Oral Oncology 123 (2021) 105627

Contents lists available at ScienceDirect

Oral Oncology

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

Detecting head and neck lymph node metastases with white light reflectance spectroscopy; a pilot study

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A B S T R A C T

Introduction: A challenge in the treatment of patients with head and neck cancer is the management of occult cervical lymph node (LN) metastases. Single-fiber reflectance (SFR) spectroscopy has the potential to detect physiological tissue changes that occur in a positive LN. This pilot study aimed to investigate whether SFR spectroscopy could serve as an alternative or additional technique to detect cervical lymph node metastases.

Materials and Methods: We performed intraoperative SFR spectroscopy measurements of LNs with and without malignancies. We analyzed if physiological and scattering parameters were significantly altered in positive LNs.

Results: Nine patients with a total of nineteen LNs were included. Three parameters, blood volume fraction (BVF), microvascular saturation (StO_2), and Rayleigh amplitude, were significantly lower in positive LNs. They were combined into one optical parameter 'delta', using discriminant analysis. Delta was significantly decreased in positive LNs, p = 0,0006. It had a high diagnostic accuracy where the sensitivity, specificity, PPV, and NPV were 90,0%, 88.9%, 90,0%, and 88.9%, respectively. The area under the ROC curve was 96.7% (95% confidence interval 89.7–100.0%).

Conclusion: This proof of principle study is a first step in the development of an SFR spectroscopy technique to detect LN metastases in real time. A next step towards this goal is replicating these results in LNs with smaller metastases and in a larger cohort of patients. This future study will combine SFR spectroscopy with fine-needle aspiration, using the same needle, to perform preoperative *in vivo* measurements.

Introduction

Regional lymph node (LN) metastasis often occurs in head and neck squamous cell carcinomas (HNSCC) [1,2]. Regional LN metastasis at the time of diagnosis is among the most important negative prognostic factors [3]. Lymph node metastases (*i.e.*, 'positive neck' or cN +) require treatment by surgery with or without adjuvant (chemo)radiation or by primary radiotherapy [1].

The management of HNSCC patients without LN metastases (*i.e.*, 'negative neck or cN–) is still a topic of controversy [2]. Because detection of LN metastases can be challenging, a high percentage of HNSCC patients have occult LN metastases [1,2]. Two main policies are in practice when the treatment of the primary tumor is surgical. The first is to perform an elective neck dissection (ND) on all patients with clinical negative necks and a high risk of occult metastases. These elective NDs are performed to properly treat the group of "negative neck" patients with occult metastasis; patients who actually have a positive neck. However, a consequence of this strategy is that 60–70% of the patients that did not have occult LN metastasis will receive unnecessary surgery. This could cause morbidity, among other negative consequences [2]. The alternative policy is 'watchful waiting'. In this strategy, the clinician carefully monitors the patients' necks and only performs NDs in patients that develop manifest LN metastases. However, the existing techniques to monitor the neck all have limitations in detecting metastatic deposits in LN at the earliest stage [1,2,4]. A solution for this issue in clinically negative necks is a more accurate staging of the neck to personalize treatment. Sentinel lymph node (SLN) biopsy in HNSCC diagnostics is an intra-operative technique that is used to assess whether the primary tumor has metastasized to the LN(s). The SLN(s) is/are the first LN(s) likely to harbor metastasis and they provide information on the rest of the nodal basin. The SLN biopsy is performed during surgery of the primary tumor and meticulously examined by the

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https://doi.org/10.1016/j.oraloncology.2021.105627
Received 16 June 2021; Received in revised form 8 September 2021; Accepted 9 November 2021
Available online 23 November 2021

histopathologist. This SLN examination has a promising reported sensitivity of 86% and negative predictive values (NPV) of 95% in patients with early-stage oral cavity tumors [4]. When a SLN harbors metastatic cells, a ND is recommended. However, this needs to be performed in a second stage, due to the time needed for the pathologic examination of the sentinel LN. This is undesirable, because it could cause distress for the recovering patient and lead to OR-, or post-operative radiotherapy-planning difficulties [2,5,6]. Other disadvantages could be an increased risk of injury of the marginal mandibular branch of the facial nerve and a higher risk of morbidity and possibly infection associated with second-stage surgery.

Single-fiber reflectance (SFR) spectroscopy has the potential to serve as an additional technique, or 'companion diagnostic', for the SLN biopsy. Several researchers have reported physiological tissue changes in LNs harboring metastases [7–9]. These changes indicate a compromised vascular network within the center of the LN, which affects microvascular blood oxygen saturation (StO_2) and blood volume fraction (BVF). Furthermore, these changes occur throughout the entire LN and not just in the part of the metastasis. Our group has previously shown that SFR spectroscopy measurements can be used to measure and quantify physiological and scattering properties of *in vivo* tissue [10–12]. Consequently, SFR might be useful to differentiate between metastatic and non-metastatic LNs in head and neck cancer [13]. Diagnosing LN metastases with SFR spectroscopy could give the head and neck surgeon an intraoperative tool to detect a (sentinel) LN metastasis in real-time. In this approach, a potential therapeutic ND could be performed in the same session, since the SLN would not have to be excised to be diagnosed by a histopathologist.

Another potential advantageous use of SFR spectroscopy is to combine it with (preoperative) ultrasound-guided fine-needle aspiration (FNA) [14,15]. Fine-needle aspiration is widely used in the diagnostic workup of HNSCC to acquire material for cytology of lymphatic tissue. Although the diagnostic accuracy of FNA is high, false negatives do occur [16]. This could be explained by the needle 'missing' the metastasis in the LN and only extracting normal tissue. Single-fiber reflectance spectroscopy might not have this problem, since its aim is to detect physiological changes that occur in the entire LN. The SFR spectroscopy measurement could be performed through the same needle as used for the FNA.

Kanick et al. investigated this hypothesis in a study of ten patients with lung cancer [13]. They performed SFR measurements in 7 malignant and 7 non-malignant mediastinal LNs and found significant ($p < 0.01$) differences in StO_2 and BVF of the LNs. By combining these two parameters it was possible to predict a malignant LN with high accuracy. A recent study, using a similar technique, showed that at least one point measurement per LN is enough to accurately estimate the tissue properties of the entire LN [17].

In this proof of principle study, we took the first step towards the use of SFR spectroscopy to detect cervical LN metastases. We aimed to investigate whether SFR spectroscopy measurements could differentiate cervical LNs harboring metastases from healthy LNs [13]. For this purpose, intraoperative SFR measurements of LN with and without metastases were performed. It was analyzed whether the optical properties of the metastatic LNs were altered compared to the non-metastatic LNs.

Materials and Methods

This prospective study was approved by the Medical Ethics Committee of the Erasmus MC (MEC-2017–551) and performed in accordance with the ethical standards of the Helsinki Declaration.

Subjects

Patients were recruited from the Department of Otorhinolaryngology and Head and Neck Surgery of the Erasmus MC Cancer Institute. Adult patients with a primary mucosal head and neck tumor (all subsites and stages) who had an indication for a neck dissection and at least 1 preoperatively diagnosed positive LN were included in this study. Patients with prior tumor treatment ([chemo]radiation or surgery) or another illness that could affect the anatomy of their LNs were excluded. All patients signed an informed consent form before enrollment in this study. Patient and tumor specific data such as age, sex, and tumor stage were collected using the electronic medical patient records.

SFR spectroscopy device

The measurements were performed with a SFR spectroscopy device, which has been described in detail previously (Figure 1) [18]. In summary, the setup utilizes a sterilized single-use optical fiber (Light Guide Optics International, Latvia) had a core diameter of 230 μm, an outer diameter of 400 μm, an SMA905 connector, a distal polished fiber for wide-angle beam, a numerical aperture of 0.22, and a length of 3 m (±0.2 m). Its sampling depth is approximately 165 μm, which is the same as half the core diameter of the fiber. This optical fiber is connected to a bifurcated optical fiber, of which one arm delivers light from a halogen light source (HL-2000-FHSA, Ocean Optics, the Netherlands) to the tissue and the other arm collects light from the tissue to a spectrometer (SD-2000, Ocean Optics, the Netherlands) to measure white light reflectance. A calibration procedure to account for internal reflections, variability in lamp-specific output and in fiber-specific transmission properties has been described in detail in a previous paper [19].

Examination procedure

The optical LN measurements were performed during neck dissection surgery with the patient under general anesthesia. They were performed in an *in vivo* environment after the surgeon had exposed the cervical lymph nodes. The LNs were chosen based on their likeliness to harbor metastases by visual and manual examination and preoperative clinical and radiologic information. Per patient, measurements of a positive and negative LNs were performed. Measurements were performed on both sides of the neck in patients with probable bilateral metastatic disease. We aimed to perform a peripheral and a central measurement of each LNs. For the measurements, a 'Spinocan' needle (Braun, Germany) (20 Gauge, outer diameter 0.91 mm, inner diameter 0.60 mm) was first inserted in the peripheral part of the lymph node. The surgeon aimed to place the tip of the needle approximately 1 mm inside the LN. The SFR fiber was then guided through the needle until it was in contact with the LN tissue. Finally 5 repeated SFR measurements were performed with a total duration time of approximately 10 s. This procedure was respectively repeated for the central part of the LN to ensure a peripheral and center measurement per LN. All measured LNs were individually marked to assure accurate correlation with final histopathologic assessment. After obtaining the SFR measurements, the surgeon continued the procedure including excision of the cervical LNs. All measurement were led by an investigator (OB, DR) and performed by experienced head and neck surgeons (JH, SKe, AS).

Analysis of Spectra

The SFR spectra were analyzed using a previously described analytical model to describe the wavelength-dependent optical properties, with the goal to extract physiological (absorption) and morphological (scattering) information from the sampled tissue [18,20].(see Fig. 2).

The four physiological parameters extracted were: 1) microvascular blood oxygen saturation (StO_2 [%]), the fraction of oxygen-saturated hemoglobin relative to total hemoglobin in the blood; 2) blood volume fraction (BVF [%]), which is the percentage of blood in the measured volume; 3) mean vessel diameter (VD [mm]), which is the mean estimated mean of the vessel diameters; and 4) tissue bilirubin concentration ($[BIL]_{tis}$ [μmol/L]).

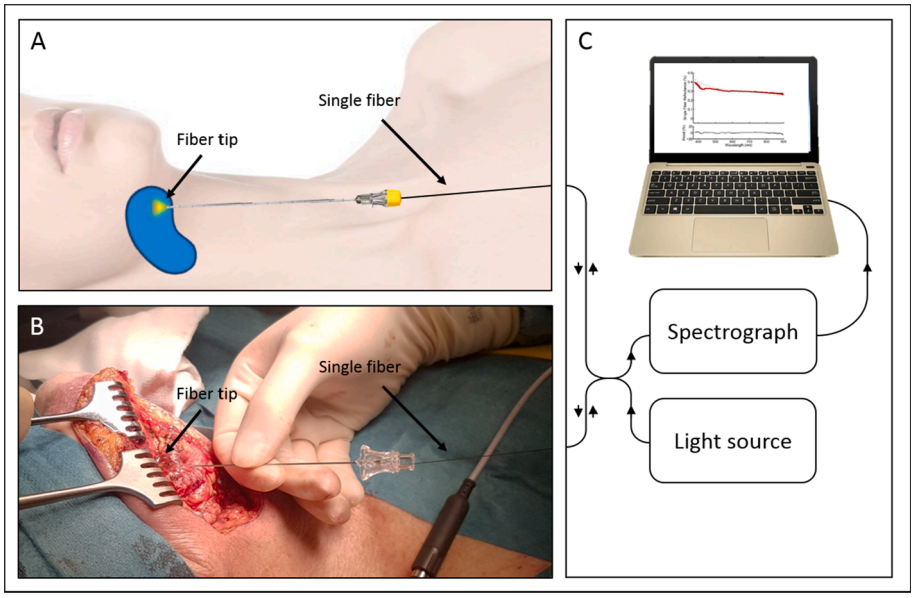

Fig. 1. Schematic (A) and real life (B) representation of a single fiber reflectance spectroscopy measurement of a cervical lymph node. Patient consent was giving to take and use this intraoperative picture for scientific purposes.

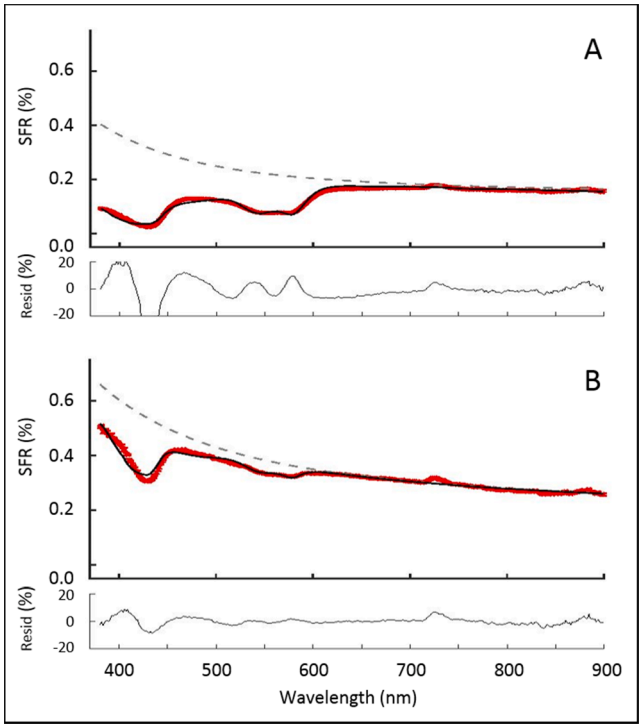

Fig. 2. Spectra of the A) negative and B) positive lymph node of patient 9. Patient A features a higher absorption coefficient in the wavelength region from 500 to 600 nm. The spectrum from patient A correlates with a higher blood oxygen saturation and blood volume than patient B.

Part of the model is the background scattering model that is a combination of Mie and Rayleigh scattering, given by wavelength-dependent power law functions with fitted parameters specifying the Mie amplitude (S1), Mie slope (S2), and Rayleigh amplitude (S3).

Histopathologic assessment of LNs

All the measured LNs underwent histopathologic examination. To start, the LNs were visually and manually examined and any irregularities were described. Representative tissue samples were fixed by formalin 4% and embedded in paraffin. Thin 4 μm slices are examined by confocal microscopy. All LNs were examined by a single experienced head and neck histopathologist (SKo). The stage of differentiation of the metastasis, the presence of keratin deposits and the presence of fat cells were determined.

Statistical analysis

Confidence intervals on optical parameter estimates were calculated from the square root of the diagonal of the covariance matrix [21]. Parameter values were averaged over five repeated measurements, weighted by the confidence interval of individual spectral fits. Spectra that showed evidence of a blood pool within the detection volume (blood volume fraction > 40%) were excluded from the analysis. Seven parameters were analyzed: StO_2, BVF, VD, $[BIL]_{tis}$, Mie amplitude, Mie slope, and Rayleigh amplitude. Continuous data was reported as median value and interquartile range (IQR) (non-normally distributed data and $n < 30$ per group). Categorical data was reported as counts and percentages. Differences in the continuous data (parameters obtained from the spectral analysis) between the two groups were analyzed using the Mann-Whitney *U* test. We standardized our data to a standard normal distribution ($x_{new} = (x - \mu) / sd$) where μ is the mean and sd is the standard deviation of parameter × to compute a biomarker, delta, to identify positive LNs. A discriminant analysis of the significantly different parameters was performed to create a combined biomarker 'delta'. A ROC-curve of delta was created to test its diagnostic accuracy. There were no missing data. Statistical analysis was performed using SPSS version 21 (IBM Co., USA) and the cut off point for significance was $p < 0.05$.

Results

Twenty two LNs in 9 patients were initially included in this study (Table 1). Seven patients were male (77.8%) and two female. Their mean age was 67.9 years (IQR 59.0–74.7). The primary HNSCC tumors were located in the oral cavity [5], larynx (1), and hypopharynx (1). Two patients had an unknown primary tumor. All tumors had metastasized to regional LNs. In two patients (study ID 5 and 6), both sides of

Table 1
Lymph nodes included in analysis (n = 19/22), patients (n = 9), index tumor and baseline characteristics.

Lymph nodes								Patient			Index tumor		
#	Pathology	Side	Level	Size (mm)*	Diff.	Keratin	Fat	#	Sex	Age	Location	cT	cN
1	Positive	Left	2a	29	Well	Yes	Yes	**1**	Male	64.1	Oral cavity	3	2b
2	Negative	Left	1a				No						
3	Positive	Right	2	25	Poor	No	Yes	**2**	Female	77.7	Oral cavity	0	1
4	Negative	Right	4				No						
5	Positive	Right	3	55	Poor	No	No	**3**	Male	67.9	Unknown pr.	0	2a
6	Negative	Right	1a				Yes						
7	Positive	Left	2a	20	Well	Yes	No	**4**	Female	70.4	Oral cavity	3	1
8	Negative	Left	4				No						
9	Positive	Right	3	40	Mild	No	No	**5**†	Male	72.4	Larynx	3	2c
10	Negative	Right	5				Yes						
11	Positive	Left	2a	37	Mild	No	No						
12	Negative	Left	1a				Yes						
x	Negative	Right	2b				Yes	**6**†	Male	59.7	Oral cavity	3	2b
x	Negative	Right	4				Yes						
x	Negative	Left	2a				Yes						
13	Negative	Left	4				Yes						
14	Positive	Left	2a	20	Poor	No	Yes	**7**	Male	77.0	Hypopharynx	2	3b
15	Negative	Left	1b				No						
16	Positive	Right	1b	14	Well	Yes	No	**8**	Male	58.2	Oral Cavity	3	3b
17	Positive	Right	3	10	Well	Yes	Yes						
18	Positive	Left	2a	44	Poor	No	No	**9**	Male	45.8	Unknown pr.	0	2a
19	Negative	Left	1b				Yes						

Diff. = stage of differentiation of the LN metastasis, Keratin = the presence of keratin deposits in the metastasis, Fat = the presence of fat cells in the LN, Unknown pr. = unknown primary tumor. * All negative lymph nodes were < 15 mm; † Both sides of the neck were measured in these patients.

the neck (4 LNs) were measured.

The histopathologic assessment of the LNs did not confirm our pre-operative assessment (based on physical examination and imaging techniques) in two patients (study ID 6 and 8). In patient 6 all four measured LNs turned out to be negative (without metastasis) and in patient 8 both measured LNs turned out to be positive (with metastasis). In total 10/22 LNs showed metastases: four LNs showed well differentiated metastases with keratinization, two LNs showed mild differentiation, and the last four were poorly differentiated of which two were cystic. Fat absorption was observed in 12 out of 22 LNs of which 8 were normal LNs.

The measurements of three LNs of patient 6 were excluded prior to the analysis of potential differences in optical properties between the LNs with and without metastases (LNs are marked with an 'X' in Table 1). The results from our spectroscopy measurements, together with histopathological examination of the tissue, showed that these measurements were not performed in lymphatic tissue, but in surrounding fat tissue. After the exclusion of these three LNs, a full statistical analysis was performed 19 LNs of which ten were positive and nine negative.

The LN optical properties of the central measurements were best predictive of the presence of a LN metastasis. Three parameters, BVF, StO_2, and Rayleigh amplitude, were significantly different between the negative and positive LNs (Table 2). The BVF was lower in the positive LNs (0.920 [IQR 0.780–4.720]) than in the negative ones (4.835 [IQR 2.035–18.480], p = 0.022). The StO_2 was also decreased in positive LNs, p = 0.034. Finally, the median Rayleigh amplitude was lower in the positive LNs (0.140 [IQR 0.035–0.235]) than in the negative LNs (0.305 [IQR 0.235–0.435], p = 0.011). The parameters VD, $[BIL]_{tis}$, Mie amplitude, and Mie slope were not significantly different between the two groups of LNs. The peripheral measurements, while not significantly different from the central measurements, were not able to discriminate between positive and negative LNs.

Table 2
Values of Rayleigh amplitude, blood volume fraction, and blood oxygen saturation values in 10 positive and 9 negative lymph nodes.

		Median	25th %	75th %
S3 (-)	Pos	0.140	0.035	0.235
	Neg	0.305	0.235	0.435
BVF (%)	Pos	0.920	0.780	4.720
	Neg	4.835	2.035	18.480
StO_2 (%)	Pos	40.0	15.8	53.8
	Neg	62.9	43.7	85.8

S3 = Rayleigh amplitude, BVF = blood volume fraction, StO_2 = blood oxygen saturation, Pos = positive, Neg = negative.

The three significantly different parameters, BVF, StO_2, and Rayleigh amplitude were combined into one optical parameter 'delta', using a discriminant analysis. This resulted in an optical parameter that was significantly lower in positive LNs, p = 0.00061. It had a high diagnostic accuracy. The sensitivity, specificity, positive predictive value, and negative predictive value of delta were 90,0%, 88.9%, 90,0%, and 88.9%, respectively. The area under the ROC curve was 96.7% (95% confidence interval 89.7–100.0%).

Discussion

With the technique described in this paper, it could be possible to detect positive LNs by looking into the physiological changes caused by the malignant cells, instead of the malignant cells themselves. We analyzed whether SFR spectroscopy measurements were able to detect these tissue changes in cervical LNs of HNSCC patients. For this purpose, we performed intraoperative SFR measurements of positive and negative LNs.

Main results

Our measurements showed that the BVF was decreased in positive LN. This finding is in accordance with the study from Kanick et. al. in mediastinal LNs of lung cancer patients. This study used the same SFR spectroscopy technique. Interestingly, the BVF values of the cervical LNs

in the present study were approximately a 2-fold lower than those of the mediastinal LNs. This might be because mediastinal LNs are better vascularized. However, we found no literature to support this hypothesis. An explanation might be that central region/organs are more vascularized than peripheral ones. Another similarity with both studies is that we both found a decreased blood oxygen saturation in LNs harboring a metastasis. Moreover, it is surprising that both the lower BVF and StO_2 were also seen in our 'Optical Screening' study in the measurement of the field cancerization (in the buccal mucosa) of laryngeal cancer patients [22].

The scattering parameter Rayleigh amplitude was also decreased in positive LNs. This finding is not in line with the two previous studies from our group. However, it does confirm our pre-study hypothesis that LNs with metastases undergo ultrastructural tissue changes.

Liu et. al. also found spectroscopic changes, in the higher infrared wavelengths, in positive cervical lymph nodes of patients with thyroid cancer [23]. These changes were caused by alterations of nucleic acids, proteins, lipids and carbohydrates. Their *ex vivo* measurements of 68 metastatic and 123 non-metastatic LNs provided a sensitivity of 80.3% and a specificity of 91.9%.

Challenges and limitations

During the execution and analysis phase of this pilot-study we encountered some challenges we did not account for in the design phase. This provided us with very useful information to design a future study.

Our study design was to perform the measurements on LNs in an "as *in vivo* as possible" environment: during surgery, when visible for the surgeon. During the measurements we noticed the LNs were not as *in vivo* as we initially thought they would be. In attempt to visualize the LNs, the surgeons had to remove a considerable amount of surrounding tissue. Although attempts were made not to disturb the LN's vasculature, it is very plausible that this was not achieved. This may have had an influence on the physiological parameters of the measured LNs compared to a real *in vivo* measurement.

It also proved to be difficult to take SFR spectroscopy measurements from the periphery and center of each LN, without additional radiological guidance, which our study design dictated. This was especially the case for the, small, negative LNs. Despite efforts to carefully insert the needle in the desired locations in the LNs, the surgeons found it cumbersome to determine the exact position of needle tip (and thus the measurement point of the fiber tip). In our analyses we even had to conclude that some measurements were not performed inside an actual LN. In these cases, fat tissue might have been misclassified as LNs. With the use of ultrasound guidance, it is possible to overcome this challenge. Radiologists already widely use this technique of ultrasound-guided needle placement for fine-needle aspiration cytology. It is also possible to adjust our SFR spectroscopy device to also detect fat tissue, by increasing the wavelength range.

LNs with cysts bring another challenge. In the present study, the positive LNs of two patients (study ID 2 and 3) were cystic. Insertion of the needle into the cyst for an SFR spectroscopy measurement will unfortunately result in a non-reliable measurement. The fluid inside of the cyst will have a completely different "optical signature" than lymphatic or metastatic tissue. This should be avoided by either measuring the wall of the cyst or a non-cystic part of the LN. Again, ultrasound guidance could assist in the needle placement in these types of LNs. Another option is to not use this method on LNs suspected to have cystic metastases.

This pilot study had a modest number of included patients and lymph nodes. This prevented us from making definitive statements. On the other hand, finding significant differences given the number of patients is promising. A future study will show whether these differences will remain in larger groups as this might increase the discriminative power of the method. It will also allow to cross-validate our test. Also, a larger cohort of patients will allow us to investigate the influence of other factors such as the relationship between HPV status, the physiology primary tumors and their associated LNs and the performance of SFR spectroscopy.

For this pilot study we chose patients with a high susceptibility of harboring malignant LNs (physical examination, imaging) or proven metastases (cytology). The real clinical value lies in detecting smaller, or even micro-, metastases. In this proof of concept study our primary goal was to determine if SFR spectroscopy measurements could discriminate positive LNs from negative. Future research should focus on detecting physiological LN changes caused by smaller metastatic deposits.

In conclusion, this proof of principle study showed that SFR spectroscopy of cervical LN has the ability to differentiate between positive and negative LNs. This may lead to a useful tool to detect LN metastases. It might eliminate the need for a two-stage surgery by analyzing the SLN in real-time. Another potential use is to implement SFR spectroscopy as an addition to FNA cytology. We aim to perform a future study in which we will perform the SFR spectroscopy measurements during, or just prior to, the FNA procedure, using the same needle, thus taking another step towards the implementation of this technique in clinical practice.

Declaration of Competing Interest

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

Acknowledgements

The authors would like the thank Iris Schmidt from the University Medical Center Groningen for assistance with calibration and data analysis.

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