the tumour under US guidance, in four patients. All margins were clear in those 4 patients; therefore, the sensitivity was not calculable, and the specificity was 100%.

Four studies compared an intervention group to a control group. De Koning, 2022 et al. found the largest reduction in positive margins, from 15% in the control group to 5% in the intervention group, and the most increase in free margins from 16% to 55%, respectively (113). However, the frequency of free margins in the control groups of the studies of de Koning et al. (16% and 17% free margins) were relatively low compared to the control groups of Nilsson et al. and Bulbul et al. (41% and 48% free margins respectively), meaning there was more room for improvement in the study of de Koning et al.

Bekedam et al. had a sensitivity of 100% when taking the smallest distance of different US measurements into account; this was the highest sensitivity of the studies investigating US. However, they only investigated 8 tumour margins. The position of the transducer was tracked, making a 3D reconstruction of the US images possible. However, multiple US acquisitions were performed, introducing variability in the 3D volumes of the same specimen, and the successive manual segmentation also introduced variability (103). Furthermore, the range of the average resection margin was large.

Fluorescence

Four studies investigating the use of fluorescence were included in this systematic review (Table 3) (149–152). Two studies used indocyanine green (ICG) fluorescence intravenously (150,151). They used ICG as a contrast agent, which accumulates in abnormal tissues with enhanced permeability and retention, such as tumour tissue (150,151) When excited by light of a specific wavelength, ICG re-emits light in the near-infrared spectrum, which can be detected by near-infrared fluorescence (NIF) imaging equipment (151). There are also contrast agents that use antibodies that bind to tumour tissue. These antibodies are coupled with a fluorescent dye. Cetuximab-800CW is an epidermal growth factor receptor targeting tracer, consisting of cetuximab, an antibody, and IRDye800CW, the fluorescent dye that can be detected with NIF imaging equipment (149,153). De Wit et al. pre-administered unlabelled cetuximab intravenously to prevent rapid plasma clearance and occupy off-target receptors, after which cetuximab-800CW was administered (149). Filip et al. used 5-aminolevulinic acid (5-ALA), which was administered orally. It concentrates in mitochondria as the fluorescent metabolite protoporphyrin IX, which emits light in visible red-pink light when exposed to blue light (152). Malignancies with high metabolic activity might be more fluorescent compared to the surrounding structures. All four studies used a variety of oral SCC subsites.

Table 1. Included studies investigating ultrasound											
Author	Methodology	Deep or superficial margin assessed	Tumour location	In- or Ex-vivo	Number of tumours and/or margins	Acquisition time (minutes)	Surgical margin (mm)	Immediate revision	Definition histological positive margin (mm)	Definition histological free margin (mm)	Probes
Al, 2023 (148)	Retro	Both	Tongue	Both	29	N.G.	≥ 5	Yes	< 5	≥ 5	N.G.
Adriaenssens, 2023 (147)	Pros	Both	Buccal mucosa	Both	13 (65 margins on histology, 62 imaged)	N.G.	5-10	Yes, based on US or surgeon	< 1	≥ 5	in-vivo: L16-4Hs, ex-vivo: L20-5s (Mindray)
Nilsson, 2022 (114)	Pros vs. Retro	Both	Tongue	Both	110 (intervention: 34 control: 76)	5-10	5-10	Yes, based on US or FSA	< 0.01	≥ 5	18 MHz high frequency linear 8870 probe (BK Medical)
de Koning, 2022 (113)	Pros vs. Retro	Both	Tongue	Both	136 (intervention: 40 tumours, 193 margins) control: 86 tumours	5-10	5-10	Yes, based on US or surgeon	< 1	≥ 5	in-vivo: L16-4Hs, ex-vivo: L20-5s (Mindray)
Bekedam, 2021 (103)	Pros	Both	Tongue	Ex-vivo	8	33	N.G.	No	< 1*	≥ 5*	10 MHz intraoperative convex transducer (T-shape, BK Medical)
Bulbul, 2021 (90)	Retro vs. Retro	Both	Tongue	Both	44 (intervention: 23 control: 21)	5-10	10-15	N.G.	0	≥ 5	L15-7to; (Philips)
de Koning, 2021 (83)	Pros vs. Retro	Both	Tongue	Both	101 (intervention: 10 control: 91)	no longer than frozen sections	5-10	Yes, based on US or surgeon	< 1	≥ 5	in-vivo: L16-4Hs, ex-vivo: L20-5s (Mindray)
Brouwer de Koning, 2020 (66)	Pros	Deep	Tongue	Ex-vivo	29 (2/31 excluded because base of tongue)	< 5	N.G.	No	< 1*	≥ 5*	5-10 MHz Pro-Sound SSD-AL-phi 5 (Alkoka)
Tarabichi, 2018 (57)	Retro	Deep	Tongue	Both	12	N.G.	> 10	N.G., FSA was an option	< 1*	≥ 5	L15-7to; (Philips)
Kodama, 2010 (55)	Pros	Deep	Tongue	Both	4	N.G.	10	No	< 1	≥ 5	7.5-MHz sector probe (Alkoka)

Abbreviations: pros: prospective, retro: retrospective, N.G: not given. * values in study adjusted to review.

Wu et al. used fluorescence to image the specimen in-vivo for three purposes: 1) before the start of the resection to determine the margins, 2) during the resection to check the cutting plane 3) after the resection to image the wound bed and the specimen ex-vivo (151). Pan et al., de Wit et al. and Filip et al. imaged the tumour in-vivo but did not use fluorescence to guide the surgical margin. Moreover, they imaged the wound bed and imaged the specimen ex-vivo (149,150,152). De Wit et al. used two closed-field devices to image the specimen ex-vivo. These closed-field devices eliminate ambient light and enable standardisation of imaging between specimens. They also pre-dosed the patient with unlabelled cetuximab to prevent rapid plasma clearance of the tracer and occupy off-target receptors in normal tissue, to improve contrast between the tumour and healthy tissue. De Wit et al. also imaged bony margins with their technique (149). Pan et al. only imaged mucosal and deep soft tissue margins (150). Wu et al. probably used their technique for both bony and soft tissue margins (151). It was unclear if bony margins were imaged in the study of Filip et al. (152).

The percentage of tumours or patients that had positive margins varied between 8% and 25% (median: 15%). The percentage of tumours or patients that had free margins varied between 28% and 90% (median: 75%). However, the studies used different definitions for positive and free margins, which makes it difficult to compare them (Table 3).

The sensitivity and specificity were calculated for all four studies (Table 2, Figure 3). In only a few cases in the studies of Wu et al., Pan et al., and Filip et al., the wound bed was fluorescence positive, resulting in a sensitivity ranging from 0 to 100% and a specificity of 89 to 100%. When fluorescence was used to assess the wound bed for residual cancer in the study of de Wit et al., only one fluorescent spot was found. However, no residual cancer was found at this spot, resulting in a positive predictive value (PPV) of 0%. This false-positive fluorescent spot turned out to be an artery. De Wit et al. did not mention if they expected a positive spot in the wound bed in the remaining 64 patients, for example in the case of a 0 mm margin (cut-through), resulting in an incalculable sensitivity and specificity. The PPV of a fluorescent spot in the wound bed was also 0% in the study of Wu et al. (151). They explained that this could be due to the ability of ICG to accumulate also in dense tissue, normal glands, gingiva, or inflammatory tissue, besides tumour tissue (151).

Table 2. The true positives, false negatives, false positives, and true negatives per study

Imaging technique	Author	Assessed margin	Single or multiple margins measured per specimen	P	FN	FP	TN
Ultrasound	Au, 2023 (148)	O	S	0	11	0	18
		D	S	0	8	0	21
	Adriaansens, 2023 (147)	O	M	14	15	6	27
	Nilsson et al, 2022 (114)	O	S	0	14	0	20
		D	S	0	8	0	26
	de Koning, 2022 (113)	O	M	9	29	15	140
	Bekedam, 2021 (103)	O	S	3	0	2	3
	Bulbul, 2021 (90)	O	S	0	7	0	16
		D	S	0	5	0	18
	de Koning, 2021 (83)	O	S	2	2	0	6
	Brouwer de Koning, 2020 (66)	D	S	7	3	1	18
	Tarabichi, 2018 (57)	D	S	0	1	0	11
	Kodama, 2010 (55)	D	S	0	0	0	4
Fluorescence	Wu, 2022 (151)	O*	S	0	1	1	11
		W*	N.A.	0	1	1	11
		O*	M	0	1	1	127
	Pan, 2020 (150)	O*	S	2	0	2	16
		W*	N.A.	2	0	2	16
	de Wit, 2023 (154)	D	M [#]	40	11	17	54
	Filip, 2023 (152)	W*	N.A.	1	0	0	3
MRI	Giannitto, 2021 (155)	O	S	1	0	0	9
	Heidkamp, 2020 (59)	R1: O	M	5	9	8	95
		R2: O	M	1	13	20	87
	Steens, 2017 (146)	O	S	6	2	0	2

Abbreviations: TP: true positives, FN: false negatives, FP: false positives, TN: true negatives, N.A.: not applicable. R1: Reader 1, R2: Reader 2, O: deep and superficial margins of the specimen were assessed (overall), D: only deep margin of the specimen was analysed (deep), W: wound bed was assessed, S: the narrowest margin (one) per specimen was analysed (single), M: multiple margins per specimen analysed (deep and superficial in multiple directions).

*TP, FN, FP, TN are not based on the definition of inadequate margin of < 5 mm or based on an unclear definition, see Table 3 for the used definition.

[#]Based on an SBR ≥ 1.5, the optimal cutoff for the detection of close margins.

The sensitivity and specificity of the ex-vivo assessment of the specimen ranged from 0 to 100% and 76 to 99%, respectively. However, the definition of a free margin was not mentioned by Filip et al. and Wu et al. and was 0 mm in the study of Pan et al. (150–152). Therefore, the sensitivity of 78% and specificity of 76% reported by de Wit et al. are most applicable for this review and these results can be compared to other techniques. To calculate the sensitivity and specificity, we used the by the authors recommended signal-to-background ratio (SBR) of > 1.5 to identify positive and close margins.

The applicability of fluorescence, scored according to the QUADAS-2 tool, was intermediate because of potential interobserver variability. At the moment, an experienced clinician is required to execute the procedures for margin assessment with a SBR (149). However, with the application of a standardized imaging interpretation, fluorescence could be less interobserver-dependent than the other imaging techniques. De Wit et al. used the SBR to identify close or positive margins, although the SBR was not predefined. None of the other studies defined an SBR to identify positive or close margins. The study of de Wit et al. was the only non-dose-escalating study investigating fluorescence molecular imaging (FMI) (149). The extent of the margin can be estimated ex-vivo, which, in combination with a closed-field device, also enables standardisation of imaging (156).

Compared to the other studies, the use of 5-ALA had practical disadvantages. There was a need to cover the skin for up to 72 hours after administration and the operating room lights also needed to be covered with a filter to prevent the transmission of light below 470 nm, to prevent the consequences of photosensitivity. Within a small sample size ($n = 4$), they found a sensitivity and specificity of 100%.

Magnetic Resonance Imaging

Magnetic resonance imaging (MRI) has been used to visualize the tumour-free margins intraoperatively in three studies with a small sample size ($n = 10$) per study (Table 4). The whole specimen is scanned in an ex-vivo setting, and the goal is to visualize the mucosal and deep margins, which can provide feedback for the surgeon during the same session. Giannitto et al. (155) used a 1.5 Tesla (T) MRI-system, Heidkamp et al. (59) a 3 T MRI-system, and Steens et al. (146) a 7 T MRI-system. None of the studies used the margins measured by the MRI-system for intraoperative feedback. The percentages tumours with a ≥ 5 mm margin varied between 20% and 90% (median: 70%). The acquisition time ranged from 16 to 90 minutes. All three studies immersed the specimen in perfluoropolyether to eliminate magnetic susceptibility artifacts arising from the air-to-tissue transition. Furthermore, the three studies investigated this technique only for SCC of the tongue, therefore information about bony margins is lacking.

The sensitivity and specificity for the three studies were calculated for a 5 mm margin determined by the MRI-system and histology (Table 2, Figure 3). Heidkamp et al. analysed the sensitivity and specificity for identifying < 5 mm mar-

gins on MRI by analysing only 3 margins (cranial, caudal, and deep). Two readers analysed the MRI-scans, and those measurements were compared to histology (59). Giannitto et al. and Steens et al. looked at only one margin per specimen (146,155). The sensitivity of Giannitto et al. and Steens et al. was much higher than the sensitivity found by Heidkamp et al. This could be because Steens et al. and Giannitto et al. looked at the whole specimen, while Heidkamp et al. looked at multiple margins within multiple slices per tumour. Moreover, it is unclear if the narrowest margin measured on histology was located at the same site on MRI in the studies of Giannitto et al. and Steens et al. Also, variation in the MRI systems, sequences used, and bore size could play a role in the observed sensitivity differences. Presumably, the lower the field strength of the MRI system and the wider the bore, the lower the signal-to-noise ratio, which can hamper the visualisation of fine details (59,146). Also, the different radiofrequency coils used in the studies, which are important in MR image quality, could play a role in the found differences in sensitivity (157).

Distinguishing oral mucosa from the resection plane was difficult and thereby mentioned as a limitation in two out of three studies (59,146). Also, the acquisition times varied between 16 and 90 minutes, which is relatively long for intraoperative implementation, especially when compared to the other imaging techniques.

General discussion

In oral SCC, the tumour-free margin is an important prognostic factor that can be influenced by the surgeon (158). Unfortunately, obtaining free resection margins is challenging, and a technique that can determine the extent of the tumour-free margin intraoperatively is needed. Three different imaging modalities emerged after a literature search and were analysed in this systematic review, which can potentially determine the extent of the tumour-free margin in the deep resection plane during cancer resection. US is the most investigated technique, with ten publications. Four publications investigated fluorescence, and three publications focused on MRI. Most studies focused on the narrowest margin of the entire specimen instead of multiple margins at different locations of the specimen. We believe that it is more accurate to look at the agreement per location of the predicted margin by the imaging technique and the corresponding histological margin. Focusing on the narrowest margin per specimen could be less accurate because if there is a margin of < 5 mm on histology, it could be at an entirely different location than the < 5 mm margin indicated by the imaging technique. However, the narrowest margin is probably more interesting for the individual patient and clinical practice.

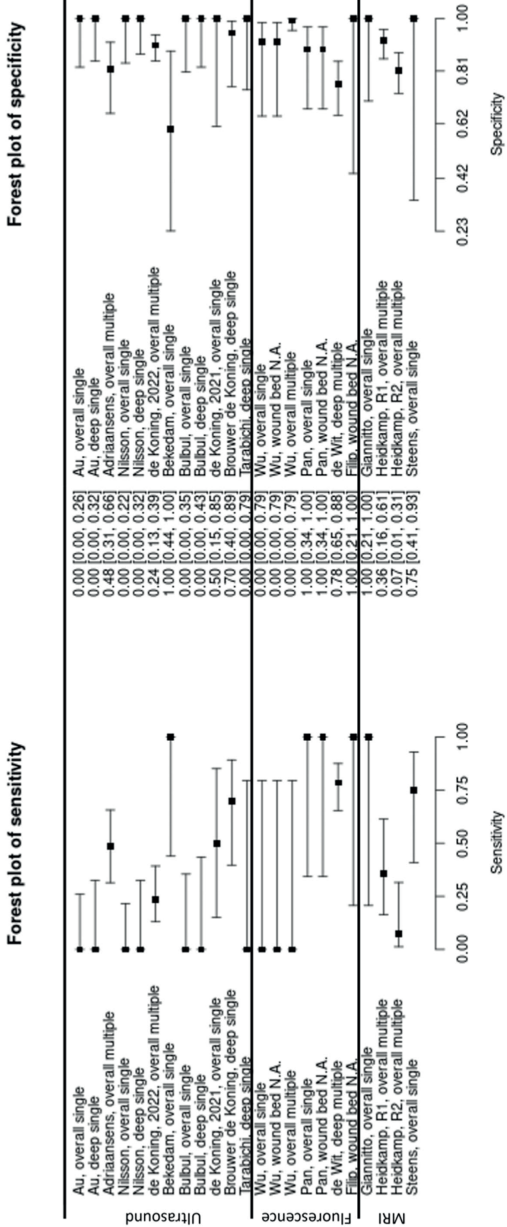


Figure 3: Forest plots of sensitivity and specificity for the included studies. It was not possible to calculate the sensitivity for Kodama et al., the specificity was 100 %.

Table 3. Included studies investigating fluorescence													
Author	Methodology	Deep or superficial margin assessed	In- or ex-vivo	Number of tumours and/or margins	Fluorescence & dose	Acquisition time (minutes)	Surgical margin aim (mm)	Immediate revision	Definition histological positive margin (mm)	Definition histological free margin (mm)	Number of positive tumours and/or margins (%)	Number of free tumours and/or margins (%)	Device
Wu, 2022	Retro vs. Retro	Both	Both	Tumours: intervention: 13 control: 16; margins: intervention: 129 control: 112	ICG: 0.75 mg/kg 10 h before surgery	N.G.	15–20 from fluorescence	Yes, based on fluorescence after FSA confirmation	N.G.	N.G.	Tumours: intervention: 1/13 (8) Control: N.G.; margins: intervention: 1/129 (1) control: 7/112 (6)	N.G.	NIF imaging equipment (Nanjing Nuoyuan Medical Devices, Co., Ltd.)
Pan, 2020	Pros	Both	Both	Tumours: 20	ICG: 0.75 mg/kg 6–8 h before surgery	N.G.	N.G.	Yes, based on fluorescence	0	> 0	Tumours: 2/20 (10)	Tumours: 18/20 (90)	REAL-IGS, Nuoyuan Medical Devices Co., Ltd, Nanjing, China integrated with a hand-held NIF spectrometer (Maya 2000 Pro, Ocean Optics, Dunedin, FL, USA).
De Wit, 2023	Pros	Both	Both	Tumours: 66 (patients: 65) margins: 122	75 mg unlabelled cetuximab and 15 mg cetuximab-800CW 2 days before surgery	5	10	Biopsy of fluorescent positive wound bed	< 1	≥ 5	Patients: 13/65 (20), margins: 14/122 (11),	Patients: 18/65 (28), margins: 71/122 (58),	In-vivo: SurgVision Explorer Air® (SurgVision GmbH) Ex-vivo: SurgVision Explorer Air® coupled to a dedicated closed-field imaging box (Vault, SurgVision GmbH) and the Pearl-Triology® (LI-COR Biosciences, Lincoln)
Filip, 2023	Pros	Deep	Both	Tumours: 4 (3/7 excluded because no oral cavity)	20 mg/kg 5-ALA 3–5 h before surgery	N.G.	N.G.	No	N.G.	N.G.	Tumours: 1/4 (25)	Tumours: 3/4 (75)	405 nm blue light fluorescence-guided headlight system, the operating microscope with blue light capabilities and a handheld camera with an external filter

Abbreviations: pros: prospective, retro: retrospective. N.G.: not given. For de Wit et al. the number of patients with free margins were noted because one patient had two tumours.

Table 4. Included studies investigating MRI

	Methodology	Deep or superficial margin assessed	In- or ex-vivo	Number of tumours and/or margins	Acquisition time (minutes)	Surgical margin aim	Immediate revision	Definition histological positive margin (mm)	Definition histological free margin (mm)	Number of positive tumours (%)	Number of free tumours and/or margins (%)	Coil and MRI-system
(Giannitto et al., 2021)	Pros	Both	Ex-vivo	Tumours: 10	23 (range: 16–40)	N.G.	Yes, based on FSA	< 1	≥ 5	1/10 (10)	9/10 (90)	A four-channel phased array surface carotid coil (Magnetom Aera, Siemens) and a 1.5 T MRI system (Magnetom Aera, Siemens)
(Heidkamp et al., 2020)	Pros	Both	Ex-vivo	Tumours: 10 margins: 160 (R1:117, R2:121)	30	N.G.	No	< 1	≥ 5	N.G.	Tumours: 7/10 (70), margins: 143/160 (89)	A bilateral four-channel phased array surface carotid coil (Machnet BV, Roden) and a 3 T MRI system (Magnetom Skyra, Siemens Healthineers)
(Steens et al., 2017)	Pros	Both	Ex-vivo	Tumours: 10	< 90	N.G.	No	< 1*	≥ 5*	1/10 (10)	2/10 (20)	An integrated circular polarized transmit/receive 1H volume coil and a Bruker ClinScan horizontal-bore MR system, interfaced to a Siemens Syngo VB15 console (Bruker BioSpin)

Abbreviations: Pros: prospective, retro: retrospective, R1: reader 1, R2: reader 2.

*values in study adjusted to review.

Seven studies did not perform a re-resection to improve margin status based on the findings on ex-vivo images (55,59,66,103,146,148,152). Four studies used FS or a biopsy to analyse margins, whether or not guided by the imaging technique, to identify or confirm positive or close margins (57,149,151,155). All studies had the intention of improving the deep margin status, whether for immediate implementation or for eventual application. However, the definition of 'deep margin' was not always clear. Some studies defined the deep margin as the central part beneath the tumour (83,113,147). But most studies did not mention their definition of the deep margin. The definition of a deep margin is important to be able to compare the performance of different studies and techniques.

Intraoperative intraoral US and MRI tend to have higher specificity than sensitivity. Fluorescence molecular imaging (FMI) with cetuximab tends to have a higher sensitivity than US or MRI. The sensitivity of these techniques could be enlarged by increasing the cutoff margin for re-resection. US and ICG fluorescence were the only techniques that enabled real-time in-vivo guidance, which is preferable over a re-resection, probably due to relocation problems (60,61,139). However, until now the application of US for bony resections in oral SCC was not investigated. In the future, US could guide the soft tissue resection in-vivo, while FMI, ex-vivo US, and MRI could identify the positive or close margins and FSA (relatively high specificity) could confirm these margins. FMI is the only technique which was investigated to aid in obtaining information about the bony margins. Furthermore, to improve the comparability of the imaging techniques and studies, we would advise to define a generally accepted clear definition of a deep margin or for each author to at least mention what definition was used. Also, more subsites in the oral cavity could be investigated. Most of the included studies focused on the oral tongue; larger cohorts are needed to prove the superiority of the imaging technique over visual inspection and palpation.

We deliberately did not discuss the intraoperative assessment of resection margins method (158), diffuse reflectance or Raman spectroscopy (159–161), Spider Mass spectroscopy (162), hyperspectral imaging (163,164), touch imprint cytology (165,166), and 3D positron emission tomography and X-ray computed tomography (3D-PET-CT) (167) in this review, as these techniques only image a small proportion of the resection margin, margins were not correlated to histology, or the technique was only able to identify tumour cells at the cut surface of the specimen.

Conclusion

For oral SCC currently three imaging techniques are used intraoperatively which can image the entire tumour-free soft tissue margin: US, fluorescence, and MRI. Overall, the sensitivity of these techniques is currently insufficient for identifying < 5 mm margins, and further research on larger cohorts is needed to improve the sensitivity by determining cutoff points for a re-resection. This would improve the diagnostic value needed for the clinical implementation of these much-desired additional techniques to obtain adequate resection margins.

Chapter 8

Intraoperative techniques that define the mucosal margins of oral cancer in-vivo: a systematic review

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Abstract

Background: This systematic review investigates techniques for determining adequate mucosal margins during the resection of oral squamous cell carcinoma (SCC). The primary treatment involves surgical removal with ≥ 5 mm margins, highlighting the importance of accurate differentiation between SCC and dysplasia during surgery.

Methods: A comprehensive Embase and PubMed literature search was performed. Studies underwent quality assessment using QUADAS-2.

Results: After the full text screening and exclusion of studies exhibiting high bias, eight studies were included, focusing on three margin visualisation techniques: autofluorescence, iodine staining, and narrow-band imaging (NBI). Negative predictive value (NPV) was calculable across the studies, though reference standards varied. Results indicated NPVs for autofluorescence, iodine, and NBI ranging from 61% to 100%, 92% to 99%, and 86% to 100%, respectively. Autofluorescence did not significantly enhance margins compared with white light-guided surgery, while iodine staining demonstrated improvement for mild or moderate dysplasia. NBI lacked comparison with a white light-guided surgery cohort.

Conclusions: We recommend studying and comparing the diagnostic accuracy of iodine staining and NBI in larger cohorts of patients with oral SCC, focusing on discriminating between SCC and (severe) dysplasia. Furthermore, we advise reporting the diagnostic accuracy alongside the treatment effects to improve the assessment of these techniques.

Introduction

Approximately one-third of all head and neck cancers are oral squamous cell carcinoma (SCC) (168). The preferred choice of treatment is complete surgical removal with histopathological adequate resection margins of the primary tumour to establish local control (2,112).

There is still a discussion about the definition of an adequate margin. Several studies investigated the ideal histopathological cutoff margin (30,43,78,104,106,112). Most guidelines define a free margin as ≥ 5 mm between the SCC and the resection plane (27,28). There is a general consensus that margins between 0 and 1 mm from the resection plane adversely affect locoregional survival (30,61,107) and are an indication for adjuvant treatment. This could either be radiotherapy or a re-resection, both having their drawbacks. Radiotherapy has several side effects (44), while a re-resection requires extra operating time and sometimes general anaesthesia during a second procedure. Furthermore, problems in localizing the inadequate margin in an already closed wound bed introduce uncertainty about the definitive margin status (42).

The existence of (severe) dysplasia in the resection margin adds a different aspect to the discussion of adequate margins. In many patients, the oral SCC develops in an area of (severe) dysplasia, also known as 'field cancerisation' (35). There is evidence that when there is residual severe dysplasia after SCC resection, there is a high chance of local recurrence or new primaries (169,170). There is little consensus about the appropriate treatment in case of severe dysplasia in the resection margin. This could either be CO₂-laser evaporation or an additional surgical resection (171). However, surgical resection of all mucosal dysplasia in the case of extensive field cancerisation may be an unnecessary overtreatment, potentially leading to increased morbidity. Nevertheless, it is important to differentiate between SCC and (severe) dysplasia in the resection margins, given the varied consequences of residual dysplasia in the resection margins. These consequences encompass differences in locoregional recurrence and the severity of adjuvant treatment.

In the past decade, an increasing amount of research into intraoperative margin assessment has been conducted that could improve the final margin status. For example, frozen section analysis (FSA) can be used to identify SCC and distinguish it from (severe) dysplasia. This technique uses tissue samples of the wound bed or specimen, which are rapidly assessed for SCC or dysplastic cells through histopathological examination. This allows for the immediate revision of surgical margins, if necessary. However, only 0.1-1% of the specimen and/or wound bed is sampled; therefore, a frozen section may lead to sampling errors, resulting in a low sensitivity for inadequate margins (4,61,62). Bulbul et al. concluded in a meta-analysis that margin revision indicated by FSA does not lead to better local control (60).

In our centre, the application of an intraoperative ultrasound has been investigated for SCC of the buccal mucosa and oral tongue (113,147). Although it contributed to an enhanced assessment of deep and submucosal margins, it proved difficult to differentiate the tumour and (severe) dysplasia from normal mucosa. Also, intraoperative ex-vivo MRI, which is able to image deep and submucosal margins, has limitations in imaging the mucosal resection plane (59). However, a margin visualisation technique that ensures adequate mucosal margins is equally crucial as achieving adequate submucosal and deep margins. This is preferably a technique that determines the mucosal margin with a high sensitivity for both SCC and dysplasia.

There are several systematic reviews evaluating margin visualisation techniques that may contribute to a higher number of adequate resection margins (95,96,140,141). However, these reviews discuss only deep margins (26,141) or a combination of deep and superficial margins (96). Some also include preclinical research, research that includes technologies that require sampling of the resection specimen and/or wound bed, or ex-vivo examination of the resection specimen (26,96,140).

This systematic review aims to provide an overview of publications evaluating the diagnostic accuracy of recently investigated mucosal margin visualisation techniques that aim for adequate mucosal margins, both in the context of SCC and dysplasia. These techniques should be combined with deep margin visualisation techniques. We specifically focus on in-vivo technologies that are already applied in clinical practice and are suitable for defining the mucosal margin before incisions are made.

Materials and Methods

This systematic review was conducted following the guidelines outlined in the Preferred Reporting Items for Systematic Reviews and Meta-Analyses (PRISMA) (142) and has not been registered in PROSPERO.

Eligibility criteria

The criteria for inclusion were: 1) the study population consisted of patients with a SCC of the head and neck area with a sub-group of oral SCC; 2) an in-vivo intraoperative technique (i.e. directly before the incision, during the resection or directly after the resection) was studied that was able to visualize the entire extent of the mucosal margin during surgery; 3) it aimed to assess or improve resection margin status; and 4) the sensitivity, specificity, positive predictive value (PPV), negative predictive value (NPV), number of free margins, or number of positive margins (in terms of SCC or dysplasia) were mentioned or could be extracted from the publication.

The criteria for exclusion were: 1) non-clinical studies; 2) publications before 2010; 3) publications that described techniques that only used white light (WL) for tumour/margin visualisation e.g. trans-oral robotic surgery without visual enhancement; 4) publications that described head and neck cancers with < 50% oral cancers or without subgroup analysis of oral cancer in the intervention group; 5) publications that described margin visualisation techniques that only work with samples of the resection specimen; 6) publications that described techniques that only identified the presence of SCC or severe (dysplasia) rather than defining a positive or free margin 7) reviews, case reports, book chapters, editorials, oral presentations, technical notes, and scientific posters; and 8) publications in a language other than English, Dutch, or German.

Search strategy

A systematic search for relevant publications was performed on PubMed and Embase on 31 August, 2023 (KJK). The main focus was to find margin visualisation techniques that helped the surgeon identify adequate SCC-free and/or dysplasia-free margins, during surgery. Therefore, search terms focused on the title, abstract, and MeSH-terms and included 'carcinoma', all subsites of the oral cavity and 'margins of excision'. The same search terms were used in Embase, but instead of the Mesh-terms, the 'explode function' was used. Records predating 2010 were excluded from the search based on the assumption that techniques emerging before 2010 lack clinical relevance in the absence of subsequent publications after 2010. The search syntax is shown in supplementary materials.

De-duplication was conducted using the method described by Bramer et al. in EndNote (Version 19.3.3, Clarivate Analytics, Philadelphia, PA, USA) (143). Afterwards, data were exported to Rayyan QCRI (Hamad Bin Khalifa University, Al Rayyan University, Qatar). Two of the three screening authors (CMA, KJK, RN) independently assessed the relevance of all titles and abstracts based on the predetermined in- and exclusion criteria. Consensus was reached through discussion. Two screening authors (CMA, KJK) reviewed the full texts to determine inclusion or exclusion. Additionally, a reference and citation check were conducted on the selected publications to ensure comprehensive coverage of the entire field of interest.

Data extraction

The information extracted from the included publications included the following: year of publication, study methodology (i.e. intervention vs. control or diagnostic accuracy test), sort of index tests (i.e. margin visualisation technique), sort of reference-standard (i.e. frozen section analysis or final histopathology), consistency of the cohort (i.e. types of SCC), number of included tumours and/or mar-

gins, safety margin distance around the SCC and/or (severe) dysplasia visible under white light safety margin around the area showing positive for the index test, immediate revision based on imaging modality, use of FSA (and whether it was guided by the technique), definition of histopathological positive margin and number of histopathological free margins.

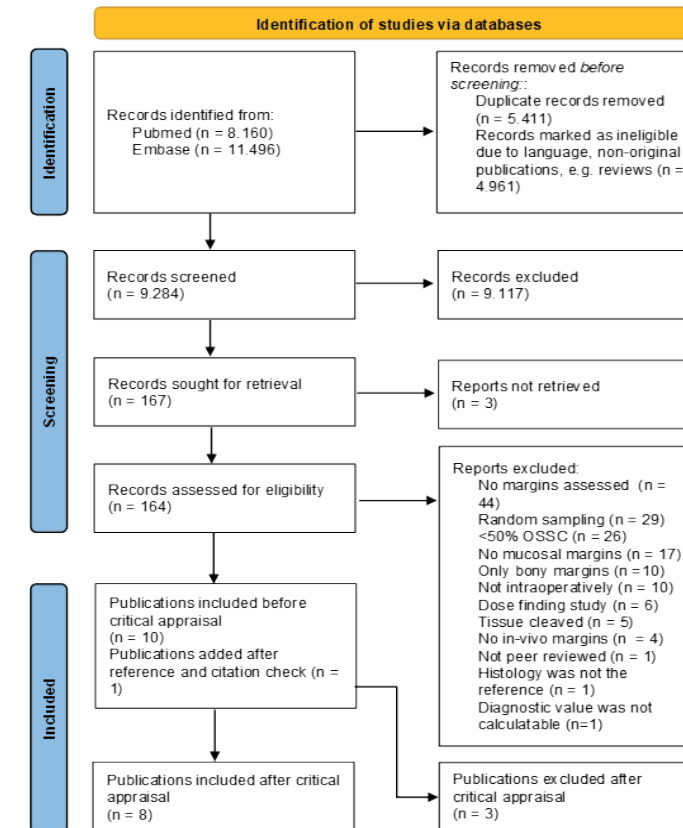
Areas that were indicated by the index test as positive and showed a SCC and/or (severe) dysplasia in that area during the histopathological examination were considered 'true positive' (TP), and in the case that no SCC and/or dysplasia was found, 'false positive' (FP). Areas beyond the positive index test were considered negative (Figure 1). Depending on whether or not this index-negative area showed SCC and/or (severe) dysplasia it was deemed false negative (FN) or true negative (TN), respectively. We registered when these variables were determined per resected specimen (specimen-based) or with multiple FSA samples per specimen (sample-based).

If possible, sensitivity (TP cases divided by positive cases according to histopathology), specificity (TN cases divided by negative cases according to histopathology), positive predictive value (PPV) (TP divided by positive cases according to the index test), and negative predictive value (NPV) (TN divided by negative cases according to the index test) were calculated. This was performed, if possible, for the detection of 1) SCC only, 2) SCC in combination with severe dysplasia, and 3) SCC in combination with all types of dysplasia.

Critical appraisal

Two screening authors (CA, KK) separately critically appraised the included publications using the Quality Assessment of Diagnostics Accuracy Studies (QUADAS-2) tool (172). Elements making part of the following categories were assessed to score the risk of bias: 1) 'patient selection': a consecutive cohort of patients had been used, the optional control cohort was relevant and inappropriate exclusions had been avoided; 2) 'index test': the index test was interpreted without knowledge of the reference-standard; 3) 'reference-standard': the reference-standard was the final histopathology and the pathologist was blinded for the index test; 4) 'flow and timing': the reference-standard and index test were executed equally in each patient and all included patients were analysed. Applicability was evaluated on the following categories by their elements: 1) 'patient selection': oral SCC of both small (T1-T2) and large (T3-T4) tumours were included; 2) 'index test': there was a definition of a positive index, i.e. it used an observer-independent cutoff value and the needed devices and or doses had been described; 3) 'reference-standard': a clear definition of a positive margin was given and the reference-standard (i.e. final histopathology) was not affected by additional frozen sections that were not indicated by the margin visualisation technique. All items were scored as sufficient: 2 points, unclear: 1 point, or bad: 0 points. The score for each category was determined by summing the points

and then dividing the total by the number of items. Overall scores were categorized as 'insufficient' within the range of 0-0.5, 'intermediate' within the range of 0.6-1.4, and 'sufficient' within the range of 1.5-2.0.



From: Page MJ, McKenzie JE, Bossuyt PM, Boutron I, Hoffmann TC, Mulrow CD, et al. The PRISMA 2020 statement: an updated guideline for reporting systematic reviews. *BMJ* 2021;372:n71. doi: 10.1136/bmj.n71

Figure 1: Prisma chart for inclusion and exclusion of publications. From: Page, 2021 (142).

Results

Search strategy and article selection

The search revealed 19,656 citations (Figure 1). After removing duplicates and records that were marked ineligible because of language (e.g. non-English, non-Dutch or non-German) or not being an original journal paper (e.g. conference abstract, review, book chapter) 9284 records remained and were screened on title and abstract, leading to 164 records that were screened full text. Eventually ten records were included and used for the reference standards and citation checks.

This led to one additional inclusion, resulting in eleven articles considered eligible for this review.

Critical appraisal

An overview of the critical appraisal can be found in Table 1. Considering the risk of bias, none of the studies had a risk of bias for the category ‘index test’. For ‘patient selection’ and ‘flow and timing’, an intermediate risk of bias was found. Regarding the category ‘reference-standard’, Baj et al. (173) and Sun et al. (174) scored insufficiently since only FSA or small samples were used to determine diagnostic accuracy, and no final histopathology was used.

Considering applicability, only two studies scored sufficiently in the category ‘patient selection’; Baj et al. (173) and Sun et al. (174) included both early and advanced-stage oral SCC. Durham scored insufficiently for this category, as they did only include small (T1 and T2) tumours or ‘high grade lesions’ defined as dysplasia or in situ carcinoma.

Tirelli et al.’s 2019 study (175) scored insufficiently for the category ‘index test’. They did not clearly define the definition of a positive index test while using narrow band imaging (NBI), possibly because the validation of the NBI technique was not the primary goal of this study. Other studies thoroughly described the definition of a positive index test. However, their description was still observer-dependent and subjective, leading to an ‘intermediate’ score.

Both studies of Morikawa et al. (176,177) scored insufficiently for the category ‘reference-standard’, considering applicability. Both studies did not give a clear definition of a ‘positive’ margin. Moreover, they applied frozen sections in addition to their margin visualisation technique but did not discriminate the contribution of the FSA-indicated revisions from the margin visualisation technique to the frequency of free margins. The latter issue was also the case for the 2019 study of Tirelli et al. (175). However, they gave a clear definition of a positive margin. Therefore, they scored ‘intermediate’ for this category.

The fact that the studies of Morikawa et al. (176,177) and Tirelli et al. from 2019 (175) did not discriminate the contribution of the FSA-indicated revisions from the margin visualisation technique, made it impossible to determine the diagnostic accuracy of the margin visualisation technique. Therefore, these studies were excluded from further analysis (Figure 1). Despite other studies scoring ‘insufficient’ on other categories as well (122,173,174), we decided to evaluate their margin visualisation technique in this systematic review, since it was possible to determine their diagnostic accuracy. This left eight studies for final evaluation. An overview of all studies and their methods of conducting their research can be found in Table 2.

Table 1. Critical appraisal of included studies after text screening											
	Risk of Bias			Applicability			Reference				
	Patient selection	Index test	Reference	Flow and timing	Patient selection	Index test	Reference	Index test	Reference	Index test	Reference
Morikawa, 2019 (176)	●●	●	●	●●	●●	●	●	●●	●	●●	●
Durham, 2020 (122)	●●	●	●●	●	●	●	●●	●●	●	●●	●
Sun, 2021 (174)	●	●	●	●●	●	●	●	●●	●	●●	●
Morikawa, 2023 (177)	●	●	●●	●●	●●	●	●	●●	●	●●	●
McMahon, 2010 (178)	●	●	●	●	●●	●	●	●●	●	●●	●
Umeda, 2011 (179)	●●	●	●	●	●●	●	●	●●	●	●●	●
Tirelli, 2015 (180)	●	●	●●	●	●●	●	●	●●	●	●●	●
Tirelli, 2017 (181)	●	●	●●	●	●●	●	●	●●	●	●●	●
Tirelli, 2018 (182)	●●	●	●●	●	●●	●	●	●●	●	●●	●
Tirelli, 2019 (175)	●	●	●	●	●●	●	●	●●	●	●●	●
Baj, 2019 (173)	●●	●	●	●	●	●	●	●●	●	●●	●

Green check marks: ‘sufficient’. Yellow exclamation marks: ‘intermediate’. Red cross marks: ‘insufficient’.

Margin visualisation techniques

Two included studies investigated autofluorescence (122,174). Two studies assessed iodine staining (178,179). Four included studies analysed NBI (173,180–182). In general, methodology of all studies could be categorized as follows (Figure 2):

- **Method A:** Interventional studies (with or without a WL-safety margin control group). Surgical margins were enlarged when the index-positive area exceeded the WL-safety margin. SCC and/or dysplasia determined the TN or FN in the index-negative areas surrounding the index-positive areas. Index-positive areas were not analysed; hence, only the NPV could be calculated. Three studies used this methodology (122,178,179).
- **Method B:** Interventional studies with diagnostic accuracy. In these studies, the index test was either smaller or larger than the WL-safety margin, and a specimen was either considered index test negative (index $\leq$ WL) or positive (index $>$ WL). Tumours were excised according to the largest area. Histopathology determined the diagnostic accuracy in these areas. In contrast to Method A, the TP and FP could also be evaluated. In case the index-positive area was as large as the WL-safety margin, the case was considered negative. Two studies used this methodology (181,182).
- **Method C:** Diagnostic accuracy studies. In these studies, all tumours were excised according to the WL-safety margin. Index-positive areas extending beyond the WL-safety margin were sampled and assessed on the TP or FP. Areas not extending further than the WL-safety margin were also sampled, indicating either the TN or FN. The overlap between the WL-safety margin and positive index test was considered a plausible situation, in contrast to 'Method C'. Three studies used this methodology (173,174,180).

Autofluorescence

Autofluorescence is one of the multiple imaging techniques that use the fluorescent properties of certain biomaterials. These materials can be excited by absorbing light of a particular wavelength and subsequently emitting this light by a different wavelength. These wavelengths are visible using fluorescence cameras. Instead of external contrast agents with fluorescent properties, autofluorescence margin visualisation techniques use the fluorescent properties of biomaterials found within the body, especially those of collagen crosslinks and flavin adenine dinucleotide. When blue light (wavelength 400–460 nm) is absorbed by normal tissue, it subsequently re-emits light that appears green when observed through a filter. Abnormal tissue, such as neoplastic, dysplastic and inflammatory tissue, cannot be excited and does not emit green light but appears brown through the filter (183). These so-called fluorescence visualisation loss (FVL) areas can be delineated with a certain margin to obtain the free margin status.

One interventional study by Durham et al. ('Method A') performed a randomized controlled trial with a minimal 10 mm WL-safety margin and minimal 10 mm FVL-safety margin (122). They included OSCCs (n = 261) and high-grade lesions (i.e., severe dysplasia, n = 182). This study only reported the "first-pass margin"; margins found "positive for severe dysplasia or greater histopathologic change" and thus seemed not to make a difference between SCC and (severe) dysplasia. Additional revisions were possibly conducted but not described, resulting in an unknown number of free margins in final histopathology. The NPV of their test cohort (70%) was similar to that of their conventional cohort (70%).

One study by Sun et al. performed a 'Method C' study on autofluorescence by applying a demarcation on the boundary of the FVL-positive area (174). They included only SCC patients. Then, they resected the specimen with a 15–20 mm WL-safety margin. In all cases, the FVL-positive area fell within this WL-safety margin. Samples (n = 126) collected from random locations between the FVL-based demarcation and resection plane, were assessed on the frequency of SCC and/or (severe) dysplasia beyond the FVL-positive area. For SCC in the samples, this frequency was 0% (NPV 100%). For severe dysplasia, the frequency was 18% (NPV 82%). For mild dysplasia, the frequency was 21%. As no moderate dysplasia was found, for all types of dysplasia the frequency was 39% (NPV was 61%).

An overview of autofluorescence's diagnostic accuracy can be found in Table 3

Iodine staining

Iodine staining has been widely used for the detection of intraepithelial neoplasia of the oesophagus but can also be used to detect oral SCC and dysplasia (178). Iodine stains healthy tissue and creates an iodine unstained (IU) area on the SCC or dysplastic tissue. Similar to autofluorescence, an IU-safety margin around the IU boundary can be applied to achieve free margin status. Only two interventional studies using 'Method A' were included that assessed this method (178,179).

One study by McMahon et al. used a 10 mm WL-safety margin and a 0 mm IU-safety margin (178). They compared their prospective iodine-guided surgery cohort, consisting of 40/50 (80%) patients with oral SCC, with a retrospective WL-guided surgery cohort, consisting of 42/50 (84%) patients with oral SCC. They found no SCC-positive margins in the iodine-guided cohort (NPV of 100%) and 2/50 (4%) SCC-positive margins (NPV of 96%) in the WL-guided surgery cohort. They found 1/50 (2%) severe dysplasia and 1/50 (2%) other types of dysplasia in the iodine-guided cohort and 1/50 (2%) severe dysplasia and 13/50 (26%) other types of dysplasia in the WL-guided cohort. The NPV for dysplasia (all types) was 96% in the iodine cohort and 68% in the WL-guided cohort..

Table 2. Methods for included studies											
Author	Method	Technique	Cohorts / survival analysis	No. tumours / No. margin samples	Consistency	Demarcation of safety-margin	Positive margin defined by publication	Definition free margin by authors	Acquisition time	Technique Influence on final histopathology	Free margin status on final histopathology
Durham, 2020 (122)	A	Autofluorescence	Autofluorescence (R) vs. WL-guided surgery (R) / survival analysis	443	Autofluorescence: 277 OSCC + HGL, control: 216 OSCC + HGL	At least 10 mm from boundary of WL-positive area and FVL-positive areas	"Positive margin for severe dysplasia or greater histologic change"	Not given	Undefined	Yes	Undefined, only "first pass margin" given, defined as positive margin before re-resections are taken from the tumour bed.
Sun, 2021 (174)	C	Autofluorescence	Diagnostic accuracy of autofluorescence (P)	30 / 126	30 OSCC	15-20 mm from boundary of WL-positive area, no resections based on FVL-positive area	Carcinoma in situ, invasive carcinoma, and severe dysplasia in mucosal samples	Normal epithelium in mucosal samples	Undefined	No	Undefined, only margins within random samples reported
McMahon, 2010 (178)	A	Iodine	Iodine (P) vs. WL-guided surgery (R)	100	Iodine: 40 OSCC and 10 OPSCC, control: 42 OSCC, 8 OPSCC	10 mm from boundary of WL-positive area and 0 mm from IU-positive areas were included	"Intraepithelial neoplasia in mucosal margin"	"Mucosal margins free from dysplasia, carcinoma in situ and invasive carcinoma"	Undefined	Yes	Iodine: 96% (48/50), WL 96% (34/50)
Umeda, 2011 (179)	A	Iodine	Iodine (R) / Survival analysis.	93	93 OSCC of the tongue	At least 10 mm from boundary of WL-positive areas and at least 5 mm from boundary of IU-positive areas	"Positive for SCC" or "positive for dysplasia"	SCC ≥ 1 mm from deep or mucosal margin	Undefined	Yes	81/93 (87%)
Tirelli, 2015 (180)	C	NBI	NBI (P)	16	8 OSCC, 8 OPSCC	15 mm from boundary of WL-positive areas and including the boundary of NBI-positive areas.	SCC < 0.1 mm from mucosal margin	SCC > 3 mm from mucosal margin	5 min	Yes, technique directed FSA, did not influence diagnostic accuracy	94% (15/16)/yes
Tirelli, 2017 (181)	B	NBI	NBI (P)	31	20 OSCC, 11 OPSCC (of 2 the result of the reference test was not clear)	15 mm from boundary of WL-positive areas and including the boundary of NBI-positive areas.	SCC < 0.1 mm from mucosal margin	SCC > 3 mm from mucosal margin	5 min	Yes, FSA in addition to technique, did not influence diagnostic accuracy	77% (24/31)/yes
Tirelli, 2018 (182)	B	NBI	NBI (P)	61	39 OSCC, 22 OPSCC	15 mm from boundary of WL-positive areas and including the boundary of NBI-positive areas.	SCC < 0.1 mm from mucosal margin	SCC > 3 mm from mucosal margin	5 min	Yes, FSA in addition to technique, did not influence diagnostic accuracy	85% (52/61)/yes
Baj, 2019 (173)	C	NBI	NBI (P)	16/ 88	16 OSCC	15-20 mm from boundary of WL-positive area, no resections based on NBI-positive area	Tumour or dysplasia in FSA biopsy	no tumour or dysplasia in FSA biopsy	Undefined	No	Undefined, only FSA biopsy status reported

Abbreviations: WL: white light, NBI: narrow band imaging, FVL: fluorescence visualisation loss, OSCC: oral squamous cell carcinoma, OPSCC: oropharyngeal squamous cell carcinoma, SCC: squamous cell carcinoma, R: retrospective, P: prospective, FSA: frozen section analysis, HGL: high-grade lesions.

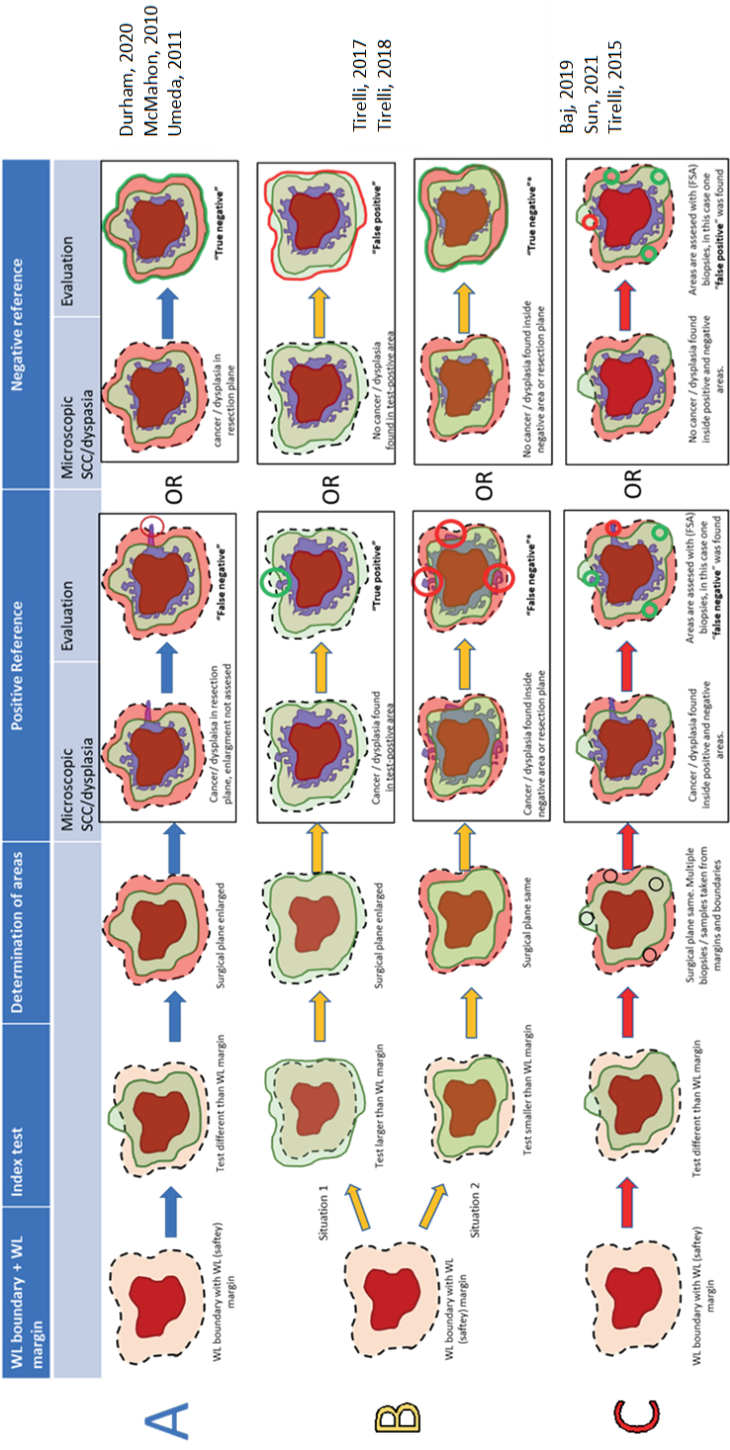


Figure 2: Scheme of the analysed methods in this review: interventional study (method A, blue arrows), interventional with diagnostic accuracy (method B, yellow arrows) and diagnostic accuracy (method C, red arrows). Dotted line: planned resection margin, which may be changed by the index test in case of method A and B), light green: positive index test (area outside positive area). Dark red: macroscopic tumour. Purple: microscopic tumour or (severe) dysplasia. Bright red: false tests. Bright green: true tests. * In type B studies, in case the positive-index test was as large as the WL-safety margin, the specimen was denoted as 'negative' (122, 173, 174, 178-182).

One single-arm study by Umeda et al. used a 10 mm WL-safety margin and a 5 mm IU-safety margin in a cohort consisting of 93 SCCs of the tongue (179). They found in their retrospective cohort that only 1/93 (1%) of the patients had SCC-positive mucosal margins, leading to an NPV of 99% for SCC. They found that 6/93 (6%) of the patients had mucosal margins positive for mild dysplasia, leading to an NPV of 94%. The NPV for dysplasia and SCC combined was 86/93 (92%).

Both studies suggest that using iodine is excellent for determining mucosal safety margins and results in most margins free of SCC and dysplasia. The NPV for SCC and dysplasia (all types) of McMahon et al.'s iodine-guided surgery cohort (178), suggest that iodine has the potential to rule out moderate and mild dysplasia in the resection margin when compared to the results of the WL-guided surgery cohort. However, these results assessed the impact of iodine staining in conjunction with the IU-safety margin, lacking specific information on the sensitivity and specificity of the IU area alone.

An overview of iodine's diagnostic accuracy can be found in Table 4.

Narrow band imaging

NBI is a technique where the surgical field is illuminated by WL, but the reflection is filtered to only two specific wavelengths (415 and 540 nm) that enhance the visualisation of the capillary bed and the intrapapillary loop pattern in the superficial mucosa (181). Changes in the architecture of the capillaries may indicate SCC or dysplasia in the oral cavity. NBI can be applied to an endoscope and is therefore applicable in surgeries of both the oral and oropharyngeal mucosa. Two 'Method B' (181,182) studies and two 'Method C' (173,180) assessing NBI were included.

The two 'Method B' studies were conducted by Tirelli et al.: one from 2017 (181), and one from 2018 (182). In their 2017 study, Tirelli et al. (181) evaluated a cohort that consisted of 20/31 (65%) oral SCC patients. In 28/31 (90%) of the patients, the safety margin was expanded, as the NBI-positive area was larger than the 15 mm WL-safety margin, which was considered to be a positive index test. Of these 28 cases, 20 were TPs (i.e. SCC and/or dysplasia of all types found in the extended margin), and 8 were FPs (i.e. no SCC and/or dysplasia of all types found in the extended margin). In 2/31 cases (7%), the NBI-positive area was similar to the 15 mm WL-safety margin and in only 1/31 (3%) cases, the NBI-positive area was smaller than the 15 mm WL-safety margin. For these three cases, an extension of the safety margin was not needed. Hence, there were three negative index tests, although the authors only reported the presence of SCC and/or dysplasia (all types) in the case with the smaller NBI margin, resulting in one TN case and no FN case. These results yielded a sensitivity of 100% (CI: 83%-100%), specificity of 11% (CI: 0%-29%), PPV of 71% (CI: 66%-76%) and NPV of 100% (CI: 3%-100%), for SCC and dysplasia (all types).

Tirelli et al.'s 2018 study (182), used exactly the same method as their 2017 study (181) in a cohort of 39/61 (64%) oral SCC patients. Of 43/61 (70%) cases, an extension of the safety margin was needed, as the NBI-positive area was larger than the 15 mm WL-safety margin (i.e. positive index test). Of these 43 cases, 34 were TPs (i.e. SCC and/or dysplasia of all types in the extended margin), and 9 were FPs (i.e. no SCC and/or dysplasia of all types in the extended margin). In 18/61 (30%) cases, no extension of the safety margin was indicated by NBI, i.e. a negative index test. Sixteen of these 18 cases were TNs and 2 were FNs. These results yielded a sensitivity of 94% (CI: 81%-99%), specificity of 64% (CI: 42%-82%), PPV of 79% (CI: 69%-87%), and NPV of 89% (CI: 67%-97%) for SCC and dysplasia (all types).

Two 'Method C' studies analysed the diagnostic accuracy of NBI, one by Baj et al. (173), and one by Tirelli et al. from 2015 (180). Baj et al. (173) assessed a cohort that consisted entirely of oral SCC patients (n = 16). They varied the distance of the WL-safety margin between 15 and 20 mm and took three to eight biopsies per specimen, situated at the border of the NBI-positive areas and of those of the WL-safety margin. After the FSA examination, biopsies were classified as positive or negative for 'SCC or dysplasia (all types)'. The authors did not discriminate SCC from dysplasia. Three TPs, 5 FNs, 14 FPs, and 32 TNs were found to yield a sensitivity, specificity, PPV, and NPV of 38% (CI: 9%-76%), 70% (CI: 54%-82%), 18% (CI: 7%-37%), and 86% (CI: 78%-92%), respectively. Contours of the NBI-positive areas were within the WL-safety margin in 50% of the cases.

Tirelli et al. (180) found in their 'Method C' study from 2015, that the 15 mm WL-safety margin was surrounded by an NBI-positive area in every case. This contrasts with the results from Baj et al. (173), who reported this situation in only 50% of the cases. They performed an FSA in the NBI-positive area and extended the surgical margin according to the NBI in case dysplasia or a SCC was found. In every case, SCC and/or dysplasia were found beyond the 15 mm WL-safety margin. For SCC only, it resulted in 12 TPs, 0 FNs, 4 FPs, and 0 TNs cases, yielding a PPV of 75%, a sensitivity of 100%, and a specificity of 0%, but no calculable NPV. For 'SCC and dysplasia (all types)', it resulted in 16 TPs, 0 FNs, 0 FPs, and 0 TNs cases, yielding a PPV of 100%, a sensitivity of 100%, but no calculable specificity or NPV. Although the safety margins were enlarged when FSA confirmed TP, there was still one specimen with SCC-positive margins (6%) and one specimen with margins positive for dysplasia.

NBI is the only assessed technique in this review, of which three out of four studies report both a calculable PPV, NPV sensitivity and specificity. However, a wide variety of methods are employed to obtain these outcome measurements across the studies.

An overview can be found in Table 5.

Table 3. Results from studies about autofluorescence.

Author	Evaluation	Reference results based on	Test positive / negative	Reference positive / negative	NPV SCC (test / WL control group)	NPV SCC + severe dysplasia (test / WL control group)	NPV SCC + dysplasia (test / WL control group)	Bias or concern
Durham, 2020 (122)	Interventional (with WL-guided control group)	Full specimens (OSCC or HGL)	NA / 10 mm from WL-positive area and 10 mm from FVL-positive area (whichever was wider)	“Severe dysplasia or greater histologic change” in resection plane on final histopathology / normal tissue in resection plane on final histopathology	Not given	Test: 70% (151/216) Control: 70% (159/227)	Not given	Unknown reason for certain exclusions, patients with small tumours and “High-grade lesions” were included as well.
Sun, 2021 (174)	Diagnostic accuracy	Samples from margin	Sample within the FVL-positive area exceeding the WL-positive area / sample within the FVL-positive area inside the WL-positive area	SCC or dysplasia (all types) in sample of FVL-positive area / normal tissue in sample of FVL-positive area	100% (126/126)	82% (103/126)	61% (77/126)	126 samples were taken and analysed from random locations between boundary of the FVL-positive area and the surgical margin of 30 tumours

Abbreviations: WL: white light surgery, FVL: fluorescence visualisation loss, FSA: frozen section analysis, NPV: negative predictive value, SCC: squamous cell carcinoma, NA: not applicable.

Table 4. Results from studies about iodine.

Author	Evaluation	Reference results based on	Test positive / negative	Reference positive / negative	NPV SCC (Test group / WL control group)	NPV SCC + severe dysplasia or (test / WL control group)	NPV SCC + dysplasia (test / WL control group)	Bias or concern
McMahon, 2020 (178)	Interventional (with WL-guided control group)	Full specimens	NA / 10 mm from boundary of WL-positive area 0 mm from IU-positive area	Dysplasia (all types) or SCC in resection plane	Test: 100% (50/50) Control: 96% (48/50)	Test: 98% (49/50) Control: 96% (47/50)	Test: 96% (48/50) Control: 68% (34/50)	None
Umeda, 2011 (179)	Interventional (no WL-guided surgery control group)	Full specimens	NA/ 10 mm from boundary of WL-positive area 5 mm from IU-positive area	Dysplasia or SCC in resection plane	99% (92/93)	Not given, only mild dysplasia in resection plane	92% (86/93)	None

Abbreviations: WL: white light, FSA: frozen section analysis, NPV: negative predictive value, CIS: carcinoma in situ, IU: iodine unstained, SCC: squamous cell carcinoma, NA: not applicable.

Table 5. Results from studies about NBI.

Author	Evaluation	Reference results based on	Test positive / negative	Ref positive / negative	Sens / spec cancer	PPV / NPV cancer	Sens / spec SCC + Sdys	PPV / NPV SCC + Sdys	Sens / spec SCC + dys (all types)	PPV / NPV SCC + dys (all types)	Bias of concern
Tirelli, 2015 (180)	Diagnostic accuracy	FSA-samples	NBI-positive area beyond the 15 mm WL-safety margin/ NBI-positive area between boundary of WL-positive area and 15 mm WL-safety margin	SCC and/or dysplasia / no SCC and/or dysplasia in the NBI-positive or negative area	Sens: 100% (12/12) Spec: 0% (0/4)	PPV: 75% (12/16) NPV: undefined (0/0)	NA	NA	Sens: 100% (16/16) Spec: undefined (0/0)	PPV: 100% (16/16) NPV: undefined (0/0)	Only NBI-positive areas were assessed with biopsies, while NBI-negative areas, mucosa within the 15 mm W-safety margin, but outside the boundary of the NBI-positive area) did not receive a biopsy. Also, the NBI-positive area seemed still too small, since dysplasia and SCC were found in the resection plane. Possible overlap with Tirelli 2017 and Tirelli 2018.
Tirelli, 2017 (181)	Interventional with diagnostic accuracy	Final histopathology	NBI-positive area beyond the 15 mm WL-safety margin/ NBI-positive area between boundary of WL-positive area and 15 mm WL-safety margin	SCC and/or dysplasia / no SCC and/or dysplasia in the NBI-positive or negative area	Sens: 100% (12/12) Spec: 6% (1/17)	PPV: 43% (12/28) NPV: 100% (1/1)	Sens: 100% (16/16) Spec: 8% (1/13)	PPV: 57% (16/28) NPV: 100% (1/1)	Sens: 100% (20/20) Spec: 11% (1/9)	PPV: 71% (20/28) NPV: 100% (1/1)	Only one specimen with NBI-negative findings (specimen with the boundary of the NBI-positive area within the WL margin). Of two specimens, the NBI-positive areas were as large as the WL-safety margin, but it was unclear whether the resection plane were free from SCC / dysplasia. Hence, only 29 cases could be evaluated. Possible overlap with Tirelli 2015 and Tirelli 2018.
Tirelli, 2018 (182)	Interventional with diagnostic accuracy	Final histopathology	NBI-positive area beyond the 15 mm WL-safety margin/ NBI-positive area between boundary of WL-positive area and 15 mm WL-safety margin	SCC and/or dysplasia / no SCC and/or dysplasia in the NBI-positive or negative area	Sens: 96% (23/24) Spec: 46% (17/37)	PPV: 53% (23/43) NPV: 94% (17/18)	Sens: 93% (28/30) Spec: 52% (16/21)	PPV: 65% (28/43) NPV: 89% (16/18)	Sens: 94% (34/36) Spec: 64% (16/25)	PPV: 79% (34/43) NPV: 89% (16/18)	Possible overlap with Tirelli 2015 and Tirelli 2017.
Bai, 2019 (173)	Diagnostic accuracy	FSA-samples	NBI boundary outside 15-20 mm from WL boundary / NBI-positive area between boundary of WL-positive area and 15 mm WL-safety margin	Dysplasia or SCC in FSA biopsy from a positive test situation / no dysplasia or SCC in FSA biopsy	Not given	Not given	Not given	Not given	Sens: 38% (3/8) Spec: 70% (32/46)	PPV: 18% (3/17) NPV: 86% (32/37)	Only small biopsies were taken for certain areas. The WL-safety margin was not from a consistent distance from the WL-positive boundary (varying between 15 and 20 mm).

Abbreviations: WL: white light, NBI: narrow band imaging, FSA: frozen section analysis, SCC: squamous cell carcinoma, Sens: sensitivity, Spec: specificity, NPV: negative predictive value, PPV: positive predictive value, Dys: dysplasia, Sdys: Severe dysplasia, NA: not applicable.

Discussion

This systematic review highlights techniques that try to define the optimal mucosal surgical resection margins in the treatment of oral SCC. The demarcation of the mucosal surgical margin is an essential part of oral cancer surgery, because it serves as a critical reference point for the surgeon to achieve tumour-free (i.e. ≥ 5 mm) histopathological margins in all dimensions. In the past years, more attention has been given to margin visualisation techniques that aid the surgeon in estimating the deep extension of the tumour. Although several systematic reviews assess these techniques, to our knowledge, no reviews specifically illuminate the currently evaluated techniques that enhance the demarcation of the mucosal surgical margin in oral cancer surgery. This systematic review tries to fill in this gap in the literature.

During the setup of this review's methodology, we attempted to assess the visualisation techniques by their diagnostic value in identifying positive margins and free margins as defined by the Royal College of Pathologists (28) i.e. < 1 mm and ≥ 5 mm SCC free margins, respectively. However, no studies were found assessing the diagnostic accuracy for close margins with respect to SCC (1-5 mm). Instead, all studies seemed to focus on the presence of SCC or (severe) dysplasia in the resection plane, some of them not making a difference between the SCC or (severe) dysplasia. Indeed, several studies suggest that residual dysplasia has similar effects on disease-free survival as close margins (169,170). Hence, dysplasia is preferably resected during SCC surgery. However, when compared to residual dysplasia, residual SCC has a far greater impact on patient survival. Moreover, residual SCC requires adjuvant treatments (radiotherapy or re-resections) with higher risks and complication rates compared to CO₂-laser evaporation for residual dysplasia (44,61,108,171). Unfortunately, none of the included studies discussed the incidence of close mucosal resection margins (1-5 mm free of SCC), and some did not differentiate between SCC and (severe) dysplasia in the resection plane.

This systematic review included studies to examine the benefits of margin visualisation techniques in a surgical context. Consequently, studies that specifically reported negative or clear margins were included, while those that solely assessed the presence of tumours were not included. As a result, three of the selected studies primarily consisted of interventional research ('Type A' studies) (122,178,179). These studies do not generate a positive index test, as the surgical goal is to achieve a negative index test. Therefore, calculating a meaningful sensitivity, specificity, or PPV is impossible. For these studies, we cannot determine whether the implementation of these margin visualisation techniques will result in potential overtreatment, i.e. unnecessary wide resection margins. Nevertheless, although strongly dependent on the incidence of histologically positive margins, the NPV indicates the effectiveness of the margin visualisation technique for the resection of SCC and/or dysplasia.

In one 'Method C' study that investigated autofluorescence, conducted by Sun et al., NPV was the only measurement for diagnostic accuracy that could be reported, as the authors found that all FVL-areas were smaller than the 15-20 mm WL-safety margin (i.e. negative-index test) (174). This means that also for this study, no valuable comparison between the diagnostic accuracy for identification of SCC-positive margins and dysplastic-positive margins was possible. While the authors used the WL-safety margin during the resection, their NPV of 100% for SCC in the resection plane showed that if an FVL-safety margin had been used, no SCC would have been found in the resection plane. However, for severe dysplasia and all types of dysplasia, the NPV would have been 28% and 39%, respectively. The presented numbers are comparable with the multicentre randomized controlled trial of Durham et al. (122), who found severe dysplasia in the resection plane in 30% when autofluorescence-guidance was used. The frequency of positive margins and 5-year local recurrence were not lower in the autofluorescence-guided cohort when compared to the WL-guided cohort. According to the authors, these unexpected results were most likely caused by the relative inexperience in using autofluorescence of the participating centres outside the coordinating centre. In the studies by Morikawa et al., larger FVL-safety margins were used (in combination with iodine), yet there was a considerable amount of FSA-positive rate for SCC and/or dysplasia (all types), namely 19% and 18%.

Two interventional ('Method A') studies using iodine-guided surgery reported a positive margin rate per specimen. McMahon et al. (178) compared an iodine-guided cohort, with a WL-guided control cohort. They only found a significant difference between both cohorts when all types of dysplasia were considered positive (96% in the iodine-guided cohort vs. 68% in the WL-guided control cohort), which suggests that iodine-guided surgery makes the most difference in the detection of moderate or mild dysplasia. Umeda et al. found comparable results and reported no local recurrence in their single-arm study (179).

All studies examined NBI-guidance assessed dysplasia (all types) in the resection plane, but only several studies did this specifically for SCC and/or severe dysplasia (180-182). Baj et al. (173) reported a lower sensitivity for SCC and dysplasia (combined) in the resection plane (38%) compared to Tirelli et al.'s studies, which ranged from 94% to 100%. The reduced TP rate in Baj et al. may be subject to their sampling strategy—taking samples from the borders of NBI-positive areas, unlike Tirelli et al., who sampled within NBI-positive areas. In the diagnostic accuracy study ('Type B') of Tirelli from 2017 only one negative index test was found (181). Interestingly, their subsequent study showed a much higher number of negative index tests (182). This figure might have been the result of a learning curve.

Based on the included studies, it is impossible to determine whether autofluorescence, iodine guidance, or NBI- is more accurate than WL-guided surgery to determine a safe surgical mucosal margin, and also in terms of distinguishing (severe) dysplasia from SCC. There are several reasons.

Firstly, there is a high variety in the definition of a positive reference-standard dysplasia: i.e. SCC, SCC in combination with severe dysplasia, or SCC in combination with all types of dysplasia in the resection plane. Several studies do not differentiate between SCC and (severe) dysplasia.

Secondly, the index tests of all studies were not designed to distinguish (severe) dysplasia from SCC, but rather tissue that was divergent from normal mucosa. For autofluorescence, neoplastic, dysplastic, and inflammatory tissue all show FVL (183). Staining with Lugol's iodine is based on the fact that iodine is glycophilic and does not bind to cells that lack glycogen, leading to iodine unstained areas. However, SCC and dysplasia both lack glycogen; therefore, Lugol's iodine cannot differentiate between tissue types (178). Finally, NBI is based on detecting alternations in the interpapillary capillary loops, which can underlie histopathologic changes, but this accounts for both SCC and all types of dysplasia (180).

Thirdly, all studies are possibly subject to a high inter- and intra-observer variability, requiring a certain expertise and experience to achieve a sufficient diagnostic value. None of the studies presented a clear cutoff value to define a positive or negative index test. In the studies of Tirelli et al., NBI-experts needed to be consulted to determine the NBI-safety margin, suggesting that finding alterations in the intrapapillary capillary loop patterns is difficult. Hence, they have found a variety in the diagnostic accuracies (180–182).

Fourthly, the included studies have a relatively small number of included patients or conducted retrospective studies. Only Durham et al. (122) conducted a randomized clinical trial and may pose the highest level of evidence that autofluorescence-guidance does not influence obtaining more adequate margins or more local control than WL guidance. However, the inexperience of certain observers and the surgeon's awareness of obtaining adequate margins in the WL-guided control cohort might have influenced the results.

Lastly, in most studies, only the NPV could be calculated. The sensitivity, specificity and PPV remain unknown for autofluorescence and iodine guidance. The lack of this information complicates the assessment of their potential impact on a 'tailor-made' approach. Without these data, it remains unclear how the adjustment of the safety margin around a positive index test could affect surgical margins, either by expanding or reducing them. Only two studies suggested that NBI-guidance could lead to more tailor-made resections. Tirelli et al. have shown a specificity of 64%, meaning that 64% of the margins positive for SCC or dysplasia, were rightfully made smaller if only a resection plane free of SCC or dysplasia is considered acceptable (182). For Baj et al., this number was 70% (173).

There are several other margin visualisation techniques that could lead to new insights when investigated in a surgical setting. Optical coherence tomography (OCT), for instance, works essentially in the same manner as ultrasound,

but uses light instead of sound waves. Because of the short wavelength of light, its penetration depth is not more than 0.5 mm for mucosa, but it can provide highly detailed images (184). At the moment, the setup of OCT devices mostly does not allow intraoral assessment (185). In one study by Sunny et al. (186), a hand-held OCT device was introduced for intraoral use. The authors captured images of multiple zones around the tumour and compared them with the histopathological report. The observers of the OCT data were blinded for the surgical procedure. They found that OCT was able to detect SCC inside the tumour and the area around the visible tumour with a sensitivity and specificity of 100%. For dysplasia, the sensitivity and specificity were 93% and 69%, respectively. The study was not included in this review because of the limited field of view of the device (186). Further development is needed to eventually assess the whole mucosal part of a tumour with OCT.

Other fluorescence-guided techniques exist besides autofluorescence. Contrast-agent-based fluorescence uses a near-infrared fluorescent label for SCC-specific antigens, such as cetuximab (149) or panitumumab (187). This technique can be used intra-orally, but mostly to check the wound bed on any residual fluorescent signal (149). The scope of most studies researching this technique is an ex-vivo assessment of the resection specimen. FSA biopsies can be taken from the spot with the highest fluorescent signal and analysed to determine whether this margin is close or positive. If not, it may suggest that the other fluorescent spots on the specimen are free margins as well (187). One major advantage of this technique is that it can produce objective values for the index test, i.e. the signal-to-background ratio of the fluorescence signal, which eliminates inter-observer dependence, as presented by de Wit et al. (149). As autofluorescence does not yield significant improvements in obtaining mucosal margins when compared to WL-guided surgery, it would be interesting to investigate the impact of contrast-agent-based fluorescence on mucosal margins in randomized control trials, following a similar setup as Durham et al. (122).

Apart from iodine staining, staining with toluidine blue has also been researched. However, the studies of concern (188,189) stained the resection specimen, but only after the resection was completed. These studies concluded that this stain is highly sensitive to SCC in the resection margins but has a low PPV. Kerawala et al. (190) performed a study on the intra-oral use of toluidine blue as a margin visualisation technique, but this study was also not included since it was published before 2010. They concluded that Toluidine blue is a suitable adjunct in identifying invasive tumours but has no benefit in identifying dysplastic tissue at the surgical margins. Unfortunately, their findings did not result in further research on the intraoperative application of Toluidine blue in the past decade.

Several limitations should be acknowledged in this review. Firstly, the inclusion of various methodologies (such as ‘Method A’) and diverse outcome measures (including diagnostic accuracy for both ‘SCC and dysplasia’ or ‘SCC alone’) poses a challenge in assessing potential publication bias. This complexity makes it difficult to employ standard methods like funnel plots or Egger’s test for a comprehensive evaluation. Secondly, as some included articles have the same author (i.e. Tirelli et al.) and were published within four years while assessing the same technique, it cannot be ruled out that there may be some overlap between the described cohorts. However, evidence is lacking to confirm or refute this possibility.

We suggest that future studies on margin visualisation techniques should focus more on the differentiation between (severe) dysplasia and SCC. Moreover, the evaluation of diagnostic accuracy should go beyond the goal to achieve only a negative index test. Ideally, a setup as presented by Sunny et al. (186), would give a broader insight into the diagnostic accuracy for SCC and severe dysplasia. Independent observers designated the images obtained from the OCT device as ‘normal’, ‘potentially malignant’ and ‘malignant’. This was conducted at different zones from the tumour border, which makes it feasible to determine the diagnostic accuracy for SCC and/or dysplasia in the resection plane, but also for close margins (SCC at 1-5 mm from the resection plane). If technically possible, the margin visualisation technique should also be as inter-observer independent as possible. An example is the signal-to-background ratio-based fluorescence of de Wit et al. (149), where the author used an objective value to determine tumour-presence.

Conclusions

Three margin visualisation techniques for oral SCC have been reviewed in a pre-incision surgical setting to determine a safe mucosal margin demarcation: autofluorescence, iodine staining and NBI. Most of these studies did not assess the frequency of free margins (≥ 5 mm) but only the presence of dysplasia and SCC in the resection plane. Apart from fluorescence, the margin visualisation techniques found a wide variety in diagnostic accuracy, possibly due to learning curves and inter- or intra-observer variability. Autofluorescence-guidance seems to make no difference in obtaining better margins than WL-guidance. However, contrast-agent-based autofluorescence might be more effective, and testing this technique in large randomized controlled trials is advisable. We also recommend continuing to investigate iodine- and NBI-guided surgery in more extensive cohorts, with a larger focus on differentiation between (severe) dysplasia and SCC, as the consequences of the treatment of residual dysplasia and SCC are highly different. Apart from reporting the treatment effect of the technique in terms of margins ‘free from SCC and (severe) dys-

plasia’, the presence of close (1-5 mm) or free (≥ 5 mm) margins should be reported as well, according to the standard guidelines. Finally, we recommend a larger focus on actual diagnostic accuracy rather than treatment effect only. This strategy would allow for determining a meaningful sensitivity, specificity, and PPV, in addition to negative predictive value (NPV). Such an approach will lead to a better understanding of the value of these techniques.

Chapter 9

Feasibility of an MR-based digital specimen for tongue cancer resection specimens: a novel approach for margin evaluation

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Abstract

Objective: This study explores the feasibility of ex-vivo high-field magnetic resonance (MR) imaging to create digital a three-dimensional (3D) representations of tongue cancer specimens, referred to as the 'MR-based digital specimen' (MR-DS). The aim was to create a method to assist surgeons in identifying and localizing inadequate resection margins during surgery, a critical factor in achieving locoregional control.

Methods: Fresh resection specimens of nine tongue cancer patients were imaged in a 7 Tesla small-bore MR, using a high-resolution multislice and 3D T2-weighted Turbo Spin Echo. Two independent radiologists (R1 and R2) outlined the tumour and mucosa on the MR images whereafter the outlines were configured to an MR-DS. A colour map was projected on the MR-DS, mapping the inadequate margins according to R1 and R2. We compared the haematoxylin-eosin-based digital specimen (HE-DS), which is a histopathological 3D representation derived from HE stained sections, with its corresponding MR images. In line with conventional histopathological assessment, all digital specimens were divided into five anatomical regions (anterior, posterior, craniomedial, caudolateral and deep central). Over- and underestimation 95th-percentile Hausdorff-distances were calculated between the radiologist- and histopathologist-determined tumour outlines. The MR-DS' diagnostic accuracy for inadequate margin detection (i.e. sensitivity and specificity) was determined in two ways: with conventional histopathology and HE-DS as reference.

Results: Using conventional histopathology as a reference, R1 achieved 77% sensitivity and 50% specificity, while R2 achieved 65% sensitivity and 57% specificity. When referencing to the HE-DS, R1 achieved 94% sensitivity and 61% specificity, while R2 achieved 88% sensitivity and 71% specificity. Range of over- and underestimation 95HD was 0.9 mm - 11.8 mm and 0.0 mm - 5.3 mm, respectively.

Conclusion: This proof of concept for volumetric assessment of resection margins using MR-DSs, demonstrates promising potential for further development. Overall, sensitivity is higher than specificity for inadequate margin detection, because of the radiologist's tendency to overestimate tumour size.

Introduction

An estimated 3.5 new cases of tongue cancer occur annually in the United States for every 100,000 individuals. Squamous cell carcinoma of the tongue is the most predominant type of oral cancer (191). Complete surgical removal of the primary tumour is the first treatment choice to prevent local recurrence (31).

After surgical excision, the margins of the resection specimen are analysed during histopathological examination to verify that the minimal margin distance is adequate (≥ 5 mm) or inadequate (< 5 mm) (28). Unfortunately, inadequate margins are frequently encountered (4). A retrospective analysis of 96 SCCT patients treated at our centre revealed that 84% of the resection specimens had inadequate margins (113), which is in line with the literature (4). These patients may be considered as candidates for local adjuvant treatment, (chemo)radiotherapy or secondary resection. Local radiotherapy has several side effects, including mucositis, xerostomia, and osteoradionecrosis (44,108). Furthermore, conducting secondary surgery not only requires additional operating time and anaesthesia but it also introduces uncertainty regarding the anatomical relationship between the newly obtained resection specimen and the original specimen (42).

By assessing margins intraoperatively, surgeons can make immediate adjustments, eliminating the drawbacks of a second surgery. Several techniques exist for assessing margins: either during the resection (in-vivo) (57,113,122,179,189,192) or immediately after (ex-vivo) (66,103,113,156,187,192,193). Frozen section analysis is the most frequently used ex-vivo method. However, it is prone to sampling errors as only a small portion of the resection specimen and/or wound bed is sampled (194). Further complicating this is the challenge in linking the frozen section sample to the resection specimens (82). Over the past decade, several publications have addressed using magnetic resonance (MR) for intraoperative margin assessment (59,146,155). While only a few institutions have a clinical MR-machine in the operating room, a high-field small-bore MR, typically located outside the operating room, produces high-quality images (59). Despite the inability to assess margins in-vivo, MR has the potential to generate three-dimensional (3D) representations of the resection specimen. The fact that such a 3D representation allows examiners to view the resection specimen from multiple angles and perspectives, contrasts with the small sampling rate of frozen section analysis.

This study serves as a proof of concept for using a high-field ex-vivo MR as an alternative to frozen section analysis for improved localisation of inadequate margins. Digital MR-based 3D representations of the specimen, mapping the inadequate margins, were validated in two ways: 1) with conventional histopathological assessment as reference and 2) with a 3D representation of the specimen based on histopathology.

Methods

MR image acquisition and qualitative assessment.

Nine patients who underwent surgery for cT1-T3 squamous cell carcinoma of the tongue were prospectively included between January and June 2021. Sutures were applied to the specimen to facilitate orientation during scanning and pathological examination. Directly after surgery, the fresh resection specimens were transported to a small-bore 7 T MR-machine (BioSpec 7T, Bruker, Ettlingen, Germany), with a 0.9 T/m gradient system, interfaced with a Philips console (Philips Medical Systems, Best, Netherlands R.5.4). Additional re-resections were not analysed. The resection specimens were placed on a support made of thermoplastic material and were fixated with a gauze. On this support, the resection specimens were placed in a poly-methyl methacrylate cylindrical container (outer diameter: 70mm, inner diameter: 59mm). The container was filled with perfluoropolyether fluorinated fluid (Galden, Solvay Solexis, Thorofare, NJ, USA) to prevent susceptibility artifacts during scanning. The container was placed in a transmit-receive volume coil with a 72 mm inner diameter and 112 mm outer diameter (Bruker) (Figure 1). The B0-field homogeneity was enhanced with shimming up to the second order. For each case, four scanning sequences were used: a 3D T2-weighted (T2W) Turbo Spin Echo (TSE) with an isotropic voxel size of 0.3 mm³ (referred to as MR 3D-images) and a T2W TSE with an in-plane resolution of 0.125 mm² and 1.0 mm slice thickness in three orthogonal directions (referred as MR multislice-images). Details of these sequences are provided in Table 1. Two radiologists assessed image quality by independently rating the images using a 5-point Likert scale on four parameters: 1) overall image quality, 2) visibility of the tumour, 3) visibility of the transition between mucosa and resection plane, and 4) certainty of margin status.

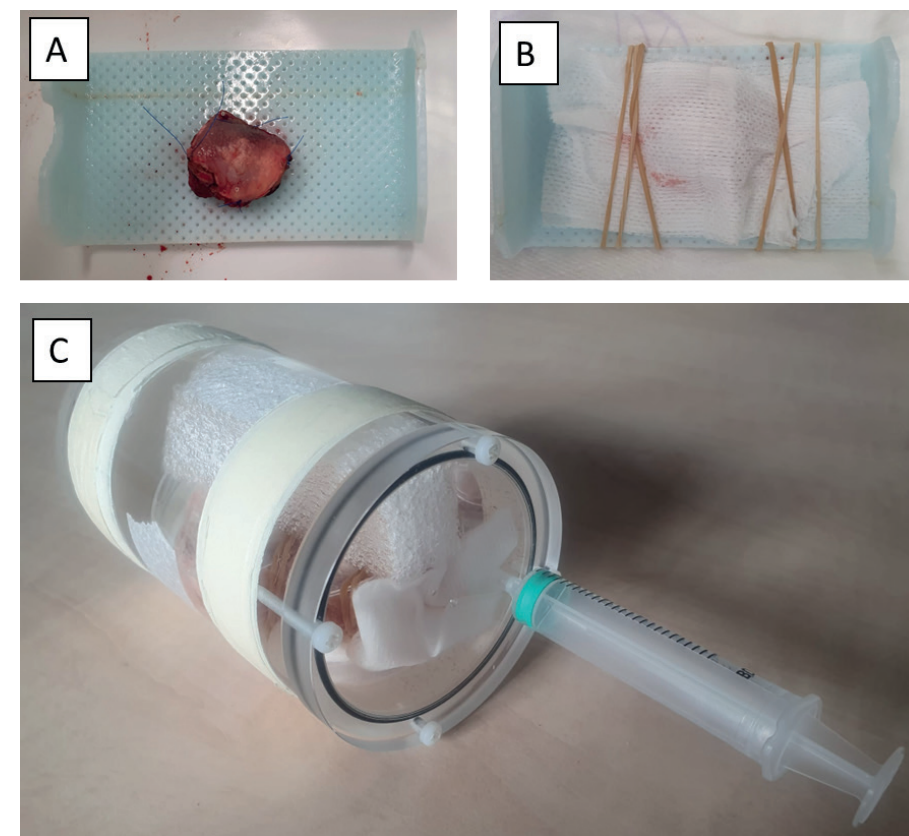


Figure 1: Set-up for MR image acquisition. (A) Resection specimen is placed on thermoplastic material. (B) Fixation of the specimen with gauze. (C) Resection specimen is placed in a PMMA cylindrical container.

Histopathological assessment

After MR-imaging, the fresh resection specimens underwent fixation in a 4% formaldehyde solution for a minimum of 24 hours before histopathological examination. The specimens were sliced into cross-sectional tissue blocks of approximately 3 to 5 mm thick, oriented perpendicular to the anterior-posterior axis. From each section, a 4 µm thick microscopic section was obtained and stained with haematoxylin and eosin (HE). After staining, the sections were converted to digital images (referred to as HE images) using the methods of Stathonikos et al. (102). The margins of the resection specimens were determined at five specific locations: anterior, posterior, craniomedial (towards the dorsal surface of the tongue), caudolateral (towards the floor of mouth), and deep central (directly under the tumour). The anterior and posterior margins were determined by multiplying the average thickness of a single slice (derived from the specimen's length divided by the number of slices taken) with the count of microscopically

tumour-free slices in the respective anterior and posterior directions. The craniomedial and caudolateral locations were defined as the space between two 45-degree lines originating from a line parallel to the mucosa through the middle of the tumour. The deep central location encompassed the region between the craniomedial and caudolateral slices. Our centre's department of pathology adopted this method to distinguish the margins of the five locations, introduced during one of our previous studies (Figure 2A-B) (113). The margins and tumour thickness were measured using a digital ruler within dedicated software to assess microscopic images (Sectra IDS7, version 23.1, Linköping, Sweden).

Creation of the MR-based Digital Specimen and HE-based Digital Specimen

Registration

The MR- and HE images were imported in in-house built viewing and contouring software (Volumetool, version 1.30.39) (195). The coronal T2W images were used for registration with the HE images. The choice of using this particular sequence had two reasons. Firstly, tumours exhibit better contrast with normal tissue in T2W multi-slice MR images than in T2W MR 3D images. This is because in a 3D T2W TSE-scan small refocusing angles need to be used to preserve signal and prevent blurring due to T2 decay. Therefore, the T2 contrast is different from a multi slice T2W image. Secondly, the orientation of the coronal plane aligns with our institution's recommended approach for resection specimen slicing to obtain HE sections, which is specifically perpendicular to the anterior-posterior axis. Matching pairs of T2W slices and HE images were selected. A point-based registration technique was used, where two observers (KK and JR) selected corresponding anatomical points (e.g., mucosa, tumour-protrusions, and arteries). Subsequently, the rotated and scaled HE images were digitally stacked. This resulted in a volumetric representation of the histopathological situation, comparable to a coronal MR dataset. The observers could also adjust the distance between each HE image within the specimen (Figure 3, 4A, 5). This procedure is similar to the methods described in the work of Caldas Magalhaes et al. (196).

Outline procedure

The radiologists contoured the tumour and mucosa by outlining both structures on the coronal T2W images. The pathologist was asked to perform the same procedure on the HE images. This resulted in a volumetric outline for both the tumour and the mucosa, each determined independently by radiologists and a pathologist (Figure 3, 4A). Pathologist-determined volumetric outlines were propagated to the available MR sequences and slightly modified by three authors (KK, JR and MP) until mutual agreement was achieved. These modifications were considered justifiable because the relatively large variance

of the HE images' spacing (1-10 mm) and because histopathological processing may affect the accuracy of the HE images (i.e. shrinkage, tissue deformation, missing tissue) (Figure 5). Radiologist-determined volumetric outlines were propagated to the other MR-sequences (i.e. sagittal, transversal and 3D images) and to the stacked HE images. In this way, the volumetric outlines of the radiologist could be compared with those of the pathologist (Figure 3, 4A). Using Volumetool, a threshold was applied to distinguish the background from the specimen on the images of the MR 3D-sequence to obtain a volumetric outline of the specimen's contour.

MR-based and HE image based digital specimens

Using medical image processing software, Mimics (v24.0, Materialise, Leuven, Belgium) and computer-assisted-design and modelling software 3-matic (v17.0, Materialise, Leuven, Belgium) the volumetric outline of the tumour, mucosa and specimen's outer contour were combined to attain a 3D visual representation of the resection specimen; two as determined by the independent radiologists, referred to as the MR-based digital specimen (MR-DS) and one as determined by the independent pathologist, referred to as the HE based digital specimen (HE-DS) (Figure 4B). The outer contour of the HE-DS was obtained from the MR images to compensate for the artifacts that occur during histopathological processing.

For comparison purposes, a 3D substitute to the aforementioned conventional assessment of the five margin directions i.e. anterior, posterior, craniomedial, caudolateral and deep central was created (113) (Figure 2). Firstly, a cone with an apex angle of 45 degrees was created to designate the deep central region of the MR and HE-DS. The apex of the cone was placed manually in the middle of the tumour with its base perpendicular to a plane fitted onto the mucosa. Secondly, the portion of the MR- or HE-DS outside of the cone was divided into 4 quadrants. To define the quadrants, an anterior-posterior midplane was created perpendicular to the cone base by manually selecting an anterior and posterior point. This midplane was used as a reference system to create two perpendicular planes and divide the remaining portion of the MR- or HE-DS into the anterior, posterior, craniomedial and caudolateral quadrants (Figure 2). By applying a distance colormap on the MR- or HE-DS outline, highlighting regions where the distance between tumour and specimen's outline was < 5 mm, the MR- and HE-DS could be used to localize inadequate margins according to radiologist-determined outline on MR images and pathologist-determined outline on HE images (Figure 4C).

Analyses

Statistical analysis was performed using SPSS (version 27.0, IBM, Armonk, NY, USA).

To assess the accuracy of the radiologist-determined volumetric outlines of the tumour, when compared to its histopathologic counterpart, we utilized the 95th percentile Hausdorff distance (95HD). This statistic measures the maximum distance between corresponding points between the MR- and HE-DS' tumours, while accounting for data variability and outliers. It was calculated in two ways:

- Underestimation 95HD: We computed the 95HD between the pathologist's tumour outline and the Boolean intersection with the radiologists', revealing tumour underestimation by the MR-DS.
- Overestimation 95HD: We computed the 95HD between the radiologists' tumour outline and the Boolean intersection with the pathologist's, revealing tumour overestimation by the MR-DS.

Table 1. MRI acquisition parameters										
Sequence name	Scan direction	TR (ms range)	TE (ms)	RA (°)	Slice Thickness (mm, range)	Gap distance (mm)	No. Slices (range)	Acquired Vox-el size (mm ³)	Scanning time (min., range)	Echo train
T2W TSE Multislice	Sagittal	8554-17412	80	140	0.9-1	0.10	32-51	0.125	4:51-12:34	13
T2W TSE Multislice	Axial	6415-21066	80	140	1	0.10	23-27	0.125	5:43 -11:02	13
T2W TSE Multislice	Coronal	10692-22906	80	140	1	0.10	17-55	0.125	5:06-17:56	13
T2W TSE 3D	Coronal	2000	140	60	0.25-0.30	-	100-140	0.25-0.30	10:45-36:06	32

Abbreviations: T2W: T2 weighted signal, TSE: Turbo Spin Echo, 3D: three-dimensional, TR: Repetition time, TE: Echo time, RA: Refocusing Angle

Table 2. Case-specific characteristics

T-stage		Tumour characteristics				95 th percentile Hausdorff distance of the tumour					
		DOI (mm)	Max. diameter (mm)	No. HGP	Differentiation	Margin status	Min. margin conv. Histopath.	U95HD R1 (mm)	O95HD R1 (mm)	U95HD R2 (mm)	O95HD R2 (mm)
#											
1	pT3	15.6	44	3	±	I	1.9	1.7	0.9	1.2	1.6
2	pT3	10.0	27	0	±	I	2.5	0.7	2.5	1.5	1.8
3	pT3	12.6	28	2	±	I	1.0	1.3	6.4	0.3	5.2
4	pT1	1.2	3	0	+	A	6.7	0.5	10.0	1.4	6.6
5	pT2	7.0	7	2	±	I	3.2	0.7	0.6	0.3	1.6
6	pT1	2.4	10	1	±	I	4.5	0.9	3.1	0.4	5.5
7	pT2	6.7	30	2	±	I	2.3	0.9	1.2	0.4	1.2
8	pT1	3.5	15	1	±	I	4.3	0.9	2.0	0.5	3.1
	pT1	3.4	15	1	±	I	4.6	0.0	11.8	5.3	2.5

Abbreviations: T-stage: Tumour stage, DOI: Depth of invasion, No. HGP: Number of unfavourable histopathological growth factors, Max. diameter: Maximal diameter, Min. margin histopath: Minimal margin histopathology, U95HD: 95th percentile Hausdorff distance of underestimation, O95HD: 95th percentile Hausdorff distance of overestimation, R1: radiologist 1, R2: radiologist 2 ±: Moderately differentiated, +: well differentiated, I: inadequate, A: adequate

Furthermore, the accuracy of the MR-DS in inadequate margin (i.e. < 5 mm) prediction was assessed per location (e.g., anterior, posterior, craniomedial, caudolateral and deep) in two manners: 1) by comparing it with the margins of all five locations, as described by the conventional method of histopathological margin assessment and 2) by comparing it with the HE-DS. It was determined whether the location of the highlighted regions that indicated < 5 mm on the MR-DS corresponded with those on HE-DS (Figure 2 and 4). Accuracy was presented as sensitivity, specificity, positive predictive value (PPV), and negative predictive value (NPV).

As the conventional histopathological margin assessment is the gold standard, and not the HE-DS, we compared location of the inadequate margins according to the HE-DS with the results of conventional pathology. This allowed us to explain differences in diagnostic accuracy when using the different reference standards.

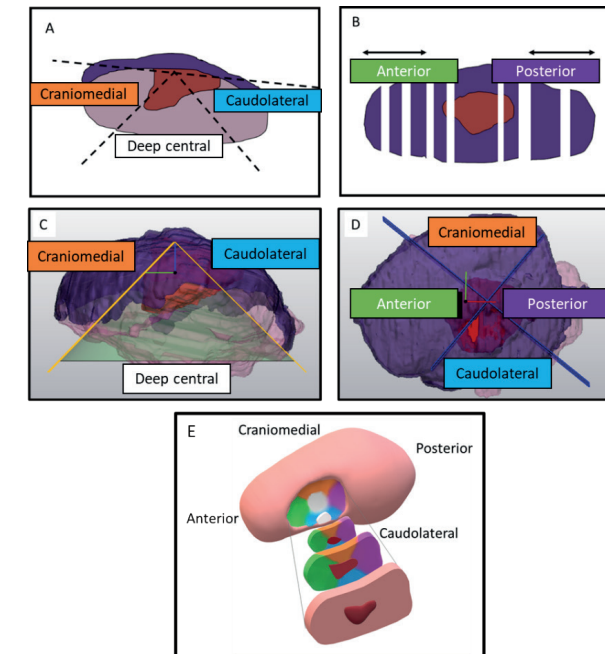


Figure 2: Division of the specimen on histopathological HE sections (according to conventional histopathological assessment) and on the digital specimens. (A) Two 45-degree lines diverge from a line parallel to the mucosa when a HE section is faced from the front. In between the 45-degree lines, the specimen's portion is defined as deep central. Outside these two 45-degree lines, the specimen's portion is either defined as craniomedial (towards the dorsum of the tongue) or caudolateral (towards the floor of mouth). (B) Top view of the specimen. Anterior and posterior margins are determined by multiplying the number of tumour-free slices with mean thickness of the tissue blocks. (C) Side view of the digital specimen which is divided by a cone from of which the apex is perpendicular to a plane fitted on the mucosa. The inclination of the cone is 45-degree. (D) Top view of the remaining portion of the digital specimen. This portion is divided by two perpendicular planes in four quadrants. The reference system for those planes is created perpendicular to the base of the cone and combined with a manually selected anterior and posterior point. (E) Anterior, posterior, craniomedial, caudolateral, deep central location of the resection specimen, depicted in a schematic figure of the tongue. The colour of the specimen's location corresponds with the colours in the text boxes of figure (A–D).

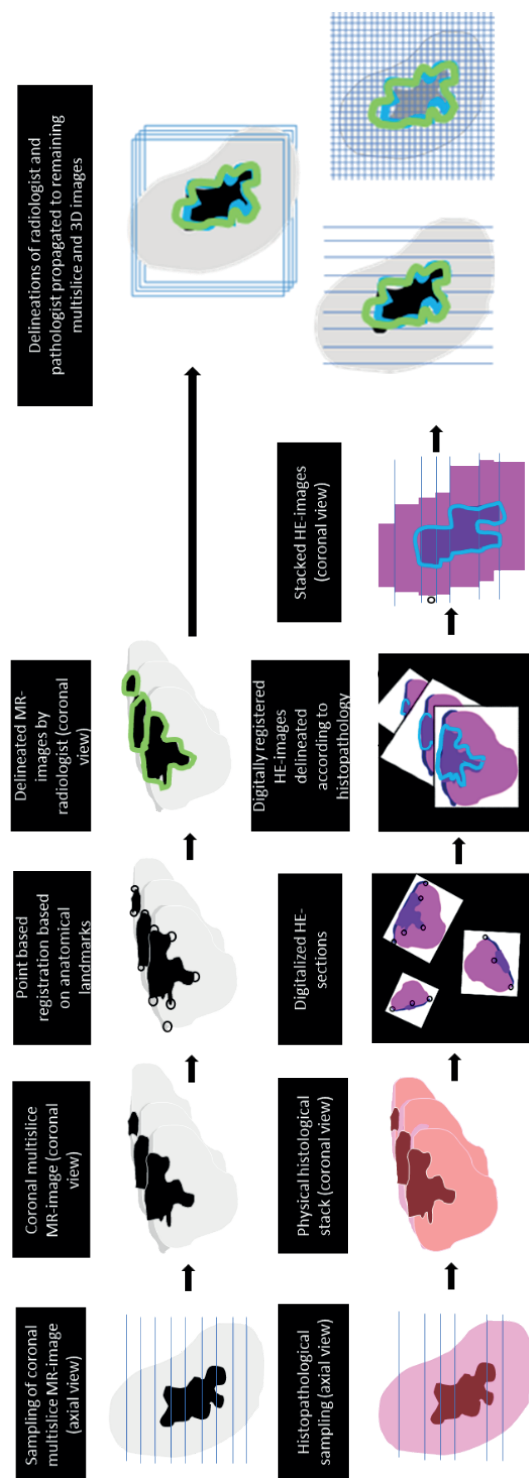


Figure 3: Workflow of the registration and mapping of the 3D outlines of MR images on all obtained sequences.

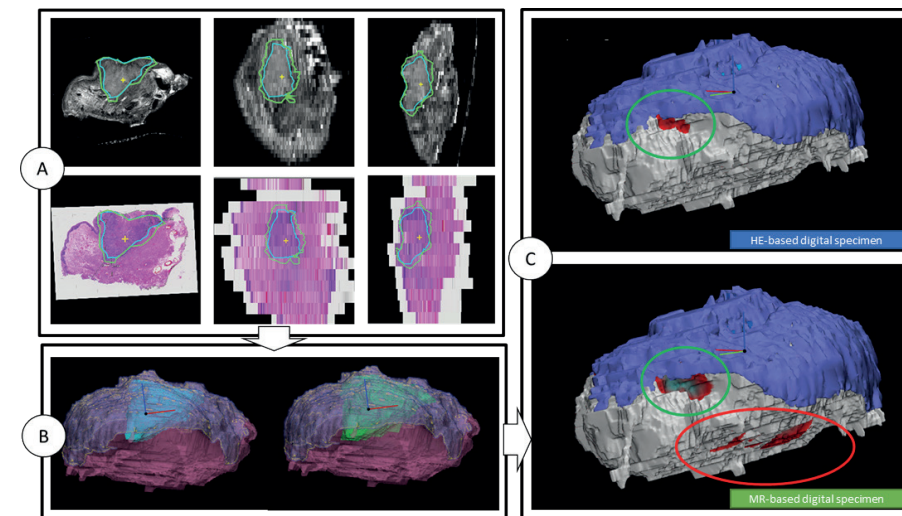


Figure 4: Workflow of the creation of the digital specimens. (A) Coronal, transversal and sagittal view of the T2W multislice MR images, while matched with the HE images. Includes tumour outlines by a histopathologist (blue line) and an outline of one of the two radiologists (green). (B) HE-DS (left) and MR-DS (right). Note that the representations of the tumours are different as the one of the HE-DS (blue) is derived from the pathologist's outline and the one of the MR-DS (green) is derived from the radiologist's outline. The mucosa (purple) and specimen's outline (pink), representing the resection plane are visible as well. (C) Colormaps projected on the HE-DS (upper) and MR-DS (lower), highlighting the regions with inadequate margins in dark red, according to respectively histopathology and radiology. Green ellipsoid is a true positive inadequate margin. Red ellipsoid is a false positive inadequate margin. For the sake of clarity, the resection plane is represented in white. The mucosa is represented in purple.

Results

Clinical characteristics

The cohort consisted of 9 cases with various tumour stages: pT1 (4 cases), pT2 (2 cases), and pT3 (3 cases). The mean (SD) of depth of invasion (DOI) was 6.9 (4.9) mm. Unfavourable histopathological growth factors (i.e. non-cohesive growth, perineural growth, and vascular invasion) were noted as follows: none for two cases, 1 for three cases, 2 for three cases, and 3 for one case. All cases exhibited moderately differentiated tumours based on histopathology, except for case 4, which had a well-differentiated tumour (Table 2).

Table 3. Diagnostic accuracy of detection of inadequate margins (< 5 mm)

	<i>Conventional histopathological assessment</i>		<i>HE-based digital specimen</i>	
	<i>Radiologist 1</i>	<i>Radiologist 2</i>	<i>Radiologist 1</i>	<i>Radiologist 2</i>
<i>Sensitivity (95% CI)</i>	77% (50%-93%)	65% (38%-86%)	94% (71%-100%)	88% (64%-99%)
<i>Specificity (95% CI)</i>	50% (31%-69%)	57% (37%-76%)	61% (41%-79%)	71% (51%-87%)
<i>PPV (95% CI)</i>	48% (29%-68%)	48% (27%-69%)	59% (39%-78%)	65% (43%-84%)
<i>NPV (95% CI)</i>	78% (52%-94%)	74% (50%-89%)	94% (73%-100%)	91% (71%-99%)

Abbreviations: HE-based: haematoxylin-eosin-based, PPV: positive predictive value, NPV: negative predictive value, 95% CI: 95% confidence interval

Qualitative assessment of original ex-vivo MR images

On a scale from 1 to 5, the median image quality was rated 4 (range: 3-4) by R1 and 3 (range: 3-5) by R2. The median visibility of the tumour was rated 4 (range: 2-4, case 3 received a score of 2) by R1 and 4 (range: 3-5) by R2. The visibility of the transition from the mucosa to the resection plane was rated 3 (range: 1-4, case 3 received a score of 1, and case 5 a score of 2) by R1 and 4 (range: 4-5) by R2. For image quality, both observers agreed with a maximum of 1 point difference in 100% of the cases. For tumour visibility this was 100% and 66%, respectively.

Diagnostic accuracy of the MR-based digital specimens

Underestimation 95HD and overestimation 95HD of the tumour extension as derived by the radiologists in MR-DSs are depicted in Table 2 and Figure 6. Range of overestimation 95HD (0.9 mm – 11.8 mm) was far larger than the range of underestimation 95D (0.0 mm – 5.3 mm). Except for the outlier of 5.3 mm, the maximal underestimation 95HD was 1.7 mm. When comparing the MR-DS with conventional histopathological assessment, R1 exhibited higher sensitivity than R2, but lower specificity. This observation remained consistent when the MR-DSs were compared with the HE-DSs. In overall, the diagnostic accuracy was higher when referred to the HE-DS than when referred to conventional histopathological assessment (Table 3).

Comparison between conventional histopathology and HE-based digital specimens

To assess the impact of the HE-DS as a 3D reference, we conducted a comparison of its margins with those obtained through conventional histopathologic assessment. Of the forty-five histopathological margins measured with conventional histopathological assessment, nine (20%) were inconsistent with the HE-DS. For five of these inconsistencies, it became evident that the subdivision of the HE-DS into the anterior, posterior, caudolateral, craniomedial, and deep central regions did not correspond to the conventional subdivision (Figure 2 and

4C). Consequently, four regions in three cases were classified as adequate (≥ 5 mm) by the conventional assessment and inadequate (< 5 mm) by the HE-DS: anterior in case 1, anterior and posterior in case 3, and craniomedial in case 5. In the deep central part of case 5, the margin was classified as inadequate by the conventional assessment and adequate by the HE-DS. In three of the nine inconsistencies, the histopathological margin appeared slightly modified due to deformation during processing of the MR- or HE images. This caused the deep central margin in the HE-DS for case 1 to be classified as inadequate due to compression during scanning. Two margins in two cases, i.e. case 2 and case 9, were classified as adequate by the HE-DS, but inadequate by the conventional method due to rupture and shrinkage during histopathological processing. As we used the specimen's outline, derived from the MR images, to compensate for these artifacts, the HE-DS's margin differed from conventional margin assessment. In the last inconsistency, the thickness of both the most anterior and posterior HE images of case 8 appeared to be greater than what was estimated by conventional assessment (Figure 5). As only these slices were tumour-free, the anterior and posterior margins were reported as inadequate, whereas, according to the HE-DS, they were judged adequate.

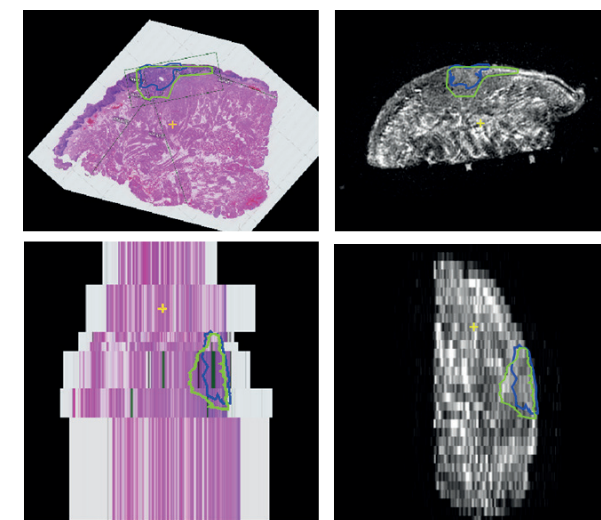


Figure 5: Coronal and sagittal views of the stacked HE stained images (left column) and coronal T2W MR image (right column) of case 8. As can be seen in this case, the HE stained image spacing can be irregular and may contribute to underestimation of the anterior and posterior margin in the histopathological report; in the clinical report the anterior margin (lower slice) has been denoted as inadequate, since only one HE stained image was tumour free and the main cross-section thickness was determined as 4.3 mm. However, after matching the HE stained images with the coronal T2W MR images, this distance seemed to be far larger.

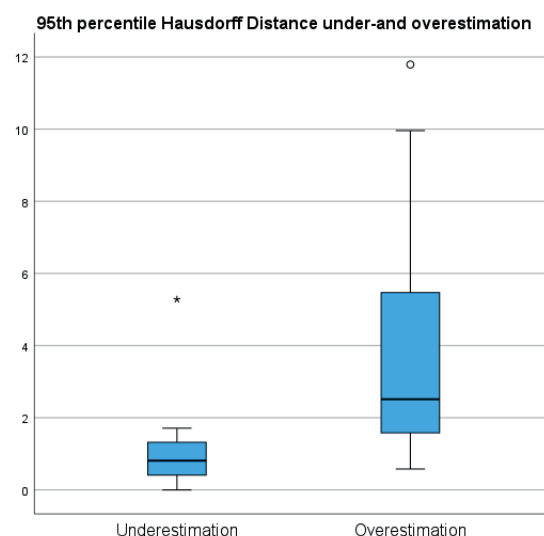


Figure 6: 95th percentile Hausdorff under and overestimation of the tumour margin in de MRI of the specimen (MR-DS)- with respect to the of the tumour in the HE-based digital specimen (HE-DS).

Discussion

In this study, we evaluated whether the volumetric assessment of resection margins using MR-DSs, as determined by radiologists, could improve the localisation of inadequate (< 5 mm) margins. We compared the inadequate margins according to the MR-DS with 1) conventional histopathologic assessment and 2) with a HE-DS. When conventional histopathological margin assessment was used as a reference, we found that the MR-DS had moderate sensitivity and specificity for inadequate margin detection. However, when compared with a HE-DS, sensitivity and specificity were higher. Generally, the NPV for detecting inadequate margins is higher than the PPV. Apparently, the tumour's volume is more often overestimated than underestimated. As the incidence of inadequate margins is relatively high, there is a relatively high frequency of false positives and a low frequency of false negatives, which results in a lower specificity but higher sensitivity.

There are several reasons why the diagnostic accuracy of MR-DS, when referred to HE-DS, was higher than when referred to conventional histological assessment. Firstly, the framework for the localisation of inadequate margins on MR-DS (i.e. determining whether there was a region of inadequate margin at the anterior, posterior, craniomedial, caudolateral and deep central portion) was defined exactly the same as the HE-DS. In contrast, conventional histopathology defines anterior and posterior margins based on the number of tumour-free

HE images multiplied by the average tissue block thickness (typically 3-5 mm). However, the actual distance between HE images may vary, especially when they originate from sections taken from the outermost distal or proximal parts of the tissue block. Hence, spacing may vary between 1-10 mm. Secondly, the colormap that was used to highlight the inadequate margins on the MR- and HE-DS led to the interpretation of the inadequate margin as an area, rather than a point, which contrasts with conventional histopathology. Thirdly, the imaging methods and histopathological processing inherently introduce forces that can cause deformation or tearing of the resection specimen. During scanning, the deep central margin may be compressed against the support of the specimen, leading to an underestimation of the margin distance. Moreover, histopathological processing can cause rupturing (e.g., case 9) and shrinkage of the specimen. Although the effect of shrinkage might be compensated by the rigid scaling that was applied during registration, shrinkage might not be uniform over the entire specimen. For instance, Umstattd et al. (67) demonstrated that specimen shrinkage predominantly occurs in the healthy tissue rather than in the tumour, which might have been the situation in case 2.

The fact that sensitivity of the MR-DS was higher than specificity may be caused by the fact that the range of tumour overestimation (95HD: 0.9 mm – 11.8 mm) was far larger than the range of underestimation (95HD: 0.0 mm – 1.7 mm, with one outlier at 5.3 mm). Radiologists' tendency to overestimate tumour volume leads to a low positive predictive value (i.e. relatively many false positives, few true positives) and a high negative predictive value (i.e. relatively many true negatives, few false negatives). However, overestimating the tumour has a more favourable clinical impact than underestimation. Despite the increased likelihood of unnecessary intraoperative re-resection, surgeons retain the discretion to disregard the indication when resecting structures that would significantly impact the patient's quality of life. At the same time, this approach increases the likelihood of a successful re-resection.

A challenge frequently encountered during ex-vivo intraoperative margin assessment of tongue cancer is the loss of the anatomical relationship between the inadequate margins and the wound bed (42). One might argue that a technique allowing in-vivo assessment (during the actual resection) is more favourable. At our institute, we investigated the application of intraoperative ultrasound during tongue cancer surgery (113). This technique enabled us to scan the entire resection specimen, both in-vivo and ex-vivo. Based on our experience (113) and that of others (53,56,57,90,147) in-vivo ultrasound can significantly enhance surgical resection margins. However, the diagnostic accuracy of the ultrasound in locating inadequate margins ex-vivo was moderate (area under the curve: 0.63). Additionally, the field of view is limited by the probe's size and its acoustic penetration depth. Most other techniques for in-vivo assessment primarily reveal only the tumour's mucosal extent (179,182,189). As no superior in-vivo

technique has emerged thus far, ex-vivo margin assessment remains crucial for margin control. The fact that MR-DSs could be projected on screens in the surgical room may facilitate a clearer understanding of the relationship between the inadequate margin in the specimen and the wound bed.

Bekedam et al. (103) attempted to develop an ultrasound-based digital specimen – with a colormap - of the resection specimen. This was achieved by stacking ultrasound images of which the reciprocal relation was determined using an electromagnetic tracker. They found that the interpretation of margins became easier. One notable limitation, however, was the limited image-quality produced by the 10 MHz probe. Additionally, they did not differentiate the mucosa in their digital specimen from the actual resection plane.

Several groups have conducted research on ex-vivo MR of tongue cancer specimens. Steens et al. (146) evaluated the visibility of tongue cancer and resection margins from ten MR scans of resection specimens using a similar small-bore 7 T MR as employed in our study. In three out of the ten specimens, the tumour was not visible, potentially due to a small depth of invasion (DOI), i.e. < 1 mm.

In another study, Heidkamp et al. (59) studied ten tongue cancer specimens using a 3 T MR scanner, situated in a surgical room, to improve logistics. The consequence of the lower field strength and larger bore was that the lower signal to noise ratio hampered the visibility of the transition between mucosa and resection plane. In our study, only a few cases received a score lower than 3 for the visibility of this transition. In case 3, the low visibility may have been attributed to the specimen being pressed against the cylindrical container. In case 5, it may have been caused by mucosal damage. Nevertheless, transition visibility did not seem to influence diagnostic accuracy significantly.

Giannitto et al. (155) performed a study with some similarities to our methods, i.e. they used a 3D-printed model of the tongue and tumour on which the resection specimen was attached for orientation of the specimen. Their 1.5 T clinical ex-vivo MR images showed a perfect diagnostic accuracy in predicting margin status (sensitivity and specificity both 100%). However, they stated their results were inconclusive due to the small sample size (n = 10) and relatively high number of true negatives.

Several limitations of our study need to be acknowledged. Firstly, the sample size was small, which implies that our results cannot be conclusive. Secondly, despite the efforts to align HE with MR images, minor inaccuracies might have been introduced (as shown in supplementary data). Thirdly, the specimen's outlines, derived from the MR images, were utilized to reconstruct the resection plane for both the MR- and HE-DSs. This implies that margin underestimation resulting from compression is reflected in the HE-DS but not in the outcomes of conventional histopathological analysis. Consequently, the HE-DS yields different results when compared to the established clinical standard, raising

questions about its accuracy as a reference standard. Finally, the radiologists are not specifically trained in tumour delineation on MR images of the resection specimens. As a result, their performance during this study might have been influenced by a learning curve.

Future studies should consider a larger sample size to strengthen the validity of the results. Other MR contrasts, such as diffusion or enhanced T2 contrast on the T2W TSE sequence used in this study, should be explored to optimize the visibility of the tumour and mucosa. Efforts to improve registration accuracy, such as setups that control the spacing between HE images, are currently being pursued. Radiologists should be provided with a training set to optimize their outline performances on MR images and improve their inter-observer agreement (especially for detecting the mucosa). Meanwhile, such a training set can also be used in deep learning to train a convolutional neural network capable of automatically outline tumours from healthy tissue (197). By saving a vast amount of time, as manual outline is a time-consuming effort, deep learning may optimize the challenging logistics faced when ex-vivo MR is used in clinical practice. At the time of writing, such a training set is currently under construction.

Conclusion

In conclusion, this proof of concept for volumetric assessment of resection margins using MR-DSs, as determined by radiologists, demonstrates promising potential for further development. This approach can enhance our understanding of the position of inadequate margins within the resection specimen. In the near future, this method could assist surgeons during tongue cancer resections by guiding them toward more precise and adequate direct intraoperative re-resection. Future studies should prioritize establishing a reliable registration with histopathology to validate the MR-DS. This step is crucial, especially if these models can be utilized in creating training sets for deep learning applications. Nonetheless, our study offers a foundational proof of principle, paving the way for subsequent studies to validate, apply, and refine this technique further toward clinical implementation.

Chapter 10

Summary, general discussion
and future perspectives

Local surgery is the preferred treatment for the primary tumour of oral squamous cell carcinoma (SCC). A complete removal with a free (≥ 5 mm) margin status (i.e. the closest margin distance in a resection specimen) is associated with better survival outcomes. A positive (< 1 mm) margin status requires adjuvant treatment, which can either be local adjuvant radiotherapy or a re-resection. Depending on other adverse histopathological factors, a close (1-5 mm) margin could also require local adjuvant treatment. Local adjuvant treatment could be a re-resection or (chemo)radiotherapy, both having their disadvantages. A re-resection requires a second scheduled surgery and comes with relocation problems, especially in a closed or healed wound bed. Local adjuvant (chemo) radiotherapy comes with morbidities such as xerostomia, mucositis and osteoradionecrosis, impacting the patient's quality of life (QoL) and oral function.

Obtaining free margin status is challenging. This accounts especially for the deep central margins, as the surgeons does not have a direct view of the tumour's deeper extent. Therefore, the surgeon must rely completely on the preoperative imaging and palpatory feedback during conventional surgery. Literature reports up to 45% close margin status and 43% positive margin status.

This thesis primarily focuses on a novel image-guided surgery technique to aid surgeons in achieving adequate resections: ultrasound (US). To a lesser extent, this thesis also discusses other image-guided surgery techniques for oral cancer, including magnetic resonance (MR) imaging.

Part 1 – Feasibility of ultrasound-guided oral cancer surgery

Part 1 introduces and tests the feasibility of US-guided surgery for oral cancer in two subsites: squamous cell carcinoma of the tongue (SCCT) and the buccal mucosa (SCCBM). This US-guided surgery workflow consisted of an in-vivo and an ex-vivo part. In-vivo US was applied intraorally with a hockey-stick shaped probe, which is able to image the deeper extent of the tumour. While performing the resection, the cutting plane and tumour border were captured in one image to measure the US-determined tumour free margin distance. This provided the surgeon with real-time feedback, while aiming for a surgical margin of at least 10 mm. Ex-vivo US was applied directly after the resection. The specimen was imaged with a high-resolution probe for a final check on inadequate margins (< 5 mm). In case an inadequate margin was found, an intraoperative re-resection could be executed.

Chapter 2 focuses on this workflow's feasibility for SCCT surgery and compared ten patients who received US-guided surgery (i.e. the US-cohort) with a retrospectively analysed cohort of patients who received conventional surgery (i.e. the conventional cohort). The baseline characteristics in both cohorts were statistically similar. The US-cohort had a much higher frequency of free margin status when compared to the conventional cohort (70% vs. 17%). One US-indi-

cated intraoperative re-resection cleared an inadequate (< 5 mm) margin to adequate (≥ 5 mm), preventing local adjuvant treatment for that particular patient. US-guided SCCT surgery posed no overtreatment (excessive resection of healthy tongue tissue) when compared with conventional surgery.

In **chapter 3**, a feasibility study comparable to the one of chapter 2 was conducted on 13 patients with SCCBM. No conventional cohort was used for comparison. In-vivo US seemed a poor discriminator between adequate and inadequate margins during the surgical procedure, while ex-vivo US performed fairly better. Switching from a 5 mm to a 7.5 mm ex-vivo US-determined margin cutoff would lead to 86% histopathological adequate margins, if an intraoperative re-resection was performed correctly. However, the notion of expanding surgical margins in the area of the buccal mucosa is a challenge by the limited amount of healthy tissue that can be excised. This limitation arises from the risk of damaging structures such as facial muscles and nerves, which could impact the patient's QoL and oral function. In several cases the surgeon declined to perform US-indicated re-resection. As a diagnostic tool for tumour-thickness, both in- and ex-vivo, US has a similar accuracy compared to MR imaging, the standard diagnostic tool. This indicates that tumour margins can be visualized by US very well.

Part 2 – Application, accuracy and impact of ultrasound-guided tongue cancer surgery

Part 2 focuses on US-guided surgery of SCCT and continues with a detailed evaluation of the application and the accuracy of it. Moreover, the impact on QoL, oral function and survival parameters are discussed.

Chapter 4 validates the results of the feasibility study performed in Chapter 2. There was a more than threefold increase in free margin status (55% vs. 16%) when comparing 40 T1-T3 SCCT patients who underwent US-guided surgery with a conventional cohort of 96 patients. Positive margin status showed a threefold decline in the US-cohort (5% vs. 15%). Baseline characteristics of the US- and conventional cohorts were comparable, although the US-cohort presented more non-cohesive growth. Ex-vivo US was a moderate discriminator between adequate and inadequate margins. Intraoperative re-resections were on the location of a histopathological inadequate margin in 46% of the cases. Of these margins, half of them were accurate enough to improve the free margin status of that location. Because several patients showed inadequate margins at multiple locations in the same specimen, the re-resections changed the margin status of only 3/21 (14%) patients to free. Switching from a 5 mm to an 8 mm ex-vivo US-determined margin cutoff would lead to 76% histopathological adequate margins, if an intraoperative re-resection was performed correctly. The combination of in-vivo and

ex-vivo US-guided surgery showed that margins were in general only 2 mm wider, meaning that unnecessarily wide resections are unlikely when US is used during surgery.

Chapter 5 evaluated the effect of US-guided surgery and other factors on local disease-free survival (DFS) in T1-T3 primary SCCT patients. During a 30-month follow-up period, patients in the US-cohort had the same local DFS probability as the conventional cohort. Local recurrence was 5% in both the US-cohort (2/38) and the conventional cohort (5/95). However, local adjuvant radiotherapy was used less often in the US-cohort, i.e. 14% (5/38) compared to 20% (19/95) in the conventional cohort. These results seem to indicate that US-guided surgery results in a similar local DFS as conventional surgery but requires less local adjuvant treatment. This may lead to a better QoL and oral function among the patients who received US-guided SCCT surgery. Moreover, local recurrences only occurred at the mucosal surface in the US-cohort, while in the conventional cohort at least 3/5 (60%) of the local recurrences occurred deep in the tongue tissue. The local recurrences in the US-cohort may be due to field cancerisation i.e. the development of a second primary, instead of residual cancer cells. Statistically insignificant differences can also be explained by the low incidence of local recurrence. A multivariate Cox regression revealed that a positive margin status is the strongest predictor for local recurrence. As US-guided surgery appears to result in a threefold reduction in positive margin status, a larger cohort might show statistically significant different outcomes.

Chapter 6 describes the method of a multicentre randomized controlled trial, in eight Dutch centres affiliated with the Dutch Head and Neck Society. It is a two-armed study in which 150 T1-T3 primary SCCT patients will be randomized in an experimental group, receiving US-guided SCCT surgery, and a conventional group, receiving conventional SCCT surgery without US. Primary outcomes are margin status, administration of local adjuvant treatment (re-resection or radiotherapy) and QoL related to the patient's disease and treatment. For the latter outcome, periodic questionnaires, and function tests about the patient's QoL related to his/her treatment will be sent out and evaluated. In the University Medical Center Utrecht, additional oral function tests will be conducted simultaneously. After 24 months, disease-specific survival, disease-free survival and overall survival will be evaluated.

Part 3 – Other image-guided surgery techniques for oral cancer

Part 3 explores alternative margin visualisation techniques for oral cancer, other than US. It discusses techniques that could be used in combination with or as an alternative for US to compensate for its limitations.

Chapter 7 is a systematic review of whole specimen imaging techniques that

are able to obtain adequate deep resection margins. Apart from US and ex-vivo MRI, fluorescence is another technique that is able to visualize the whole extent of the tumour, without the need to sample the resection specimen. The studies on these investigated techniques reported an extremely high range of sensitivity and specificity (ranging from 0% to 100%) in detecting inadequate (< 5 mm) margins. This variability was attributed to the very low or very high incidence rates of inadequate margins within the typically small study populations. However, US and ex-vivo MR tend to have a higher specificity than sensitivity, while fluorescence, specifically when labelled with cetuximab as a contrast agent, showed a rather high sensitivity for inadequate margins. For all three techniques, larger cohorts are needed to identify optimal cutoff values for an optimal diagnostic accuracy of inadequate margins.

Chapter 8 is a systematic review of whole specimen margin visualisation techniques that are able to define the mucosal margin. It is especially important that these techniques are able to differentiate between SCC and (severe) dysplasia. The techniques of contrast agent-free autofluorescence, iodine staining, and narrow band imaging were investigated. None of the studies implemented close margins (1-5 mm) as an outcome measure and some did not differentiate between (severe) dysplasia and SCC in the resection plane. Comparison between different techniques was challenging, as the methodology varied between these studies. The most reliable evidence of applicability was given by one randomized controlled trial to autofluorescence, showing that this technique was not able to outperform white light surgery in the detection of inadequate mucosal margins.

Chapter 9 explores the feasibility of a high-field MR-based three-dimensional (3D) digital specimen, which could potentially play a role in intraoperative ex-vivo assessment of the resection specimen. This digital specimen could map regions of inadequate margins on its surface, aiding the surgeon's orientation for optional intraoperative re-resections. Two digital specimens were rendered from two radiologists' segmentation of the tumour and mucosa. Each MR-based digital specimen was divided in five regions that could either be flagged as adequate (≥ 5 mm) or inadequate (< 5 mm) by colour mapping the margin distances on its surface. When compared with a digital specimen based on histopathologist's segmentations on haematoxylin-eosin (HE)-stained microscopic sections, sensitivity of the two MR-based specimens ranged between 88% and 94%. Specificity ranged between 61% and 71%. When compared with the conventional histopathological report, diagnostic accuracy was lower. MR-based digital specimens tend to overestimate tumour size (and thus underestimate margin size), which in fact leads to a relatively large amount of falsely classified inadequate margins, but also a relatively large amount truly classified adequate margins. This may lead to overtreatment, but also to more free margin status.

General Discussion

Can US-guided surgery change margin status for oral cancer patients?

In Chapters 2 and 4, it was confirmed that at the University Medical Center Utrecht, patients with squamous cell carcinoma of the tongue (SCCT) who underwent ultrasound (US)-guided surgery had a more than threefold higher frequency of free margins (≥ 5 mm histopathological margin distance in the whole specimen) compared to a historical conventional cohort (55% vs. 17%). Regarding positive margin status (i.e. at least one margin distance of < 1 mm specimen), frequency was three times lower when compared with conventional surgery.

Two other studies compared a US-cohort with a conventional cohort with SCCT patients, however without statistically significant results. Bulbul et al. (90) reported 70% (16/23) and 48% (10/21) free margins for US- and conventional cohort, respectively. They found no positive margin status in the US-cohort and only one in the conventional cohort. Nilsson et al. (114) reported 59% (20/34) and 41% (31/76) free margins for US- and conventional cohort, respectively. The insignificance of these results is possibly caused by the small patient groups and the higher percentage of free margins in the conventional group, when compared to our research in chapter 2 and 4.

Both studies also performed a subgroup analysis on the deep margins. Bulbul et al. (90) reported 78% (18/23) and 67% (14/21) adequate (< 5 mm) deep margins, while Nilsson et al. (114) reported 76% (26/34) and 59% (45/76) in the US- and conventional cohort, respectively (both not statistically significant). Our subgroup analysis on deep central margins revealed statistically significant differences: 87% (33/38) vs. 55% (21/38) adequate deep central margins in the US- and conventional cohort, respectively. The difference in our results, when compared to other studies, might be partly due to our method of dividing the resection specimen in five regions (i.e. deep central, anterior, posterior, craniomedial and caudolateral), whereas other studies only categorized the specimen in mucosal and deep subgroups. This more detailed division reduces the likelihood that the deep margin, as defined by us, contains inadequate margins. This is because the deep margin is defined as a smaller area when compared to other studies.

Considering squamous cell carcinoma of the buccal mucosa (SCCBM); chapter 3 does not compare a cohort of SCCBM patients who received US-guided surgery. However, it can be compared with a retrospectively analysed cohort from the same centre by Adriaansens et al. (198). Chapter 3 reported 8% (1/13) free margins status and 23% (3/13) positive margin status. Adriaansens et al. reported 11% (11/97) free margin status and 39% (37/96) positive margin status in conventional treated patients, respectively. As discussed in chapter 3, these results could be caused by the fact that in some cases surgeons declined to perform an intraoperative re-resection in an attempt to avoid mutilation, which otherwise may result in a decline of QoL and oral function. Although ex-vivo US had

a fair diagnostic accuracy in identifying inadequate margins, it cannot be concluded yet that US-guided SCCBM surgery results in more adequate margins.

The preference for investigating tongue cancer with US-guided surgery (53–57,90,114,148,199), has several reasons. Firstly, the tongue's flexibility allows easy placement of the US probe, unlike other subsites. Secondly, the tongue is a hydrostatic muscle without bony tissue nearby (5), preventing shadowing artifacts that hinder US imaging in other subsites such as gum, retromolar triangle and hard palate. Thirdly, the tongue contains mainly dense, homogenic muscular tissue (52). In contrast, other oral subsites contain structures with a varying echogenicity, complicating differentiation between healthy and malignant tissue. For instance, glandular tissue is also hypoechoic, similar to SCCBM. Presence of submucosal and sublingual salivary gland tissue could be the reason why studies to US-guided surgery of squamous cell carcinoma (SCC) of the floor of mouth are difficult to execute. It is also reported that sublingual tissue produces acoustic shadowing (200). The buccal subsites contain structures such as the buccal fat pad, muscles (i.e. buccinator, masseter and orbicularis oris) and fascia that impede tumour identification.

It could be argued that, currently, US-guided surgery, as presented in this thesis, is only proven effective for SCCT surgery to achieve adequate margins. Differences in surgical outcomes between centres might have other causes than surgical skills. For instance, the standard surgical margin may vary between centres, leading to differences in the extent of the resection (201). This also accounts for guidelines; recently the NCCN guidelines changed their recommended 10–15 mm of normal mucosa beyond the SCC to 15–20 mm (38). Additionally, histopathological examination practices vary between centres, leading to differences in accuracy and precision in determining adequateness of the resection. For instance, some centres do not perform microscopic evaluation of every histopathologic slide, nor do they calculate the anterior and posterior margins by multiplying the free tumour slides by the mean slice thickness, as used in the clinical studies presented in this thesis.

How does US-guided surgery change margin status in oral cancer?

US-guided surgery is a valuable method to obtain more adequate resections. However, obtaining 100% free margin status is unrealistic due to various contributing factors.

Firstly, there are technical and human limitations. For instance, the field of view is constrained by the transducer's width and the sound's penetration depth, which is especially problematic for larger T3 tumours. Although moving the transducer over the tumour's surface resolves this problem, it can disorientate the examiner. The examiner must construct a volumetric representation based on cross-sectional images. This can complicate the interpretation of the relationship between different images produced by different probe positions. An angled probe could make a margin to appear adequate (≥ 5 mm), while in reality

it is not. Additionally, the US-system has a limited spatial resolution. Even high frequency probes, which offer higher spatial resolution (48), cannot identify isolated tumour cells in extremely non-cohesive growing tumours. Larger tumour nests may be detectable with high frequency US but for an examiner it is challenging to identify such an object. Finally, even in the homogeneous muscle of the tongue, anatomical structures or anomalies can confuse the observer. Minor salivary glands, haemorrhages, scar tissue and oedema caused by (previous) treatments such as biopsies or sentinel node procedures, can also hamper adequate US imaging (202).

Secondly, margin shrinkage after resection and histopathological fixation techniques leads to smaller histopathological margin distances than observed on US (67,203). Correcting for this phenomenon is complicated and due to the variability in margin shrinkage, which depends on the histological aspects of the subsite. Studies have reported discrepancies of the mucosal margins, varying between 24% to 42% for SCCT and 21% to 67% for SCCBM (203). However, the amount of shrinkage of submucosal or deep margins have never been reported. In our experience, finding a correction for margin shrinkage with US is complex. Comparing in- and ex-vivo US-determined deep and submucosal margins with histopathological margins yields unreliable results due to the technical and observer-dependent limitations mentioned above. Umstattd et al. (67) found that substantial mucosal margin shrinkage occurs immediately after the resection, but barely during formalin fixation. However, while margin shrinkage plays an important role in oral cancer surgery, its exact effect remains unknown and may be location and patient specific.

Thirdly, factors associated with the surgical method itself may hamper the success of US-guided surgery. Surgeons receive feedback only after the initial incision. Although this feedback is valuable information for the remaining course of the resection, the surgeon still needs to adjust the cutting plane. It is essential to communicate clearly to pathology when the previous cut was a 'false' resection plane, also called a 'hesitation cut'. Several surgeons who participated in this study opted to continue with the resection without immediately changing the resection plane. Instead, they performed an intraoperative re-resection after ex-vivo ultrasound confirmed an inadequate margin (< 5 mm) at the corresponding location. However, these additional re-resections are frequently subjected to relocation errors. As found in chapter 4, re-resections were expected to be rightly located at only 23% of the cases and changed margin status in only 3/40 (8%) patients.

Despite the abovementioned limitations, US-guided surgery has multiple advantages over conventional surgery. Intraoral US is highly accurate in estimating histopathological tumour thickness in oral cancers (26,52) and even depth of invasion for SCCT (51). Once the US probe is placed directly onto the tumour, the surgeon gains a clear understanding of its deeper extension. While US captures the tumour and resection plane simultaneously, it enhances the surgeon's awareness of the surgical situation and possibly his/her surgical technique. This

is likely the greatest advantage of intraoral US-guided surgery.

Although ex-vivo US has only a moderate accuracy and intraoperative re-resections improve margin status by only 8%, there are notable considerations to continue using ex-vivo US.

Firstly, the standard method for assessing surgical margins intraoperatively in oral cancer surgery is frozen section analysis (FSA). The majority of FSAs involve sending small, unoriented tissue samples from the wound bed to the pathologist for rapid histopathological assessment on presence of tumour (204,205). While FSA is very accurate in classifying these tissues as 'positive for SCC' or 'negative for SCC' (62,206), it is incredibly challenging to find the corresponding location in the wound bed of these tissue samples (42). As tissue samples are mostly taken from the wound bed it is also impossible to determine the presence of margins of > 0 mm yet inadequate, because FSA is only positive for SCC in case of a cut-through. Fragments that only sample a fraction of the specimen or wound bed cannot assess whether there is an inadequate margin somewhere else. This might be the reason why patients with positive margin status revised to free status still have same DFS as patients with positive margin status (42,61,107). FSA methodology can be improved by sampling from the resection specimen to identify 0-5 mm margins distances and by sampling in a more systematic way (207,208). Although ex-vivo US is probably not as accurate in identifying tumour cells in the resection specimen as FSA, ex-vivo US is able to assess the whole resection specimen at any location at any direction in a relative short amount of time and against less costs (62,109).

Secondly, although 8% additional free margin status seems a small improvement, comparable numbers are witnessed in studies to FSA. DiNardo et al. concluded that successful margin revision (from cut-through to adequate), occurred in only 4/80 (5%) head and neck cancer patients (62). Chaturvedi et al. revised 23% FSA-determined inadequate margins to 12% inadequate margins, yielding a difference of 11% (206).

In our perspective, optimizing in-vivo US to its full potential is preferable. However, until in-vivo US achieves the full potential of US-guided surgery, further exploration of the benefits of ex-vivo US is a viable option. For instance, the orientation of the intraoperative re-resection could be enhanced by the parallel tagging method, introduced by van Lanschot et al. (97). This method is implemented in the RCT presented in chapter 6.

How can the patient profit from US-guided resection in oral cancer?

There is general consensus that a positive margin status is a strong predictor of local recurrence (30,61,106,107). In chapter 5, it was concluded that, in line with literature, positive margin status is an independent predictor for local recurrence in SCCT cancer, (hazard ratio (HR): 7.94). Regarding this positive margin status, US-guided surgery shows a favourable outcome, by reducing the incidence of

positive margins threefold, compared to conventional surgery.

In this thesis we found for SCCT patients similar local DFS for US-guided surgery and conventional surgery. We would expect an improved local DFS for US-guided surgery. Reasons why we did not find a difference might be the relative low incidence of positive margin status (2/37 (5%) in the US-cohort vs. 13/95 (14%) in the conventional cohort) and the more frequently applied local adjuvant treatment for those patients. Meanwhile, neck metastasis was also identified as an independent predictor for local recurrence (HR: 6.09), which is in line with literature (116). The incidence was slightly higher in the US-cohort, i.e. 19/37 (51%) vs. 39/95 (41%).

Moreover, even though the incidence of close margins was significantly higher in the conventional cohort, i.e. 67/95 (71%) vs. 16/37 (43%), it was not shown that close margins had any significant effect on local DFS in a multivariate analysis. It could be argued that this effect is caused by the administration of local adjuvant treatment. However, none of the statistical analyses in chapter 5 proved that local adjuvant treatment was a predictor for local DFS. This is also in line with literature; the effect of local adjuvant radiotherapy is still under debate for patients with close margins and unfavourable histopathological growth factors (43,118–120). Again, this may be caused by small numbers and low incidence of local recurrences, but also some diversity in this group of oral SCC patients with close margins. A close-margin subgroup with smaller margin distances or a combination of unfavourable histopathological growth factors may benefit more from local adjuvant treatment than other subgroups.

The fact that patients in the US-cohort had less local adjuvant therapy when compared to the conventional cohort, while local DFS was similar, is a tangible benefit for patients who underwent US-guided surgery. It is well known that local adjuvant radiotherapy can be a significant burden for patients. It is reported that local adjuvant radiotherapy has the most negative effect on QoL of oral cancer patients (108), due to physical impairments such as mucositis, xerostomia fibrosis and osteoradionecrosis (44). Oral cancer patients who received local adjuvant (chemo)radiotherapy often have a high probability in developing radiation-induced neuropathies (209) and swallowing and speech impairment that require speech therapy (117).

Another finding in chapter 5 that suggested a possible benefit from US-guided surgery, is that none of the patients in the US-cohort developed deep local recurrences (i.e. recurrences originating from the deep wound bed), contrasting with the conventional cohort, at least 3/5 local recurrences appeared to be deep. This finding confirms that US-guided surgery is particularly useful for correct deep margin resection. As there is no direct visual sign during follow-up, deep local recurrences are typically noticed in a later stage. Hence, deep local recurrences are associated with worse survival and require more invasive treatments, including aggressive surgery. In contrast, mucosal recurrences are earli-

er recognized and subsequently treated.

It is yet unknown how the wider tumour free margins associated with US-guided surgery effects QoL and oral function. The majority of patients with primary closure of the tongue and early-stage tumours patients usually are expected to have a health related QoL similar to that before their diagnosis of cancer (210,211). However, the volume of resected tongue is mildly correlated with swallowing impairment, even though there are indications that this is more profound for the posterior part and base of the tongue than the anterior portion of the tongue (117,212). We do not expect that the additional overall margin distance of 2 mm in the US-cohort impacts oral function and QoL more than local adjuvant radiotherapy. However, we hope that more clear conclusions can be drawn once the proposed multicentre study in chapter 6 has been conducted.

Future perspectives

In this thesis we found that US-guided surgery has major impact on margin status of SCCT patients, particularly the deep margin directly situated under the tumour. The impact of our current workflow must be evaluated with respect to QoL, and oral function while being conducted in different centres. The study protocol proposed in chapter 6 could give more insight about this impact. Moreover, a more objective study onto evaluating the accuracy of ex-vivo US is currently conducted, involving matching cross-sectional US images of the resection specimen with microscopic haematoxylin and eosin (HE)-stained images, similar to the methods in chapter 9. Delineation of the US images and HE images could offer a more objective evaluation of the diagnostic value of ex-vivo US, possibly accounting for effect on deep and submucosal shrinkage.

The US-system used during surgery consisted solely of conventional B-mode scanning. However, technological advancements in US imaging have introduced new tools that may enhance its application in oral cancer surgery. For example, contrast enhanced US (CEUS) uses microbubble contrast agents that resonate and produce a high frequency US reflection when exposed to an initial pulse. It has been used for different cancer types, such as hepatocellular carcinoma (213) and renal cell carcinoma (214). This technique enables the visualisation of (micro)vasculature, including neovascularisation, which may enhance identification of the tumour border in when B-mode US whose only ill-defined borders (215). Although CEUS has not been used for oral cancer, a feasibility study is planned in our centre to evaluate its potential in a clinical setting.

Another development is elastography, which assesses the stiffness of tissue by measuring the shear wave velocity. Elastography has already been investigated in head and neck cancer to diagnose nodal disease by evaluat-

ing stiffness of lymph nodes (216). Sastry et al. (65) found that elastography can measure the formation of collagen fibres in the extracellular matrix of T1 and T2 SCCT correlating with cancer oncogenesis and thus tumour aggressiveness. This suggests that elastography could supplement US in identifying aggressiveness to indicate a more radical resection. Possibly, elastography could also play a role in the discrimination of SCC and salivary gland tissue during US-guided oral surgery in the floor of mouth and buccal mucosa. Elastography has been applied to target the needle tip towards the SCC during fine needle aspiration cytology, as both tissues possess comparable echogenicity when only B-mode ultrasound is used (59).

Possibly one of the most useful innovations in US is three-dimensional (3D) US, which addresses the earlier mentioned problem of relating multiple cross-sectional images. Although 3D acquisition probes exist, they are often bulky, expensive and have a limited field of view (217). Several methods have been developed to convert conventional two-dimensional (2D) images into 3D datasets of the resection specimen. Bekedam et al. used an electromagnetic tracker to reconstruct a volumetric dataset of a SCCT resection specimens from 2D US images (103,218). Additionally, motorized systems that move a conventional transducer along rails to create a stacked volumetric dataset of 2D images of ex-vivo specimen have been investigated as well (217,219).

Despite the advantages of 3D US, the resulting volumetric datasets contain far more information to process than several conventional 2D US images, obtained from a location thought to be at risk of an inadequate (< 5 mm) margin. As a solution, volumetric digital representations of the resection specimen can be used to map inadequate margins on its surface, as outlined in chapter 9. When projected on screens in the surgical room, these digital specimens facilitate a clearer understanding of the relationship between inadequate margins in the specimen and the wound bed. This relation may be enhanced further by using augmented reality to place a digital projection into the wound bed, facilitated by holographic technology that can be worn by the surgeon.

Bekedam et al. (103) created a digital specimen by manually segmenting a volumetric dataset acquired by an electromagnetic tracked US probe. However, multiple outlines of the same specimen showed a high intra-observer variability. Although acquisition of the volumetric dataset was rapid, creation of the digital specimen by manual delineation was time consuming. To solve this problem, they trained a convolutional neural network (CNN) on manually segmented tumours on the volumetric dataset (218). Although this type of deep learning enabled automatic segmentation of tumour tissue from volumetric US data, correlation with histopathology was low. This is because the training set was not validated by histopathological data. Training CNNs on delineating tumours in volumetric datasets of oral cancer resection specimens and validated by histopathological data, is now being conducted in our centre.

MR imaging may also play a larger role in image-guided surgery or oral cancer. In chapter 9 we introduced a proof of principle where inadequate margins, mapped on high-field T2 weighted (T2W) magnetic resonance (MR)-based digital specimens of SCCT, were validated on HE-based digital specimens. The MR-based digital specimen has good sensitivity (range: 88%-94%) and specificity (range: 61%-71%) for the detection of histopathological inadequate margins. Because this MR-based digital specimen was the result of manual delineation, the next possible step towards MR-guided oral cancer surgery is training radiologists or CNN for a faster identification of inadequate margins with MR imaging.

A study similar to chapter 9 will be conducted in our centre, where volumetric US datasets (acquired by a conventional US-system combined with a motorized system) will be compared with HE-based digital specimens. This study enables comparing the feasibility of the use of the MR-based digital specimen and the US volumetric dataset for image-guided surgery. A possible outcome from these studies is that (automatically segmented) MR-based digital specimens are used for more complex surgeries, e.g. necessitating a neck dissection, leaving time to transport the resection specimen from the operating room to the MR machine. An advantage of MR imaging is that specimens from different subsites can be used, as different MR sequences can be used to acquire a high imaging contrast between healthy tissue and SCC (51). On the other hand, US-based digital specimens could be used for simpler early SCCT resection, which are usually combined with sentinel node procedures.

Other imaging modalities have been investigated that could be supplemental to image-guided surgery techniques of US and MR imaging described above. EGFR-targeted fluorescence is a promising technique, using cetuximab labelled with a fluorescent agent to map inadequate margins on the resection specimen's surface. This is done by placing the resection specimen in a closed field imaging box and examine the specimens for spots that illuminate under a fluorescent camera. As all other ambient light is eliminated by the closed box, a > 1 signal-to-background ratio reveals increased cetuximab uptake several mms under the cutting plane, indicating an inadequate margin. A single-arm phase II study by de Wit et al. showed a 78% sensitivity and 76% specificity for inadequate margins, when a cutoff signal to background ratio of 1.5 was used (149). An objective cutoff value of a certain signal to background ratio was however not been used during in-vivo assessment as ambient light distorted the fluorescence camera. As fluorescence cameras are not able to image the deeper extent of the tumour, US-guided surgery might be a more suitable technique for in-vivo margin assessment while the contrast-enhanced fluorescence is suitable for ex-vivo assessment.

Because US has limited potential in the detection of (severe) dysplasia or in situ carcinoma at the mucosa, margin visualisation techniques for the oral

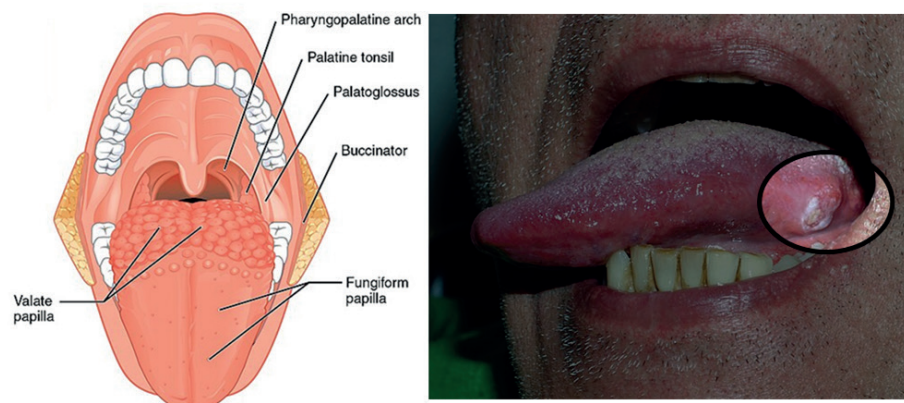
mucosa might be useful to combine with US-guided surgery. However as outlined in chapter 7, the few imaging techniques that have been investigated for this application are studied using a wide variety of methodologies. There was only high evidence that autofluorescence, which does not use any contrast agent, did not make any difference in obtaining better margins, when compared with conventional (white light) surgery. Other techniques that were suitable for whole specimen imaging, such as narrow band imaging and iodine staining, still require research in larger cohorts, focusing on the differentiation between normal mucosa, (severe) dysplasia and SCC.

Chapter 11

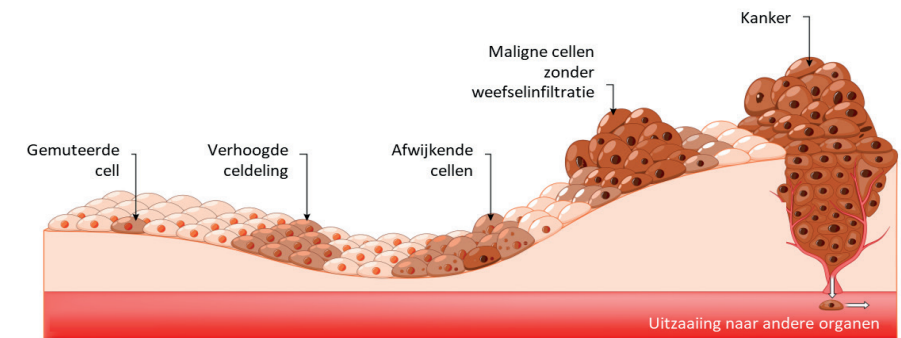
Nederlandstalige samenvatting

Wat wordt met mondkanker bedoeld?

Hoofd-halskanker is wereldwijd de op vijf na meest voorkomende kanker-soort. Mondkanker omvat verschillende typen hoofd-halskanker die in de mond ontstaan. In Nederland wordt mondkanker wordt ongeveer 920 keer per jaar vastgesteld, iets vaker bij mannen dan bij vrouwen. Dit proefschrift richt zich op de meest voorkomende soort mondkanker: het plaveiselcelcarcinoom. Deze kankersoort ontstaat uit epitheelcellen waaruit het mondslijmvlies bestaat. Het mondslijmvlies bekleedt de binnenzijde van de mond. De tong, de mondbodem, het gehemelte, het tandvlees en de binnenkant van de wang zijn allemaal gebieden van de mond waar mondkanker kan ontstaan (figuur 1). Het plaveiselcelcarcinoom dankt zijn naam aan het feit dat de epitheelcellen waaruit het ontstaat lijken op een geplaveide weg, wanneer deze worden bekeken onder de microscoop.



Figuur 1: Links: schematische anatomische afbeelding van de mondholte. Het gehele roze gebied bestaat uit slijmvlies, welke voor een groot deel bestaat uit epitheelcellen. Uit deze epitheelcellen kan een plaveiselcelcarcinoom ontstaan. Rechts: klinische foto van een plaveiselcelcarcinoom op de tongrand (omcirkeld). De zwelling op de tong is duidelijk zichtbaar. OpenStax en Own work / CC BY (<https://creativecommons.org/licenses/by/3.0/deed.en>) (bewerkt).



Figuur 2: Schematische illustratie van de ontwikkeling van kanker uit een gemuteerde cel, in verschillende opeenvolgende stadia. Aangeschaft via iStock en bewerkt.

Elke twee à drie weken wordt een epitheelcel middels celdeling door een nieuwe cel vervangen. Tijdens de celdeling is er een kleine kans aanwezig dat er een fout in het DNA van de vervangende cel ontstaat: een mutatie. Overmatig gebruik van alcohol en tabak beschadigt de epitheelcellen, waardoor deze sneller vervangen moeten worden. Daardoor wordt de kans op een mutatie groter. Een aaneenschakeling van mutaties kan tot gevolg hebben dat het DNA de cel zo programmeert dat deze zich ongeremd kan delen. Zo ontstaat een gezwel: een tumor. Deze tumor kan vanuit de mond uitzaaien naar andere delen van het lichaam (figuur 2). Dat gebeurt meestal via de lymfevaten naar de lymfeklieren in de hals en in een later stadium via de bloedvaten naar andere organen, meestal de longen en soms ook de botten of de lever. De tumor in de mond waaruit deze uitzaaiingen ontstaan wordt de primaire tumor genoemd.

Hoe wordt mondkanker gediagnosticeerd en behandeld?

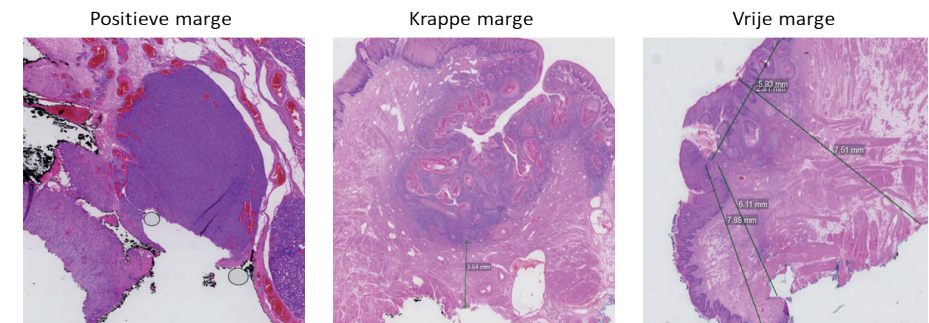
De primaire tumor veroorzaakt in een vroeg stadium niet per sé klachten. Het wordt vaak niet door de patiënt als zodanig opgemerkt. Het kan zich presenteren als een rode of witte plek op het mondslijmvlies. In een later stadium kan een patiënt ongemak of pijn ervaren; zeker als de tumor een grotere zwelling of een zweer vormt (figuur 1). Andere symptomen zijn het bloeden van de tumor, het verliezen van tanden of moeite hebben met spreken, eten of slikken. Als een patiënt zich met dergelijke klachten bij de mka-chirurg of kno-arts meldt, wordt er eerst een biopsie van de plek genomen. Het biopsie wordt onder de microscoop door de patholoog bekeken om te bevestigen dat het gaat om mondkanker. Daarnaast wordt de hals op zwellingen gecontroleerd om aanwijzingen voor uitzaaiingen op te sporen. Een zwelling in de hals, vaak veroorzaakt door een vergrote lymfeklier, kan met een naald-aspiratiebiopsie worden onderzocht op de aanwezigheid van tumorcellen.

De meest effectieve behandeling voor mondkanker is chirurgische verwijdering van de primaire tumor. Enkele weken of dagen voor de operatie wordt er pre-operatieve beeldvorming verricht. Dat is om de uitgebreidheid van de kanker te onderzoeken, ter voorbereiding van de operatie en om eventuele metastasen op te sporen. Met een MRI of een echo kan worden vastgesteld hoever de primaire tumor al in het weefsel is gegroeid en of er lymfeklier uitzaaiingen in de hals zijn. Met een röntgenlongfoto, CT-scan of FDG-PET-CT-scan kan worden bekeken of de kanker al naar de longen is uitgezaaid.

Doel van de onderzoeken in dit proefschrift

De kans op overleven en het niet terugkeren van de mondkanker is groter als er tijdens de operatie een marge van meer dan 5 millimeter (mm) aan gezond weefsel rondom de primaire tumor wordt weggenomen. Een marge van 5 mm of meer wordt gedefinieerd als een *vrije marge*, een marge van 1 tot 5 millimeter als een *rappe marge* en een marge van minder dan 1 millimeter als een *positieve marge*.

Men kan pas een definitieve uitspraak over de marge doen nádat het verwijderde weefsel (het tumorpreparaat) door de patholoog is onderzocht. De patholoog snijdt het verwijderde weefsel in plakken en bekijkt meerdere plakken onder de microscoop om de marge te beoordelen (figuur 3). Wordt er in een plak een *rappe* of een *positieve* marge gevonden, dan kan aanvullende behandeling worden overwogen. Er kan worden besloten om lokale radiotherapie te geven om eventueel achtergebleven tumorcellen uit te schakelen, wat ernstige bijwerkingen kan veroorzaken (bijvoorbeeld ontsteking van het slijmvlies of afsterven van het kaakbot). Een andere optie is een naresectie: het verwijderen van extra, mogelijk nog tumorcellen bevattend weefsel bij een tweede operatie. Omdat het lastig is te achterhalen op welke locatie in de mond de *rappe* of *positieve* marge van het preparaat gelegen is, is het lastig om de naresectie op precies de juiste locatie uit te voeren. Een laatste optie is om niets te doen en goed in de gaten te houden of de tumor niet terugkeert: een zogenaamd “recidief”. Een recidief betekent vaak dat de patiënt alsnog opnieuw geopereerd en/of bestraald moet worden.



*Figuur 3: Microscopische afbeeldingen van plakken van een tumorpreparaat. Het weefsel wordt met een roze kleurstof gekleurd om onderscheid te maken tussen kankercellen (donkerroze, paars) en gezonde cellen (lichtroze). Met behulp van een computerprogramma meet de patholoog de afstand van de kankercellen tot aan het snijvlak. Bij een positieve marge liggen tumorcellen < 1 mm van het snijvlak (in dit voorbeeld zelfs in het snijvlak). Een *rappe* marge is 1–5 mm, en een *vrije* marge ≥ 5 mm. Op basis van deze metingen wordt bepaald of aanvullende behandeling noodzakelijk is.*

Chirurgische verwijdering van de primaire tumor, waarbij de marges meteen vrij zijn, is om bovenstaande redenen dus het meest gewenst. Het komt ten goede aan de kwaliteit van leven en het vermindert de kans op een recidief, metastasen en tenslotte overlijden. Ondanks vrijwel alle primaire mondkankers op het oppervlakkige mondslijmvlies zichtbaar zijn, blijft het verkrijgen van vrije marges uitdagend. Dit geldt voornamelijk voor de locaties waar de tumor diep in het weefsel groeit. De chirurg krijgt pas na de operatie van de patholoog te horen wat de microscopische marge werkelijk is. Tijdens de operatie moet de chirurg dus varen op zicht, tast en preoperatieve beeldvorming. Uit eerder onderzoek blijkt dat door de patholoog tot 45% van de marges als *rap* (< 5 mm) en tot 43% van de marges als *positief* (< 1 mm) wordt gerapporteerd.

Naar aanleiding van bovenstaande is er behoefte aan een hulpmiddel voor het verkrijgen van meer vrije marges bij mondkankerchirurgie. Het doel van dit proefschrift is het evalueren van zowel het gebruik als de meerwaarde van echogeïde mondkankerchirurgie. Echografie heeft meerdere voordelen. De benodigde apparatuur is ten opzichte van andere beeldvormingsapparatuur goedkoop. Daarnaast is de apparatuur makkelijk te hanteren en te verplaatsen. Bovendien geeft echografie de mogelijkheid om in de diepte te kijken; als een echokop (ook wel een echoprobe genoemd) op een lichaamsdeel wordt geplaatst kan men via een beeldscherm tot op een bepaalde diepte zien wat voor weefsel zich in dat lichaamsdeel bevindt.

Dit proefschrift belicht daarnaast ook andere beeldingstechnieken die tijdens de operatie ingezet zouden kunnen worden.

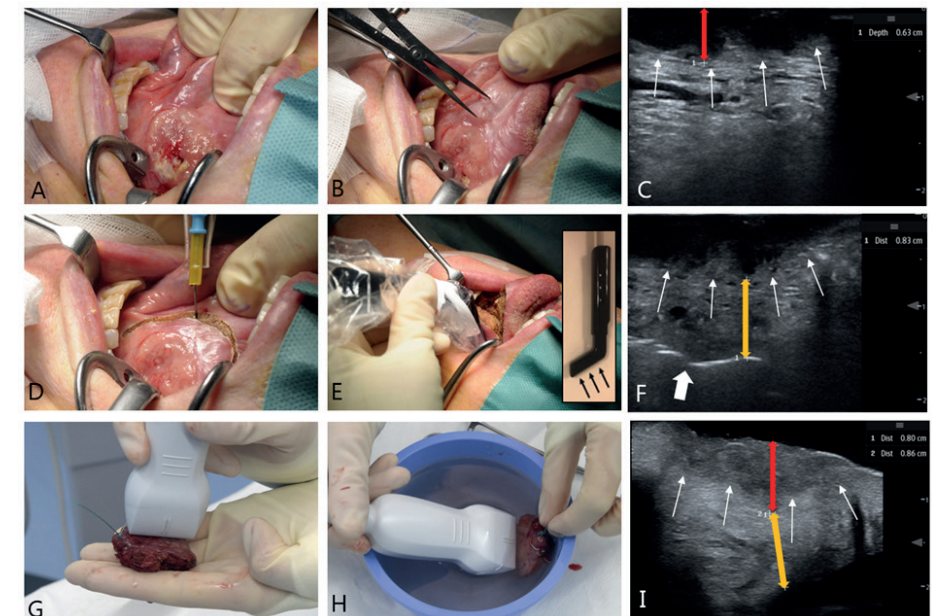
Deel 1 – De haalbaarheid van echogeïde mondkankerchirurgie

Deel 1 introduceert en onderzoekt de haalbaarheid van echogeïde mondkankerchirurgie in twee deelgebieden van de mond: de tong en het wangslimvlies. Onze werkwijze van echogeïde chirurgie omvat twee delen: een in-vivo (binnen het lichaam) deel en een ex-vivo (buiten het lichaam) deel (figuur 4).

Het in-vivo deel wordt tijdens de operatie in de mond toegepast. Tijdens het snijden wordt getracht om de diepe tumorrand (zichtbaar als een donker gebied) en het snijvlak (zichtbaar als een heldere streep) gelijktijdig in beeld te brengen met een kleine, hockeystickvormige echoprobe. Dit biedt de mogelijkheid om de afstand van de tumorrand tot aan het snijvlak te meten. Deze meting geeft de chirurg direct feedback over de afstand van het snijvlak tot het tumorfront en zo dus de margegrootte.

Het ex-vivo deel wordt direct na de resectie op de operatiekamer toegepast wanneer de patiënt nog onder narcose is. Het preparaat wordt nogmaals met een hoge-resolutieprobe echografisch onderzocht op marges kleiner dan 5 mm. In het geval een dergelijke marge wordt gevonden, kan er tijdens dezelfde operatie een onmiddellijke naresectie worden verricht op precies de juiste plek (figuur 5).

Hoofdstuk 2 beschrijft een studie waarin de haalbaarheid van deze werkwijze werd onderzocht bij tien patiënten met tongkanker (vanaf nu de “echogroep” genoemd). De hoeveelheid door de patholoog gerapporteerde vrije marges werd vergeleken met die van 91 patiënten die in het verleden met conventionele chirurgie (d.w.z. klassieke/gebruikelijke tongkanker chirurgie, zonder echografie) zijn behandeld (vanaf nu de “conventionele groep” genoemd). Het bleek dat naar verhouding de patiënten in de echogroep veel meer vrije marges hadden dan de patiënten in de conventionele groep: 70% vs. 17%. Vooral in het diepe snijvlak, dus onder de diepe tumorrand, waren de marges groter. Daarnaast leek het erop dat er, wanneer er naar het hele preparaat werd gekeken, gemiddeld 2 mm extra weefsel werd weggehaald. Dat impliceert dat, ondanks het grote verschil in vrije marges, er door echogeïde chirurgie geen overmatige hoeveelheid gezond weefsel werd verwijderd. Hoewel het kleine aantal patiënten in de echogroep slechts beperkt bewijs levert voor het succes van echografie, toont deze haalbaarheidsstudie al veelbelovende resultaten. In deel 2 wordt een studie besproken met een grotere groep patiënten met tongkanker waarbij een echogeïde resectie werd uitgevoerd.



Figuur 4: Overzicht van de werkwijze bij echogeïde mondkankerchirurgie. De rode pijlen geven de op de echo gemeten tumordikte weer. De gele pijlen geven de marge weer. Witte pijlen markeren de (vermoedelijke) tumorrand. A: Plaveiselcelcarcinoom op de tong. B: Bepalen van het oppervlakkige snijgebied. Doorgaans begint de chirurg met snijden op 10 mm van de met het blote oog zichtbare tumorrand. C: In-vivo meting van de tumordikte met een hockeystickvormige echoprobe, zodat de chirurg een idee heeft hoe diep er gesneden moet worden. D: Start van de tumorresectie. E-F: In-vivo meting waarbij de echo de afstand tussen tumorrand en snijvlak laat zien. Het snijvlak is zichtbaar als een lichte streep. G-I: Ex-vivo controle waarbij het tumorpreparaat met een hoge-resolutieprobe wordt onderzocht op krappe of positieve marges. Dit kan in de hand of in een bakje met zoutoplossing. Bron: Oral Oncology, 2022; 133: 106023

Hoofdstuk 3 beschrijft een studie waar de haalbaarheid van een vergelijkbare werkwijze werd onderzocht bij dertien patiënten met kanker van het wangslimvlies. In deze studie werd voornamelijk gekeken naar het vermogen van echografie om een onderscheid te maken tussen vrije (≥ 5 mm) en niet-vrije marges (< 5 mm). Daarvoor werden in-vivo echografie en ex-vivo echografie met elkaar vergeleken. Ex-vivo echografie bleek vrije van niet-vrije marges redelijk van elkaar te kunnen onderscheiden. Bij in-vivo echografie was deze matig. Als tijdens ex-vivo echografie een afstand van 7,5 mm in plaats van 5 mm tussen de tumorrand en het snijvlak als criterium voor onmiddellijke naresectie was gehanteerd, dan zou in theorie 86% van de marges door de patholoog als vrij zijn gerapporteerd. Dat zou echter betekenen dat er veel onmiddellijke naresecties nodig zouden zijn. Vooral in het gebied van het wangslimvlies kan dat grote negatieve gevolgen hebben voor het functioneren van de mond en de kwaliteit van leven. Om deze reden waren chirurgen terughoudend in het uitvoeren van naresecties als de marge bij de ex-vivo echo krap bleek.

Deel 2 – de toepassing, nauwkeurigheid en impact van echogelegeide tongkankerchirurgie

Deel 2 richt zich volledig op echogelegeide tongkankerchirurgie en belicht het onderzoek naar de toepassing van deze techniek, de nauwkeurigheid ervan en de impact op de patiënt.

Hoofdstuk 4 beschrijft een studie waar de resultaten van de studie in hoofdstuk 2 werden geverifieerd. Dit werd gedaan door een echogroep van 40 tongkankerpatiënten te vergelijken met een conventionele groep van 96 tongkankerpatiënten. Wederom bleek dat patiënten in de echogroep naar verhouding vaker vrije marges hadden dan patiënten die conventionele chirurgie ontvingen: 55% vs. 16%. Daarnaast bleek ook dat patiënten in de echogroep naar verhouding minder vaak positieve marges hadden: 5% vs. 15%. Een andere belangrijke bevinding was dat de patiënten in de echogroep slechts half zo vaak aanvullende radiotherapie kregen als patiënten in de conventionele groep: 10% vs. 21%.

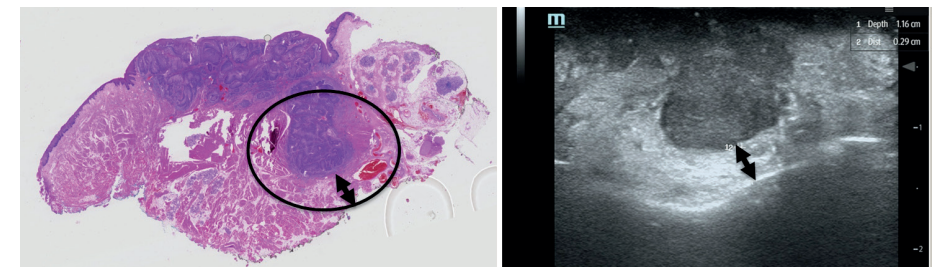
Daarnaast werd gekeken naar de toegevoegde waarde van ex-vivo echografie (figuur 5). Ongeveer de helft van de onmiddellijke naresecties die door ex-vivo echografie waren geïndiceerd, waren volgens de patholoog terecht. Echter bleek slechts een kwart van de onmiddellijke naresecties op precies de juiste locatie te zijn uitgevoerd ondanks dat deze direct tijdens dezelfde operatie werden verricht. Als de naresecties enigszins groter zouden zijn geweest, of als ze zorgvuldiger waren georiënteerd, hadden de naresecties de juiste locatie vermoedelijk wél bestreken. Uiteindelijk hadden de naresecties bij drie patiënten invloed op de door de patholoog gerapporteerde marges. Deze patiënten hoefden door echografie geen aanvullende behandeling te ondergaan. Dat zou wel het geval zijn geweest als er geen ex-vivo echografie was toegepast. Al met al is ex-vivo echografie een potentieel betrouwbare techniek om vrije van niet-vrije marges te onderscheiden.

Hoofdstuk 5 beschrijft een vervolgstudie van de studie in hoofdstuk 4. Binnen een periode van 30 maanden werd de kans op lokale recidieven in zowel de echogroep als in de conventionele groep geëvalueerd. In beide groepen kreeg slechts 5% van de patiënten een lokaal recidief. Opvallend was dat er minder aanvullende radiotherapie aan de patiënten in de echogroep werd gegeven. Dat impliceert dat de kans op een lokaal recidief bij echogelegeide chirurgie vergelijkbaar is met die bij conventionele chirurgie, maar dat de kwaliteit van leven beter behouden kan blijven doordat bestraling minder vaak nodig is. Een andere interessante bevinding was dat de patiënten in de echogroep alleen maar lokale recidieven hadden die aan het oppervlak van het slijmvlies afkomstig waren, terwijl 60% van de lokale recidieven in de conventionele groep zich in de diepte bevonden. Oppervlakkige recidieven zijn beter te detecteren en te behandelen dan diepe recidieven. Deze bevinding heeft vermoedelijk te maken met het feit dat echografie leidt tot betere tumormarges in het diepe tongweefsel.

Hoofdstuk 6 beschrijft een methode voor een “*randomized controlled trial*” (RCT) binnen acht Nederlandse ziekenhuizen die hoofd-halskanker behandelen. Een RCT vergelijkt twee patiëntengroepen:

- de testgroep: waarbij patiënten een nieuwe behandeling ondergaan (in dit geval: echogelegeide tongkankerchirurgie) en
- de controlegroep: waarbij patiënten de conventionele behandeling ondergaan (in dit geval: conventionele tongkankerchirurgie).

In tegenstelling tot de studies van hoofdstuk 2 en 4 worden beide patiëntengroepen in dezelfde periode behandeld. Een patiënt wordt willekeurig (middels een computerprogramma) aan een groep toegewezen. Wel is ervoor gezorgd dat binnen elk deelnemend ziekenhuis de verdeling van tumorstadia tussen test- en controlegroep ongeveer gelijk is. Naast de margegrootte worden ook het aantal aanvullende behandelingen, het aantal recidieven, de kwaliteit van leven en het functioneren van de mond tussen beide groepen vergeleken. De verwachting is dat echografie deze factoren in positieve zin beïnvloedt. Deze uitgebreide studie, die vanwege zijn aard en grootte een hoge bewijskracht heeft, wordt op het moment van schrijven uitgevoerd. Daarnaast wordt in deze RCT tijdens het uitvoeren van onmiddellijke naresecties meer aandacht besteed aan de nauwkeurigheid van de oriëntatie van het preparaat en waar dan precies de naresectie bij de patiënt moet plaatsvinden.



Figuur 5: Vergelijking tussen een microscopische afbeelding en een echoafbeelding van hetzelfde tumorpreparaat. De tumor vertoont in beide afbeeldingen een vergelijkbare vorm (zwarte cirkel). Tijdens het ex-vivo echografie deel van de operatie viel de krappe marge op (zwarte pijl), waarna er een naresectie werd uitgevoerd. Deze naresectie heeft ertoe geleid dat de patiënt geen aanvullende behandeling meer nodig had.

Deel 3 – andere beeldgelegeide operatietechnieken voor de behandeling van mondkanker

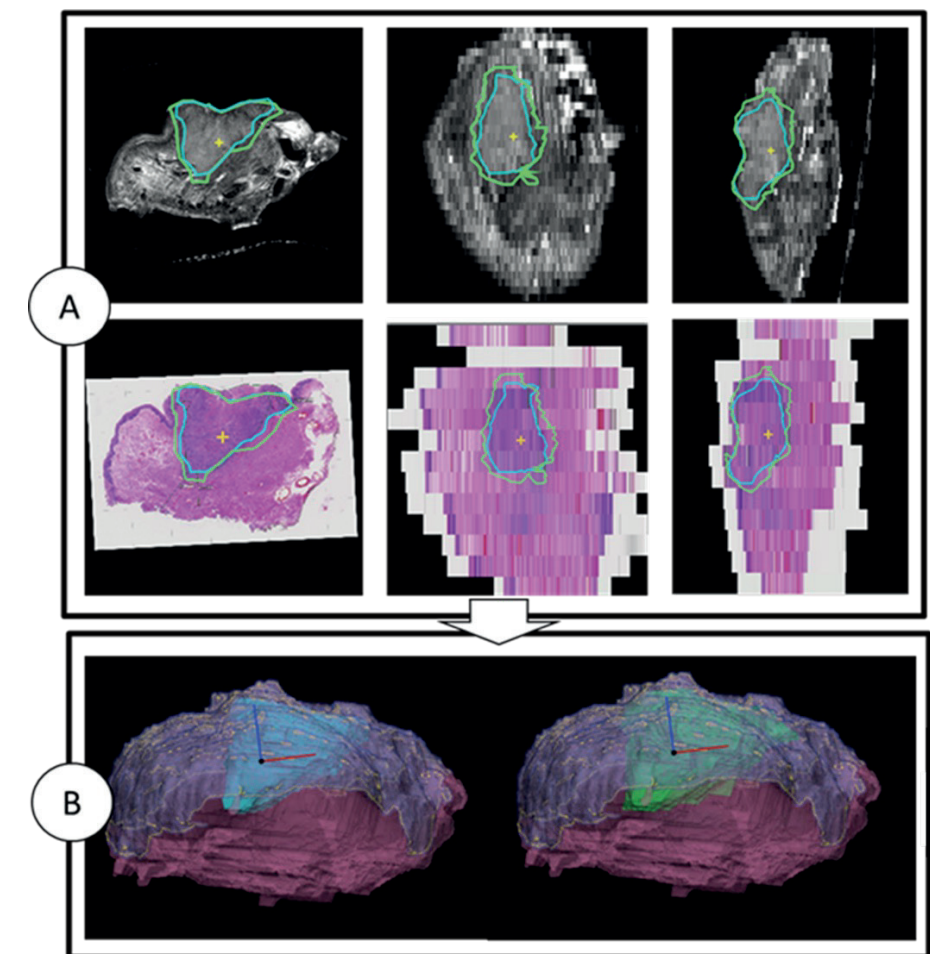
Deel 3 onderzoekt, naast echografie, andere beeldvormende technieken die de (beoogde) marges tijdens een mondkankeroperatie in beeld kunnen brengen. Daarnaast wordt ook de mogelijkheid om deze technieken met echo te combineren besproken.

Hoofdstuk 7 is een literatuurstudie (dus: een studie van andere gepubliceerde onderzoeken) naar technieken die geschikt zijn voor het in beeld brengen van diepe resectiemarges (in tegenstelling tot resectiemarges van het mondslijmvlies). De drie technieken die in de literatuurstudie zijn opgenomen zijn: echografie, MRI en fluorescentie. Fluorescentie bleek een interessante techniek waarbij een stof die zich aan tumorcellen kan hechten aan mondkankerpatiënten wordt toegediend. De aan de tumor gehechte stof kan vervolgens door een speciale camera zichtbaar worden gemaakt. De studies die de mogelijkheden van fluorescentie onderzoeken lieten een zeer grote variatie zien in het vermogen om vrije (≥ 5 mm) van niet-vrije marges (< 5 mm) te onderscheiden. Dit heeft waarschijnlijk te maken met de kleine patiëntenpopulaties en de variatie in methodiek van de onderzoeken naar fluorescentie. Verder bleek uit onderzoeken naar echografie en MRI dat deze technieken beter zijn in het bevestigen van vrije marges dan het opsporen van een niet-vrije marge. Eén fluorescentie-techniek, waarbij de toegediende stof cetuximab bevatte (wat tevens ook voor chemotherapie wordt gebruikt) bleek juist beter in het opsporen van niet-vrije marges dan het bevestigen van vrije marges. Alle drie de onderzochte studies (echografie, MRI en fluorescentie) zouden in grotere studies nader onderzocht moeten worden om meer bewijskracht te verkrijgen voor hun waarde bij mondkankeroperaties.

Hoofdstuk 8 is ook een literatuurstudie naar technieken die geschikt zijn voor het definiëren van de juiste resectiemarges op het mondslijmvlies. Het grootste belang van deze technieken is dat zij onderscheid kunnen maken tussen plaveiselcelcarcinomen en dysplasie. Dysplasie is een weefselafwijking die als een voorstadium van kanker gezien kan worden. Het grenst vaak aan een plaveiselcelcarcinoom. Desondanks hoeft dysplasie niet met dezelfde radicaliteit als plaveiselcelcarcinomen behandeld te worden (lees: met een vrije marge van 5 mm reseceren). Drie technieken die in de literatuurstudie zijn opgenomen zijn: autofluorescentie, jodine-kleuring en *narrow-band imaging*. Veel van de onderzoeken naar deze technieken maakten geen onderscheid tussen krappe en vrije marges. Het was dus erg lastig om deze technieken met elkaar te vergelijken. Het meest betrouwbare onderzoek in de literatuurstudie was een RCT naar autofluorescentie. Deze techniek leek echter niet veel toegevoegde waarde te hebben voor een mondkanker operatie.

Hoofdstuk 9 beschrijft een studie naar de geschiktheid van een digitaal 3D-model van het tumorpreparaat tijdens mondkankeroperaties (figuur 6). Het 3D-model werd gereconstrueerd uit beelden van het preparaat die door een sterke MRI zijn vervaardigd. In de toekomst is het wellicht mogelijk om het preparaat tijdens de operatie naar de MRI te brengen, om vervolgens het 3D-model te gebruiken voor het besluit tot onmiddellijke naresecties. Van tien tongkankerpreparaten werden, met de hulp van radiologen, digitale 3D-modellen gemaakt en vergeleken met de microscopische beelden van hetzelfde preparaat.

Het bleek dat het 3D-model de tumorgrootte overschatte. Dit heeft als nadeel dat met het 3D-model in de praktijk overmatig veel onmiddellijke naresecties zouden worden gedaan; de tumorrand lijkt immers dichterbij de snijrand te liggen. Een voordeel is juist dat ook een deel van deze onmiddellijke naresecties terecht zijn. Er moet meer onderzoek worden gedaan naar hoe de 3D-modellen gebruikt zouden kunnen worden en hoe er met de overschatting omgegaan zou moeten worden.



Figuur 6: Overzicht van het vervaardigen van een 3D-model van het tumorpreparaat (hoofdstuk 9). A: MRI-beelden van het tumorpreparaat werden vergeleken met de overeenkomstige microscopische afbeeldingen. De groene omlijning geeft de tumorranden weer volgens de radiologen, de blauwe volgens de patholoog. B: Deze informatie werd vervolgens bewerkt tot 3D-modellen van het tumorpreparaat. Het door de radioloog bepaalde tumorvolume (groen) is groter dan dat van de patholoog (blauw). Source: *Frontiers in Oncology*, 28;14:1342857.

Vooruitzichten voor de toekomst

In dit proefschrift laten wij zien dat echografie in potentie een grote invloed heeft op de resectiemarges bij mondkankerchirurgie. We weten echter nog niet precies wat voor invloed echografie heeft op de kans op een lokaal recidief, de kwaliteit van leven en het functioneren van de mond. Een mogelijke uitkomst is dat de grotere resecties die echogelegeide chirurgie in eerste instantie met zich meebrengt, kunnen leiden tot een tijdelijk groter verlies van mondfunctie, maar dat de kleinere kans op aanvullende behandeling uiteindelijk zwaarder weegt als voordeel – en de uiteindelijke kwaliteit van leven wellicht beter is. De studie waarvan het protocol in hoofdstuk 6 op het moment van schrijven wordt uitgevoerd, zou hier meer inzicht in kunnen bieden.

Een andere vraag die nog onbeantwoord is, is hoe nauwkeurig echografie de tumorrand kan definiëren. De oriëntatie van de echobeelden van het preparaat komt niet altijd overeen met die van de plakken die de patholoog maakt voor microscopisch onderzoek. Momenteel wordt er een studie uitgevoerd waarbij de echobeelden in dezelfde richting als het snijvlak van de patholoog worden gemaakt. De tumorranden die door de echo worden weergegeven kunnen dan beter met de tumorranden van de microscopische afbeelding worden vergeleken.

Andere, geavanceerde echografische technieken, zoals 3D echografie of echografie waar de weefselelasticiteit wordt bepaald, zijn eveneens zeer interessant om te onderzoeken. 3D-echografie zou, net zoals de MRI beschreven in hoofdstuk 9, een digitaal 3D-model van het preparaat kunnen vormen om tijdens de operatie te gebruiken. Uiteraard mag de rol van kunstmatige intelligentie in de zorg niet genegeerd worden. Kunstmatige intelligentie zou misschien razendsnel een 3D-model van een preparaat kunnen vormen zodra beelden met een MRI of echo zijn vervaardigd, zodat de informatie hiervan direct tijdens de operatie gebruikt kan worden om een eventuele naresectie op de juiste plaats te kunnen doen.

Daarnaast is het interessant om te onderzoeken welke andere technieken een aanvulling zouden kunnen zijn op echografie. Echografie kan gemakkelijk in de mond worden toegepast, en geeft de chirurg houvast over waar het snijvlak gezet moet worden. Fluorescentie met cetuximab (zie hoofdstuk 8) lijkt echter zeer goed in het ex-vivo identificeren van niet-vrije (< 5 mm) en positieve marges (< 1 mm). Een combinatie van beide technieken zou een voordeel voor de patiënt op kunnen leveren. Daarnaast is echografie minder goed in het identificeren van de juiste snijranden op het mondslijmvlies. De technieken waarvan in hoofdstuk 8 de geschiktheid voor het bepalen van de juiste resectiemarges op het mondslijmvlies werd besproken, zouden mogelijk een waardevolle aanvulling kunnen zijn.

Algemene conclusies

Dit proefschrift heeft als doel om het gebruik en de meerwaarde van echogelegeide mondkankerchirurgie in de kliniek te evalueren. Daarnaast zijn er ook andere technieken onderzocht die een mogelijk alternatief of een aanvulling voor echogelegeide mondkankerchirurgie zouden kunnen bieden. Het volgende kan worden geconcludeerd:

- Echogelegeide tongkankerchirurgie leidt tot ruimere resecties en tot meer vrije marges. Dit geldt voornamelijk voor de diepe marge.
- De echogelegeide resecties lijken niet zodanig ruimer dat dit van invloed is op het functioneren van de mond en de kwaliteit van leven. Een RCT met een grote patiëntenpopulatie zou dit verder kunnen bevestigen.
- Meer vrije marges leiden tot minder aanvullende behandelingen. Dit komt de kwaliteit van leven ten goede, terwijl de kans op een recidief in elk geval niet lijkt toe te nemen.
- Echografie in de mond (in-vivo) biedt de chirurg houvast over waar het snijvlak gezet moet worden.
- Het uitvoeren van onmiddellijke naresectie, geïndiceerd door echografie van het preparaat (ex-vivo), leidt tot een bescheiden toename van vrije marges. Een nauwkeurigere oriëntatie van het preparaat zou voor betere lokalisatie van de naresectie en daarmee grotere toename van vrije marges kunnen zorgen.
- Echogelegeide chirurgie van wangslimvlieskanker is uitdagend: chirurgen zijn terughoudend in het uitvoeren van onmiddellijke naresecties omdat dit vaak ten koste gaat van het functioneren van de mond en de kwaliteit van leven.
- Technieken die niet-vrije of positieve marges in het preparaat kunnen identificeren (ex-vivo) zouden een waardevolle aanvulling op echogelegeide mondkankerchirurgie kunnen zijn.
- Technieken die de juiste resectiemarges op het mondslijmvlies kunnen definiëren (in-vivo) zouden een waardevolle aanvulling op echogelegeide mondkankerchirurgie kunnen zijn.
- Een interessant toekomstperspectief is het gebruik van een digitaal 3D-model van het preparaat dat tijdens de operatie met behulp van een MRI of echo wordt vervaardigd.

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Appendices

Supplementary materials

Supplementary materials of chapter 7

The following search was conducted in PubMed and Embase:

Pubmed

(((((“oral cavity”[Title/Abstract] OR “gingiva*”[Title/Abstract] OR “tongue”[Title/Abstract] OR “floor of mouth”[Title/Abstract] OR “head and neck”[Title/Abstract] OR “head neck”[Title/Abstract] OR “buccal”[Title/Abstract] OR “oral mucosa*”[Title/Abstract] OR “oral*”[Title/Abstract] OR “gum”[Title/Abstract] OR “retromolar”[Title/Abstract] OR “alveolar”[Title/Abstract] OR “cheek”[Title/Abstract] OR “maxilla*”[Title/Abstract] OR “mandib*”[Title/Abstract] OR “jaw”[Title/Abstract])) AND (“cancer”[Title/Abstract] OR “cancers”[Title/Abstract] OR “carcinoma”[Title/Abstract] OR “carcinomas”[Title/Abstract] OR “tumor”[Title/Abstract] OR “tumour”[Title/Abstract] OR “tumors”[Title/Abstract] OR “tumours”[Title/Abstract] OR “malignan*”[Title/Abstract] OR “squamous cell carcinom*”[Title/Abstract])) OR (“oral cancer*”[Title/Abstract] OR “OSCC”[Title/Abstract] OR “oral squamous cell carcinom*”[Title/Abstract] OR “HNSCC”[Title/Abstract] OR “oral carcinoma”[Title/Abstract] OR “oral cancers”[Title/Abstract] OR “oral carcinomas”[Title/Abstract] OR “oral malignan*”[Title/Abstract] OR “oral tumor”[Title/Abstract] OR “oral tumors”[Title/Abstract] OR “oral tumours”[Title/Abstract] OR “oral tumour”[Title/Abstract] OR “oral tumor*”[Title/Abstract] OR “oral tumors”[Title/Abstract])) OR “mouth neoplasms”[MeSH Terms]) AND (“border*”[Title/Abstract] OR “margin*”[Title/Abstract] OR “tumor free resection*”[Title/Abstract] OR “margins of excision”[MeSH Terms] OR “thickness”[Title/Abstract] OR “depth of invasion”[Title/Abstract] OR “DOI”[Title/Abstract])) AND (2010:2023[update]))

Embase

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Supplementary materials of chapter 8

The following search was conducted in PubMed and Embase:

Pubmed

(((((“oral cavity”[Title/Abstract] OR “gingiva*”[Title/Abstract] OR “tongue”[Title/Abstract] OR “floor of mouth”[Title/Abstract] OR “head and neck”[Title/Abstract] OR “head neck”[Title/Abstract] OR “buccal”[Title/Abstract] OR “oral mucosa*”[Title/Abstract] OR “oral*”[Title/Abstract] OR “gum”[Title/Abstract] OR “retromolar”[Title/Abstract] OR “alveolar”[Title/Abstract] OR “cheek”[Title/Abstract] OR “maxilla*”[Title/Abstract] OR “mandib*”[Title/Abstract] OR “jaw”[Title/Abstract])) AND (“cancer”[Title/Abstract] OR “cancers”[Title/Abstract] OR “carcinoma”[Title/Abstract] OR “carcinomas”[Title/Abstract] OR “tumor”[Title/Abstract] OR “tumour”[Title/Abstract] OR “tumors”[Title/Abstract] OR “tumours”[Title/Abstract] OR “malignan*”[Title/Abstract] OR “squamous cell carcinom*”[Title/Abstract])) OR (“oral cancer*”[Title/Abstract] OR “OSCC”[Title/Abstract] OR “oral squamous cell carcinom*”[Title/Abstract] OR “HNSCC”[Title/Abstract] OR “oral carcinoma”[Title/Abstract] OR “oral cancers”[Title/Abstract] OR “oral carcinomas”[Title/Abstract] OR “oral malignan*”[Title/Abstract] OR “oral tumor”[Title/Abstract] OR “oral tumors”[Title/Abstract] OR “oral tumours”[Title/Abstract] OR “oral tumour”[Title/Abstract] OR “oral tumor*”[Title/Abstract] OR “oral tumors”[Title/Abstract])) OR “mouth neoplasms”[MeSH Terms]) AND (“border*”[Title/Abstract] OR “margin*”[Title/Abstract] OR “tumor free resection*”[Title/Abstract] OR “margins of excision”[MeSH Terms] OR “thickness”[Title/Abstract] OR “depth of invasion”[Title/Abstract] OR “DOI”[Title/Abstract])) AND (2010:2023[pdat])).

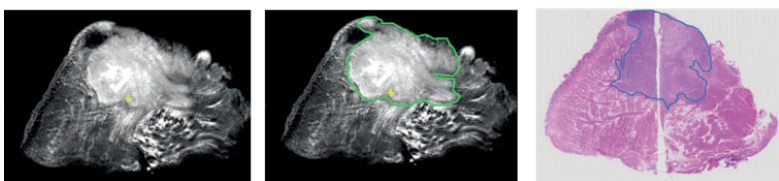
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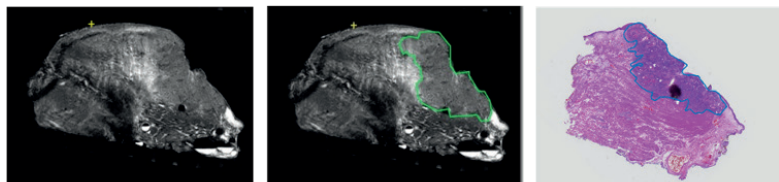
Supplementary materials of chapter 9

Images of T2W TSE sequences of the cases without segmentation (leftmost column), with the segmentation of one radiologist (middle column) and the corresponding histopathological HE-slice including the segmentation of the histopathologist (rightmost column).

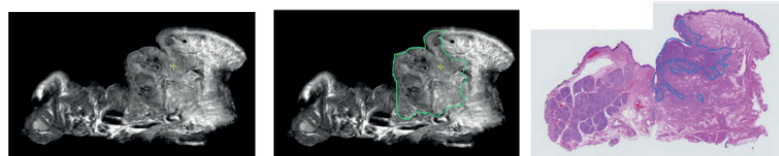
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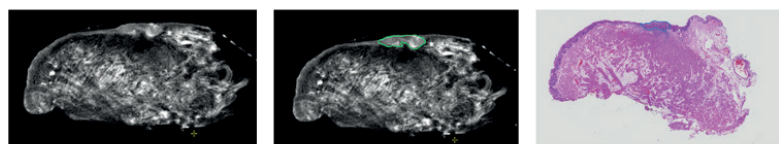
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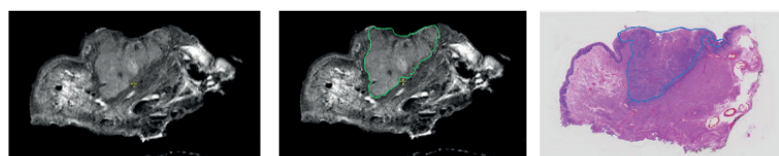
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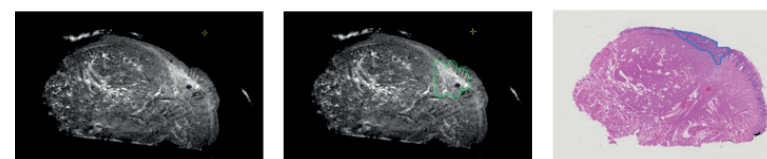
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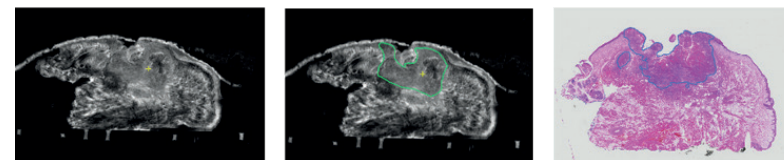
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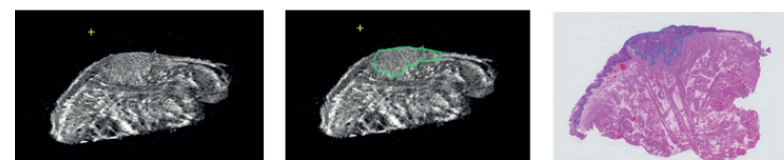
Patient 6



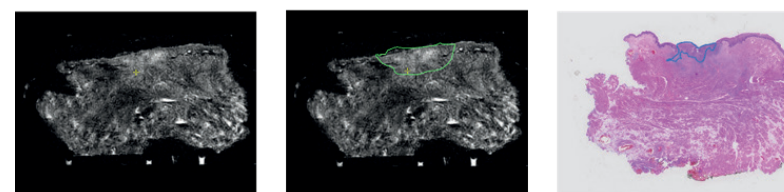
Patient 7



Patient 8



Patient 9



List of publications

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de Koning KJ, Koppes SA, de Bree R, Dankbaar JW, Willems SM, van Es RJJ, Noorlag R. **Feasibility study of ultrasound-guided resection of tongue cancer with immediate specimen examination to improve margin control - Comparison with conventional treatment**. Oral Oncol. 2021 May;116:105249. doi: 10.1016/j.oraloncology.2021.105249. Epub 2021 Mar 26. PMID: 33774501.

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Chapter 4

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Curriculum vitae



Klijs was born on September 27, 1994, in Rijen, a village between Breda and Tilburg. He grew up there, first attending CBS De Vijf Eiken primary school and later continuing his secondary education at Mgr. Frencken-college in Oosterhout.

In 2012, Klijs left the south of the Netherlands to study Technical Medicine at the University of Twente in Enschede. He found substantial distraction in student life, especially through his involvement in committees of Tennisclub Ludica, one of Enschede's largest student sports clubs. This engagement eventually led him to take a sabbatical year in 2015, after completing his Bachelor's degree, during which he served on Ludica's board as Commissioner for Events and Facilities (better known internally as "bar-commissaris").

In 2016, he began his Master's program in Medical Imaging & Intervention, which included clinical internships in Enschede, Nijmegen, and Groningen. In 2019, he moved further north to Groningen for a ten-month graduation internship at the Department of Maxillofacial Surgery, where he developed a keen interest in innovations within the field of maxillofacial surgery at UMC Groningen. Shortly before defending his Master's thesis, "A novel 3D digital procedure for prosthetic obturation after maxillectomy," he moved to Utrecht to start his PhD research at the Department of Head and Neck Surgical Oncology of UMC Utrecht, which ultimately resulted in this thesis.

Alongside his PhD research, Klijs also assisted head and neck surgeons in preparing maxillofacial reconstructions and resections through virtual surgical planning, and in designing 3D-printed tools such as drill and cutting guides at the "3D Face Lab" of UMC Utrecht's Department of Maxillofacial Surgery.

Looking ahead, Klijs chose to put his (hopefully) well-earned academic skills and his eagerness to keep learning to work by helping valorise the ideas of inventors and businesses, starting a career as a patent attorney at Arnold & Siedsma. In a four-year traineeship, he will learn to represent clients by drafting patent applications, providing strategic legal and commercial advice, and supporting them in patent litigation and prosecution.

Dankwoord

Eind 2019 begon ik als promovendus op het gebied van mondkankeronderzoek. Op dat moment had ik werkelijk geen idee wat mij allemaal te wachten stond. Het bleek een lang, intensief, soms frustrerend, maar vooral ook leerzaam, veelzijdig en boeiend traject. Tijdens mijn promotie leerde ik zelfstandig een project opzetten en uitvoeren en samenwerken met een grote diversiteit aan collega's. Het resultaat van dit traject is het proefschrift dat nu voor je ligt. Iedereen die hieraan heeft bijgedragen — van het echoën in de operatiekamers tot het (meermalen) meeschrijven aan verschillende stukken — verdient mijn oprechte dank. Op de eerste plaats wil ik mijn bedanken: prof. dr. de Bree, dr. Noorlag en dr. van Es.

Geachte prof. dr. de Bree, beste Remco, graag wil ik je bedanken voor het vertrouwen dat je mij tijdens dit project hebt gegeven. Onze werkbesprekingen heb ik altijd als bijzonder prettig ervaren. Vaak eindigden onze besprekingen met een concreet plan, bijvoorbeeld wanneer ik met een onderzoek vastliep (soms dankzij een overijverige monitor). Soms leverden ze juist een nieuw projectidee op, waarbij je mijn technische achtergrond altijd in gedachten hield. Af en toe liepen de gesprekken wat uit, omdat ook andere onderwerpen, bijvoorbeeld hardlopen en wielrennen, minstens zo belangrijk waren. Daarnaast wil ik je bedanken voor de fantastische groep jonge onderzoekers die door jouw toedoen mijn collega's zijn geworden. Ook al deden wij allemaal ons eigen project, het voelde toch ook als een team-effort, en dat is in grote mate aan jou te danken.

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