The Role of Narrowband Imaging in Laryngeal Cancer

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[REDACTION]

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Student Declaration

This is to certify that to the best of my knowledge, the content of this thesis is my own work. This thesis has not been submitted for any degree or other purposes.

I certify that the intellectual content of this thesis is the product of my own work and that all the assistance received in preparing this thesis and sources have been acknowledged.

This work has not been submitted for any other degree at The University of Sydney or elsewhere. This thesis is entirely the work of the writer. No other person's work has been used without due acknowledgement in the main text of the thesis. Approval for these studies was given by The Westmead Hospital Human Research Ethics Committee (Reference number: 5424).

Signature:

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Date: 25th January 2020

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Abstract

The survival rate of laryngeal cancer in Australia has remained unchanged over the last 30 years despite advances in treatment. Current white light endoscopy (WLE) lacks the diagnostic accuracy to detect pre-malignant and early malignant laryngeal lesions.

Narrowband Imaging (NBI) is an optical imaging modality that enhances and contrasts mucosal microvasculature. By detecting changes in the mucosal microvasculature as a marker of neoangiogenesis, NBI can detect pre-malignant and malignant mucosal lesions. NBI is increasingly being used in the diagnosis of laryngeal squamous cell carcinoma. During angiogenesis, superficial vessels arise from the sub-epithelium known as intraepithelial papillary capillary loops (IPCL). In 2011, the Ni classification system of NBI based on the morphology of IPCLs was proposed to detect pre-malignant and early malignant laryngeal lesions. This classification system has been reported to be more sensitive than WLE in detecting early laryngeal cancer. This thesis examines the role of NBI in the work-up and diagnosis of pre-malignant and malignant laryngeal lesions.

In the first study, the diagnostic accuracy of NBI using the Ni classification in laryngeal cancer is assessed. Patients with suspicious laryngeal lesions were recruited and graded according to the Ni classification. Ni grading was compared against the gold standard histopathological findings to determine the sensitivity, specificity, positive and negative predictive values of NBI. Furthermore, the correlation between higher grades of Ni classification and pathological stage of disease were analysed.

The second study expands the utility of NBI in the work-up of laryngeal leukoplakia. Laryngeal leukoplakia is a diagnostic challenge with over half of the biopsied specimens returning as normal epithelium. Leukoplakia limits assessment of IPCLs and has been reported to be a limitation of using NBI. However, recent studies have suggested that NBI can still be applied in laryngeal leukoplakia with superior accuracy compared to WLE. A systematic review and meta-analysis was performed to assess the diagnostic accuracy of NBI in differentiating between low-risk laryngeal

leukoplakia (defined as normal epithelium, simple hyperplasia, low and moderate grade dysplasia) and high-risk laryngeal leukoplakia (defined as severe dysplasia, carcinoma and invasive cancer.

The third study assesses the inter and intra-observer reliability of NBI in differentiating between benign and malignant disease and making an optical diagnosis. Furthermore, the impact of operator experience on the interpretation of the images is assessed.

Publications And Presentations Arising From This Thesis

Publications

- Ahmadzada, S., Tseros, E., Sritharan, N., Singh, N., Smith, M., Palme, C.E. & Riffat, F. (2019), The Value of Narrowband Imaging using the Ni Classification in the Diagnosis of Laryngeal Cancer. *Laryngoscope Investigative Otolaryngology* (Accepted)
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Authorship attribution statement

For All Publications and Presentations, I designed the study with my supervisors, recruited patients (where applicable), collected the data, analysed the data and wrote the manuscript.

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Glossary

NBI	Narrowband Imaging
WLE	White Light Endoscopy
HNSCC	Head and Neck Squamous Cell Carcinoma
SCC	Squamous Cell Carcinoma
Cis	Carcinoma in situ
EGFR	Epidermal Growth Factor Receptor
HPV	Human Papilloma Virus
STAT 3	Signal Transducers and Activators Of Transcription-3
HGF	Hepatocyte Growth Factor
IGF-1	Insulin-Like Growth Factor-1 Receptor
VEGF	Vascular Endothelial Growth Factor
HIF-1	Hypoxia Associated Transcription Factor-1
IPCL	Intraepithelial Papillary Capillary Loops
AFE	Autofluorescence Endoscopy
VS	Videostroboscopy
OCT	Optical Coherence Tomography
AJCC	American joint committee on cancer
UICC	Union for International Cancer Control
WHO	World Health Organization
SIN	Squamous Intraepithelial Lesion
SIL	Squamous Intraepithelial Lesion
TLM	Transoral Laser Microsurgery
RT	Radiotherapy
CRT	Chemo-radiotherapy
CT	Computed Tomography
MRI	Magnetic Resonance Imaging
PET	Positron Emission Tomography
DSR	Database of Systematic Reviews
DARE	Database of Abstracts of Reviews of Effects
QUADAS-2	Quality Assessment of Diagnostic Accuracy Studies
HSROC	Hierarchical Summary Receiver Operating Characteristics

Chapter One: Introduction

Incidence and Mortality

Laryngeal cancer is one of the most common cancers involving the head and neck region accounting for 15.6% of all head and neck cancers [2]. Although the incidence of laryngeal cancer has decreased from 4.3 per 100,000 to 2.6 per 100,000 over a 30-year period, the number of deaths have increased in Australia [2].

This trend has also been observed in United States of America and Canada with increasing mortality despite decreasing incidence and advances in treatment [3,4]. However, the mortality rate of laryngeal cancer has been decreasing in many European counties over the last 2 decades [5].

Risk Factors

Smoking is the most common risk factor for laryngeal cancer and the risk of developing cancer for current smokers increases 10-fold compared to people who have never smoked [2]. Although this risk can decrease by up to 70% after 10 years of smoking cessation it never returns to the level of someone who has never smoked [6]. This includes "smokeless" tobacco such as snuff, chewing tobacco and hookahs.

The mechanisms of smoking induced carcinogenesis are extremely complex and includes direct genotoxicity leading to sustained DNA damage, chronic inflammation and oxidative stress at the cellular level [7].

Smoking cessation remains the best method to minimize this risk and in the last 50 years, the rate of smoking in Australia has decreased in both males and females [2].

Alcohol consumption is another independent risk factor with the risk increasing proportionally with the amount of alcohol consumed [8]. Furthermore, cigarette smoking and alcohol consumption combined have a synergistic effect on laryngeal cancer risk[8].

Other risk factors implicated to play a role include having a genetic predisposition, chronic exposure to wood dust, paint fumes from occupations such as textiles industries, metalworking and woodworking [2,9].

Although the role of human papillomavirus (HPV)[10] has been established as a risk factor in oropharyngeal cancers, its role in laryngeal cancer is unclear and continues to be an area of research [11]. However, a systematic review and meta-analysis by Li

and colleagues [12] demonstrated a strong association between HPV infection (particularly HPV-16) and development of laryngeal SCC.

Pathophysiology

The pathogenesis of head and neck squamous cell cancer (HNSCC) is poorly understood and remains an area of active research.

This is in large part due to the complex milieu of the tumour microenvironment with multiple molecules that are involved in tumour proliferation, differentiation and invasion [1].

A comprehensive review of the molecular pathogenesis of HNSCC is beyond the scope of this thesis. Briefly, the key molecules and their roles in HNSCC belong to a family of oncogenes, tumour suppressor genes or mediators involved in cellular communication [13]. A graphical summary adapted from Rothenberg and Ellisen 2002 [1] is provided in figure 1.

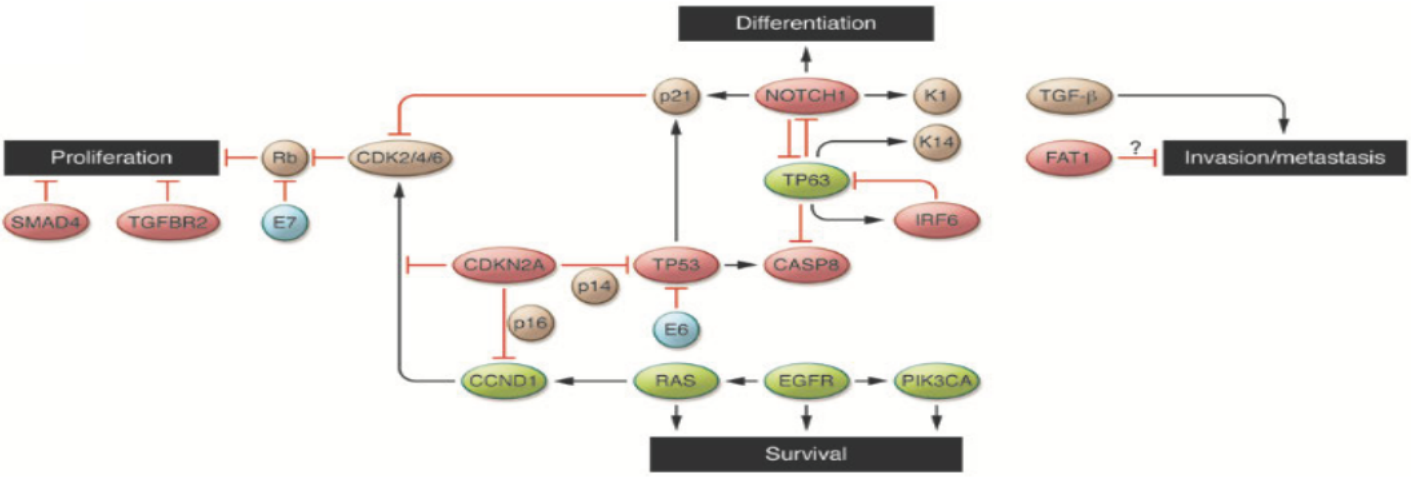

Figure 1: Pathways altered in HNSCC pathogenesis. Red: putative and established tumour suppressors; green: oncogenes; brown: other relevant genes/ proteins; blue: viral proteins. Adapted from Rothenberg and Ellisen 2012[1]

Oncogenes

Oncogenes promote abnormal cell proliferation by altering regulatory genes responsible for programmed cell growth. Mutations in these genes results in deregulated cellular proliferation and inactivation of tumour suppressor genes.

Among the many oncogenes involved in HNSCC, *ras* and *myc* genes are overexpressed in up to 9-48% of HNSCC [13]. Of particular interest are mutations in subtypes of *ras* (H*ras* and K*ras*), which are observed in SCCs in humans with a

history of tobacco exposure[13]. These mutations have also been observed following chemical carcinogen exposure in mice [1]. Overexpression of these genes lead to dysregulation of cellular proliferation through increased signal transduction to the cell nucleus [13].

Cyclin D1 is another protein found of be overexpressed in up to 49% of HNSCC particularly involving the larynx and hypopharynx [14]. Although this protein is a normal part of the cell cycle acting as a control gene, its over expression shortens the G_1 cell cycle phase [14]. This leads to accumulation of non-repaired DNA, reduction in number of cells in quiescence and cell proliferation despite inhibition from mitogen signalling [13,14]. Overexpression of cyclin D1 is associated with a poorer prognosis in patients with laryngeal cancer who have been treated surgically with or without adjuvant radiotherapy [15,16].

Epidermal growth factor receptor (EGFR) [17] belongs to the receptor tyrosine kinase family and is overexpressed in most HNSCC and is usually mutated, allowing this protein to be constantly active [18]. Among its many functions, it activates epidermal growth factor receptor [17], transforming growth factor alpha (TGF- **α**) and leads to the downstream activation of the *ras* family [13]. As with cyclin D1, EGFR overexpression has been associated with a poor prognosis independent of tumour stage with an increased risk of relapse in patients with laryngeal SCC [19]. However, in the case of early laryngeal cancer treated with primary radiotherapy, the risk of recurrence increases only with overexpression of TGF- **α,** not EGFR [20].

Other key upstream and downstream molecules playing a role in activation of oncogenes and uncontrolled cell division include signal transducers and activators of transcription-3 (STAT3), hepatocyte growth factor (HGF), insulin-like growth factor-1 receptor (IGF-1) and vascular endothelial growth factor and receptors (VEGF) [1,13,21]. STAT3 activates transcription of genes with downstream activation of cyclin D1, Bcl-X and VEGF responsible for cell proliferation, apoptosis and angiogenesis respectively [21]. Both HGF and IGF-1 are overexpressed in HNSCC and are involved in tumour progression by affecting the surrounding microenvironment, promoting angiogenesis and tumour motility [21].

Tumour suppressor genes

Unlike oncogenes, tumour suppressor genes prevent dysregulated cell proliferation by detecting mutations or DNA damage and inducing apoptosis, or preventing cell division [22].

One of the most common tumour suppressor gene involved in development and progression HNSCC is p53, which can be mutated in up to 50-80% of HNSCC malignancies [21]. This mutation as well as other genes in the p53 pathway renders the cell unable to respond to DNA damage, mainly through silencing of genes that normally activate p53 (e.g. ataxia telangiectasia mutated) [21]. Mutations in p53 are believed to play a key role in the early initiation of HNSCC evidenced by mutated p53 being detected in precursor lesions such as leukoplakia [1]. Loss of p53 function has also been associated with poor response to chemotherapy leading to decreased survival after surgical excision and adjuvant radiotherapy [1]. Furthermore, immunohistochemistry studies demonstrate that expression of mutated p53 is proportional to the stage of laryngeal cancer [23].

Cadherins are a group of proteins involved in intercellular communication and interactions. A dysfunction in a subtype of cadherins (E-cadherin) is strongly associated with invasion and metastasis [13]. E-cadherins interact with anchoring proteins forming complexes that assist with cell-to-cell adhesion. Loss of this complex and expression of E-cadherins is inversely proportional to the stage of tumour differentiation [13,24].

Angiogenesis

Angiogenesis is the process of forming new blood vessels from pre-existing vasculature and is the hallmark of tumour growth, invasion and metastasis. The process of neovascularisation or the so called “angiogenic switch” is an early step in the malignant transformation of a tumour [25].

Neovascularisation is a multi-step process dependent on over expression of oncogenes and mutations in tumour suppressor genes, which render them inactive. The vascular

endothelial growth factor (VEGF) pathway is strongly associated with neoangiogenesis in HNSCC [25]. VEGF belongs to the family of platelet-derived growth factors that promote tumour cell proliferation and increases vascular permeability, allowing growth factors to interact between endothelial cells and tumour cells. This results in migration of tumour cells and affects the immune cells in the tumour microenvironment [25]. VEGF is produced in response to upstream activators including growth factors and oncogenes mentioned above, inflammatory cytokines and tissue hypoxia [25].

Tissue hypoxia is a major stimulus in the HNSCC microenvironment, leading to hypoxia associated transcription factors (namely HIF-1 and its target proteins CA-9 and GLUT-1) that promote expression of VEGF [25]. Other promoters of angiogenesis are also overexpressed in HNSCC including Interleukin-8 (IL-8) and EGFR. The association between VEGF and immune regulation is also likely related to tissue hypoxia as high levels of cytokines that mediate hypoxia including IL-8, interleukin-4, eotaxin and granulocyte colony stimulating factor are detected in HNSCC [25,26]. This in turn promotes expression of VEGF, generating a positive feedback loop as expression of one promotes the other.

VEGF is believed to cause sprouting type neoangiogenesis, where endothelial cells from capillaries migrate towards a hypoxic angiogenic centre [27]. The sprouting endothelial cells are propelled towards the tumour by receiving maximal amount of VEGF-A evidenced by high number of VEGF receptors expressed on their surface [28,29]. These tip cells produce extensions known to filopodia, which fuse with the other filopodia from corresponding tip cells that sprout from the tumour [27]. As the two capillaries fuse together, they ultimately form tubes allowing for oxygenated blood to flow from host vessel to the newly formed vessels. Stabilisation of these vessels occurs as new basement membrane is laid down and surrounding cells called pericytes are stabilized in the periphery of these vessels [28].

Although the basement membrane is stabilised, these vessels are thin walled and "leaky" compared to their host vessels. Furthermore, they form a chaotic pattern with no morphological structure. These vessels with chaotic morphology are called

intraepithelial papillary capillary loops (IPCL) and can be distinguished from normal tissue microvasculature. Detection and classification of these IPCLs is utilised during endoscopy with narrowband imaging (NBI) to show early carcinogenesis [30]. NBI has thus been developed as a biological endoscopy method owing to its ability to distinguish and classify IPCLs to show different stages of tumour progression.

Staging and Pathology of Laryngeal Cancer

Laryngeal tumours are staged based on the TNM classification system developed in 1943 and is adopted by both the American joint committee on cancer (AJCC) and the Union for International Cancer Control (UICC) [31,32]. This system follows the Halstead theory recognising that cancer development and progression occurs in distinct stages beginning with primary tumour formation followed by loco-regional spread through the lymphatic system [33]. The TNM classification determines the stage of the tumour by determining the size and extent of primary tumour (T), the presence and degree of lymph node involvement (N) and distant metastasis (M).

The tumour may either be classified clinically through nasoendoscopy, radiological examination and laryngoscopy (*c*TMN) or pathologically (*p*TNM). Pathological staging also includes the histopathology of the resected specimen [31]. Glottic tumours are assigned 5 T categories (Tis, T1-T4) (Table 1). T1 and T4 are further subdivided into T1a/b and T4 a/b.

Although this system is widely used, some authors further subdivide T2 into T2a and T2b depending on the vocal cord mobility due to better local control following radiotherapy in T2 tumours where cord mobility is still intact (i.e. T2a) [34]. Together with nodal involvement and the presence of metastasis, this classification system can be used to stage disease, guide treatment and prognosticate.

Histological diagnosis is the gold standard test for diagnosing laryngeal SCC. Laryngeal SCC is histologically classified according to the World Health Organization histological classification of laryngeal tumours [35]. Malignant surface epithelial tumours such as conventional squamous cell carcinomas are graded by the degree of squamous cell differentiation and is divided into well-differentiated,

moderate differentiated and poorly differentiated SCC [35]. The degree of differentiation is largely determined by and is inversely proportional to formation of keratin and/or presence of intercellular bridges [35]. Laryngeal SCC subtypes also affect treatment and prognosis. These variants are classified as verrucous SCC, basaloid SCC, papillary SCC or spindle SCC [35]. Although histological grading adds prognostic value, it is observer dependent.

T Category	***Description***
Tx	Primary tumour cannot be assessed
Tis	Carcinoma in situ
T1	Tumour limited to the vocal cord(s). May involve anterior or posterior commissure with normal mobility
T1a	Tumour limited to one vocal cord
T1b	Tumour involves both vocal cords
T2	Tumour extends to supraglottis and/or subglottis, and/or with impaired vocal cord mobility
T3	Tumour limited to the larynx with vocal cord fixation and/or invasion of paraglottic space and/or inner cortex of the thyroid cartilage
T4	Moderately advanced or very advanced local disease
T4a	Moderately advanced local disease. Tumour invades through the outer cortex of the thyroid cartilage and/or invades tissues beyond the larynx (e.g. trachea, soft tissues of neck including deep extrinsic muscles of the tongue, strap muscles, thyroid or oesophagus)
T4b	Very advanced local disease. Tumour invades prevertebral space, encases carotid artery, or invades mediastinal structures.

Table 1: American Joint Committee on Cancer Classification of Glottic Carcinoma [31]

Precursor or pre-malignant lesions of the larynx are lesions with a potential to transform into malignant lesions and accurate diagnosis is critical.
The latest classification system by the WHO [35] classifies premalignant lesions of the larynx into low-grade dysplasia, severe dysplasia or carcinoma in situ (Cis) evolving from a number of different definitions including squamous intraepithelial neoplasia (SIN), squamous intraepithelial lesion (SIL), or laryngeal intraepithelial neoplasia [35].

The risk of malignant transformation is proportional to the degree of dysplasia and the risk of malignant transformation with severe dysplasia/Cis is up to 30% compared to 10.6% in mild/moderate dysplasia [10].
This simplified classification system improves inter-observer reliability and identifies key differences between moderate/severe dysplasia and Cis, which ultimately improves clinical decisions making regarding treatment [36].

Clinical Presentation

The clinical presentation of patients with laryngeal cancer depends on the site and size of the primary tumour, involvement of surrounding structures and stage of disease. The earliest and most common symptom is hoarseness and chronic voice change [37]. Other symptoms may include swallowing difficulty, globus like symptoms and in the late stage of disease, breathing difficulties [38]. This may be accompanied by constitutional symptoms of weight loss, malaise, fatigue and anorexia. Due to lack of lymphatic supply and barriers to tumour spread, glottic tumours are often detected early. However, supraglottic tumours may often present with lymphadenopathy of the neck following locoregional spread [37]. Subglottic tumours usually present at an advanced stage and are accompanied by dyspnoea, stridor and airway obstruction [37]

Examination involves a complete assessment of the head and neck including visualisation of the larynx either with laryngeal mirror or an in office nasoendoscopy. Although overt lesions are detectable with a laryngeal examination, early neoplastic or pre-neoplastic lesions of larynx (particularly glottic lesions) are difficult to detect with a laryngeal mirror and even white light endoscopy [39]. Moreover, despite a thorough history and examination, it is reported that the diagnostic accuracy of history and physical examination without nasoendoscopy alone is as low as 5% [40].

Treatment Modalities

Treatment of laryngeal SCC depends on many factors including tumour factors (i.e. stage of disease as well as the histological subtype), patient factors (age, comorbidities, performance status, patient preference) and institutional factors

(oncological expertise and experience) [37]. The ideal goals of treatment are to achieve cure while preserving laryngeal function and minimizing morbidity [37].
Treatment modalities include surgical resection either by open approach or endo-laryngeal approach, radiotherapy (RT) and/or chemotherapy [37].
The treatment options for early stage glottic cancer (defined as Tis-T2 with N0M0) include open surgical resection, transoral laser microsurgery (TLM) or RT [41]. A detailed review of open surgical techniques is beyond the scope of this thesis. Briefly, the many options for resection via open approach include laryngofissure with cordectomy, vertical partial hemilaryngectomy, frontolateral hemilaryngectomy or supracricoid laryngectomy [42].
Open surgical options are mainly reserved for salvage therapy due to their relative morbidity and poorer voice outcomes compared to RT and TLM [43]. To date, only one randomized control trial has been conducted comparing open surgery with RT [44]. There were no statistically significant differences in survival between patients treated with RT or open surgery [44]. The only statistically significant difference observed was a higher 5-year disease free survival rate for open surgery (78.7%) compared with RT (60.1%) for T2 tumours [43,44].

Although there are no randomized controlled trials to compare differences between TLM and RT, cohort studies demonstrate no significant differences in local control or recurrence rates between TLM and RT for patients with early stage glottic SCC [45]. Previous systematic reviews and meta-analysis of prospective and retrospective cohort studies comparing TLM with radiotherapy in early glottic cancer (Tis-T1a) demonstrated TLM to offer greater overall survival and increased rates of larynx preservation while RT offered greater voice quality compared to TLM [46,47]. There were no differences in local control between the two groups. A summary of comparisons is presented in Table 2.

Outcome	TLM vs. RT	Reference
Overall survival N = 685 (TLM) N = 608 (RT)	TLM OR of 1.52 (95% CI of 1.07, 2.14)	Vaculik et al, *Systematic review and meta-analysis of T1 glottic cancer outcomes comparing CO2 transoral laser microsurgery and radiotherapy*
Disease free survival N = 722 (TLM) N = 744 (RT)	TLM OR of 2.70 (95% CI of 1.32, 5.54)	
Laryngeal Preservation N = 986 (TLM) N = 929 (RT)	TLM OR of 6.31 (95% CI of 3.77, 10.56)	
Local Control N = 841 (TLM) N = 862 (RT)	No significant difference OR of 1.19 (95% CI of 0.79, 1.81)	
Voice quality (Maximum phonation time) N = 141 (TLM) N = 126 (RT)	No significant difference Mean difference -1.55 (95% CI of -4.55, 10.6)	Guimarães et al, *Comparison between transoral laser surgery and radiotherapy in the treatment of early glottic cancer: A systematic review and meta-analysis*
Voice quality (Fundamental frequency) N = 212 (TLM) N = 171 (RT)	No significant difference Mean difference 7.64 (95% CI of 0.12, 15.16)	
Voice quality (Voice handicap Index) N = 70 (TLM) N = 60 (RT)	No significant difference Mean difference (95% CI of -6.75, 19.58)	

Table 2: Comparison of outcomes between Transoral laser microsurgery versus Radiotherapy for early glottic cancer

Both treatments offer advantages and disadvantages. In addition to its lower cost, TLM provides more therapeutic options in the case of persistent or recurrent disease, leaving options open of revision laser surgery, adjuvant RT, open laryngeal surgery or total laryngectomy. However serious complications include airway fire, infection, bleeding, cutaneous fistula, emphysema and need for tracheostomy, although these

are rare [48]. In the case of anterior commissure involvement, TLM confers an additional risk of glottic webbing and scarring and radiotherapy may be a more viable option [49].

Radiotherapy offers the advantage of having outpatient treatment, is more widely available and is an option for patients with difficult exposure of the glottis. Disadvantages among many include oedema, glottic stenosis, hypothyroidism, mucositis and xerostomia [46].

Despite similar survival rates and local control, in the case of recurrent or persistent disease, surgery via open approach or laryngectomy remain the most viable options [50]. Salvage TLM for post radiation recurrence has been suggested as another option but remains technically challenging and is dependent on tumour size and location, patient factors and experience of the surgeon [51,52]. Advances in diagnostic technology such as intra-operative use of NBI is also contributing to increasing numbers of salvage TLM [52].

Treatment options for advanced glottic SCC (Stage III or stage IV disease) include surgery with or without adjuvant RT or RT +/- chemotherapy.

Treatment option of T3 tumours remain controversial owing to the heterogeneous nature of the disease [53]. T3 tumours can range from low volume tumours with invasion of vocalis muscle and fixed vocal cord to bulky transglottic tumours [37]. Traditionally, T3 tumours were treated with conventional total laryngectomy until two landmark trials - theVeterans Affairs[54] and Radiation Therapy Oncology Group (RTOG) 91-11 trial [55] demonstrated induction chemotherapy followed by radiation therapy and chemoradiotherapy to be viable non-surgical options. Evidence from cohort studies and case series suggest that there are no significant differences in the overall survival rate of T3 tumours between TLM, chemo-radiotherapy (CRT), RT alone and total laryngectomy [53]. However local control rate is worse with RT alone compared to total laryngectomy or partial laryngectomy [53].

The treatment options for T4 glottic tumours involve a total laryngectomy with or without post-operative RT or CRT [41]. Primary RT or CRT may be a viable option for patients not suitable for surgery, however local control rates are worse compared with total laryngectomy [56].

Prognosis

The 5-year survival for laryngeal cancer in Australia is 64.8% [2]. Glottic SCC has the highest 5-year survival rate at 74% followed by supraglottic SCC (47%) then subglottic SCC (30-50%) [57].

There are several clinical and molecular factors, which may affect survival. Clinically, the most important prognostic factors determining survival are the TNM stage and site of primary tumour [58]. A higher T stage, nodal involvement (N) and presence of metastasis (M) are all associated with poor prognosis [58]. Tumours with subglottic extension and/or transglottic location also have a poorer prognosis [58]. In T2 glottic tumours, patients with a fixed vocal cord have lower rates of local control and higher chance of recurrence following RT [34].
Furthermore the histologic grade of tumour, pattern of invasion (i.e. infiltrative) and perineural and/or vascular invasion are also associated with decreased survival requiring a more aggressive treatment approach [59,60]
In patients who are node positive, the presence of extranodal spread is also associated with increased recurrence and decreased survival [61].

The role of molecular factors and biomarkers to prognosticate disease remains an area of active research. EGFR overexpression and VEGF have been shown to portend a worse prognosis with decreased disease-free survival and overall survival [58]. The expression of p53 has not been demonstrated to be of prognostic value [58].

Conclusion

The treatment of laryngeal cancer is complex often requiring multimodal treatment. This is due to the intricate pathogenesis of the disease, which is incompletely understood, heterogeneous nature of the disease and lack of high-quality evidence to guide management. Given that the TNM stage is the most important prognostic factor with a potential for cure with appropriate treatment, early detection and diagnosis becomes crucial, particularly in the case of glottic cancers. With an increased understanding of the disease, vocal cord imaging has evolved from radiological imaging to high definition nasoendoscopy to newer biological imaging techniques used for early diagnosis, intra-operative resection and surveillance of disease. The next chapter will focus on reviewing currently imaging modalities of the larynx.

Chapter Two: Review of Vocal Cord Imaging

Overview

The diagnostic workup of laryngeal cancer involves a thorough history and examination, radiological evaluation and visualisation of the larynx.
Visualisation of the larynx has evolved from a laryngeal mirror examination to direct laryngoscopy in the operating theatre and in-office flexible nasoendoscopy.
Although white light endoscopy (WLE) is the most widely used optical diagnostic technique, newer modalities for vocal cord imaging have recently been developed including videostroboscopy, autofluorescence, optical coherence tomography, elastic scattering spectroscopy and narrowband imaging.

This chapter provides a literature review of the accuracy of current modalities used in the diagnostic workup of laryngeal cancer including emerging optical diagnostic techniques. These methods help diagnose and stage disease with an assessment of epithelial changes, laryngeal framework, and metabolic activity.

Assessment of Laryngeal Framework

Imaging is essential in the workup of laryngeal cancer to stage disease by determining size and site of tumour, nodal involvement and distant metastases [37].
Computed tomography (CT) and magnetic resonance imaging (MRI) are the two most common radiological investigations used [62].
The criteria used to assess for tumour involvement includes the presence of a focal mass, soft tissue thickening, invasion and infiltration of surrounding structures and/or abnormal contrast enhancement [63].

In many institutions, CT is the imaging of choice due to its fast acquisition times, higher spatial resolution and better evaluation of bony anatomy [62]. MRI is used in some cases as a complementary tool to assess for cartilage invasion, identify involvement of the laryngeal ventricles, transglottic spread or anterior commissure involvement [64]. However, the disadvantages are high acquisition times leading to motion artefact, higher cost and lower resolution compared to CT [62].
Despite the high resolution of CT, its role in detecting early laryngeal cancer is limited as it fails to detect small foci of mucosal tumours [63].

The main advantages of CT and MRI are to detect extra-laryngeal spread into the pre-epiglottic or para-epiglottic space, cartilage invasion and presence of metastatic nodal deposits [62,63,65]. Extra laryngeal spread in the pre-epiglottic space commonly occurs from supraglottic tumours arising from the epiglottis. Invasion into the para-glottic space is seen in supraglottic tumours arising from the false cord, laryngeal ventricle or aryepiglottic fold [62]. Glottic tumours involving the anterior commissure may also spread into the thyro-arytenoid muscle, contralateral cord or para-glottic space [62].

The sensitivity of both CT and MRI in detecting invasion in the pre-epiglottic space is 100% with specificity being 93% and 84-90% respectively [62].
In the case of spread into the para-glottic space, the sensitivity and specificity of CT and MRI are similar at 93-95% and 50-76% respectively [62]. This is largely due to the inflammatory changes associated with para-glottic space invasion, leading to a high false positive rate [62]. CT is also superior in detecting cervical node metastases compared to clinical examination [66] and MRI [63]. Using size criteria of more than 1cm or central necrosis, the sensitivity of CT in determining nodal metastasis is as high as 90% with a lower specificity of 73% [63].

The overall sensitivity of detecting early cartilage invasion using CT has been reported at 82% with a specificity of 79% [63]. Although MRI is more sensitive at detecting cartilage invasion (89-94%) compared to a CT scan, it is slightly less specific due to its failure to differentiate between infiltration and inflammation, oedema, or fibrosis [62]. This is particularly relevant in thyroid cartilage invasion (as opposed to cricoid cartilage invasion) with an even lower specificity of 56% [62].

Metabolic Activity

The use of nuclear imaging such as positron emission tomography (PET) is increasingly being used as part of staging disease. PET scans have a higher sensitivity of detecting laryngeal cancer compared to CT and MRI at the expense of lower spatial resolution and low specificity [63]. Combined PET-CT technique improves anatomic localization and is increasingly being used for staging and surveillance in many institutions. The main disadvantages of PET apart from low specificity and low spatial resolution are higher cost and lack of tracer uptake in some neoplasms [63]

Epithelial Diagnostic

Videostroboscopy

Videostroboscopy (VS) is an optical diagnostic tool used to examine vocal cord vibration and is commonly used in the assessment of dysphonia [67]. VS captures vocal cord movement in slow motion by synchronising flashes of light to vocal cord vibration and displaying a series of still images in a slow-motion video sequence [68,69]. Normal vocal cords display symmetrical mucosal waves and complete glottic closure. Abnormalities in vocal cord vibration may be due to scarring, polyps, vocal overuse, inflammation or early glottic cancer [69]. These abnormalities include impaired mucosal wave and amplitude resulting from invasion of the vocal ligament and the intermediate layer of the lamina propria by glottic SCC [64].

A systematic review and meta-analysis of 307 patients demonstrated an overall sensitivity of 96% and a specificity of 65% [68]. The forest plot of individual studies revealed wide variations between studies with sensitivities ranging from 86-100% and specificity ranging from 7-93% [68]. With high sensitivities, VS may be a reliable test to rule out disease in the presence of normal mucosal waves. However, reduced or absent waves may necessitate more diagnostic procedures and often cause patient anxiety. Recently the use of high-speed imaging, which captures at least 2000 frames per second to detect very early glottic cancer has been shown to have high sensitivities (100%) and higher specificity (82%) [70]. However, further studies are required to establish its role.

VS is an important adjunct to WLE in the workup of laryngeal cancer and remains the gold standard method to assess impact of disease, treatment and rehabilitation on voice.

Autofluorescence Endoscopy

Autofluorescence endoscopy (AFE) displays the natural fluorescence of tissues when they are exposed to light of a particular wavelength [64]. Normal mucosa has high levels of fluorophores such as riboflavin that are in an oxidized state [71,72]. Light with narrow wavelengths (ranging from 442nm to 520nm), activate these tissue fluorophores,

which is captured by an image-intensified camera through the endoscope, processed by the system and displayed on a video monitor [73].

As normal mucosa emits green autofluorescence, it will appear green-white where precancerous or cancerous lesions appear dark. This is because pre-malignant and malignant lesions, which have low fluorophores, emit weakly and therefore appear darker than the surrounding normal tissue[73] (Figure 1).

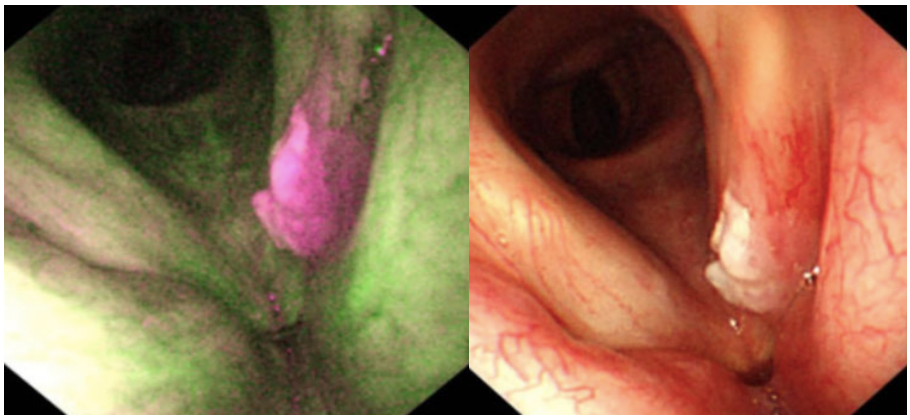

Figure 1: Comparison of White light endoscopy and autofluorescence imaging displaying left vocal cord lesion. Image adapted from Ni et al [73].

Several studies comparing AFE with indirect flexible WLE and direct microlaryngoscopy have reported AFE to have higher sensitivity in detecting premalignant or malignant glottic lesions [72,74,75]. However, similar to videostroboscopy, the specificity of AFE varies across different studies ranging from 48.1% to 87% with conflicting results as to whether its specificity is superior or inferior to WLE [72,74,75].

In addition to its role in the diagnosis of laryngeal lesions, AFE is applied intraoperatively to accurately identify positive margins of resection leading to improved local control and disease-free survival [76].

AFE is limited by the presence of blood on mucosal surfaces, hyperaemia from chronic inflammation or previous irradiation and hyperkeratosis [72]. In these situations, the contrast between normal and abnormal tissue is lost.

Optical Coherence Tomography

Optical coherence tomography (OCT) is a powerful imaging device that provides a cross-sectional image of laryngeal microstructure with 10μm resolution [74].

OCT uses infrared light reflected from normal tissue and is able to differentiate between epithelium, basement membrane and superficial lamina propria based on signal amplitude and depth penetration. This backscatter of infrared signals then generates an image similar to a vertical histologic section [75] (Figure 2). By providing real time in vivo images of laryngeal microstructure, OCT may detect very early changes in the mucosal layer of the vocal cord from both benign and malignant conditions [74].

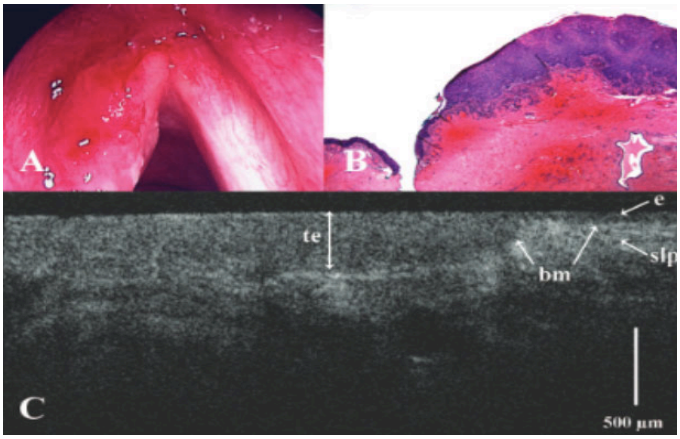

Figure 2: Comparison of A) white light endoscopy during direct laryngoscopy, B) histological section and C) optical coherence tomography. Bm = basement membrane; e = epithelium; te = thickened epithelium; SLP = superficial lamina propria. Adapted from Armstrong et al [75].

OCT is routinely used in ophthalmology to detect retinal or macular changes and has been used intra-operatively during rigid laryngoscopy to guide accurate biopsies by identifying basement membrane disruption as well as a transition zone for the tumour [74]. However, in a study of 26 patients with laryngeal cancer who underwent OCT studies, an abnormal microstructure was detected only in 18 studies (69%) during intraoperative laryngeal examination [75].

OCT is limited by a depth penetration of 1.2mm, which precludes assessment of the basement membrane in bulky exophytic tumours. This also applies in papillomatous disease, hyperkeratosis, leukoplakia, granulation tissue or ulcerated lesions where differentiation between different layers become difficult [75]. Furthermore, to generate accurate images for assessment of the microstructure, a learning curve in both probe

manipulation, positioning and interpretation of the images are required [75]. Given that the probe is required to be in close proximity or even in contact with the vocal cords, performing this on an awake patient is difficult.

Previous feasibility studies have demonstrated OCT to be possible in an awake patient following design modifications, however this is still an area of active research [76].

Although OCT seems to be a promising modality, its role is limited to assessment of flat mucosal cancers and intra-operative assessment.

Flexible Nasoendoscopy – While light endoscopy

Indirect white light endoscopy (WLE) is a routine part of laryngeal examination and is a critical component in the diagnosis and surveillance of laryngeal cancer [37]. Commonly, a flexible nasoendoscope with WLE is introduced trans-nasally to assess the nasal cavity down to the larynx. The fibreoptic technology and charge coupled-device generates a colour image with high resolution, which can assess early mucosal changes not detected on radiological imaging [77] (Figure 3).

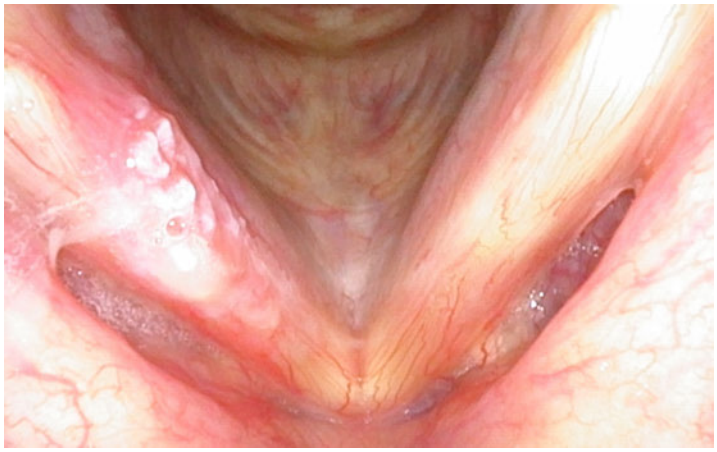

Figure 3: White light endoscopy demonstrating right vocal cord Cis

These endoscopes may also be attached to a monitor to record the examination and many commercial endoscopes are now compatible with new model processors with advanced imaging features and high-definition capability.

However, despite the high-resolution images, the sensitivity of WLE in the detection of early laryngeal cancer has been reported at 81% with a specificity of 92% [78]. This

is based on studies comparing lesions detected with WLE alone and WLE with another optical modality (e.g. WLE + narrowband imaging), against histology. By observing mucosal changes, WLE may also detect pre-cancerous or benign laryngeal lesions whereby patients may then proceed to have a direct laryngoscopy and biopsy. However due to the heterogeneity of changes observed, low sensitivity of WLE and lack of established criteria to define malignant changes, most lesions biopsied are benign on histology. In a review of 2188 leukoplakias biopsied, Isenberg and colleagues [79] reported that 53% demonstrated no dysplasia, 33% demonstrated moderate dysplasia and 15.2% demonstrated severe dysplasia.

Although flexible nasoendoscopy with WLE is a routine part of laryngeal examination and has been recommended in both workup and surveillance of laryngeal cancer [80], its relatively low sensitivity may fail to detect early neoplastic lesions necessitating the need for more accurate optical diagnostic modalities.

Narrowband Imaging and Ni Classification

Narrowband imaging (NBI) is an optical imaging modality that narrows the bandwidth of light to two wavelengths (415nm and 540nm) corresponding to the absorption peaks of haemoglobin [81]. The shorter wavelength (415nm), displayed as blue light penetrates superficial capillary networks, while the longer wavelength (540nm), displayed as green light penetrates the subepithelial vessels. When these wavelengths are combined, they provide a contrast enhanced image highlighting squamous mucosal microvasculature [30]. (Figure 4).

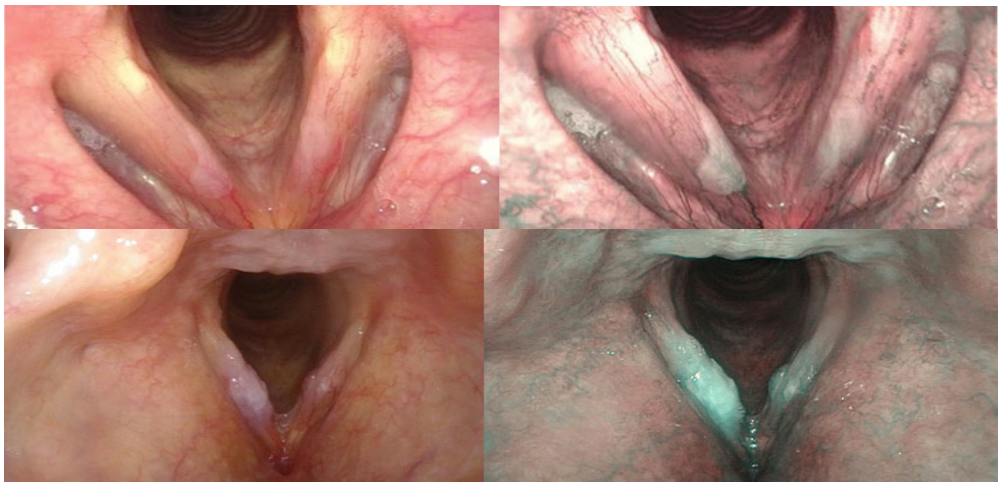

Figure 4: Comparison of white light endoscopy with narrowband imaging

By displaying a high resolution, contrast enhanced image of tissue microvasculature, subtle morphological changes can be detected raising suspicion for malignant or premalignant lesions.

Normal mucosal microvasculature appears as thin, arborescent vessels, running obliquely along the vocal cord. Changes in the microvasculature may be seen by identifying intraepithelial papillary capillary loops (IPCLs), which are small vessels that arise perpendicularly from smooth branching vessels in the squamous sub-epithelium [82]. Distinct changes in the morphology of the IPCLs are a hallmark of neo-angiogenesis, which can be observed under NBI to diagnose early SCC. By detecting and further classifying IPCL changes, NBI has been shown to be a sensitive test in the diagnosis of oesophageal, oral and oropharyngeal SCC, [82,83].

While NBI has been established in gastroenterology in the diagnosis of Barrett's oesophagus and colorectal tumours, its application in the head and neck region was first introduced in 2005 by Muto and colleagues [84] to detect synchronous cancers of head and neck during gastroscopy. Further studies have subsequently reported NBI having higher sensitivities compared to WLE in the diagnosis of laryngeal SCC [85-87]. Although changes in IPCL morphology may vary, the presence of "brown dots" using NBI has been reported to signify malignant change at the tissue surface [88]. These brown dots are believed to represent neoangiogenesis where vessels arise from deeper layers of the vocal cord in response to a carcinogenic stimulus [80].

Ni Classification

In 2011, Ni and colleagues [89] proposed a classification system for precancerous and cancerous lesions of the larynx based on laryngeal mucosal microvasculature and IPCL morphology observed under NBI. After assessing 104 laryngeal lesions, they classified IPCL morphology into 5 types [89] (Figure 5).

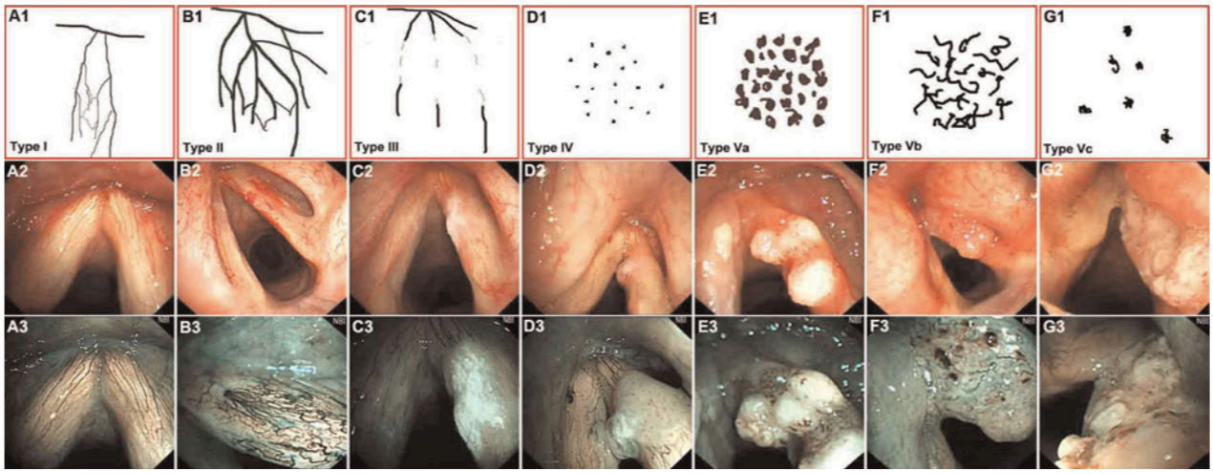

Figure 5. The Ni classification displaying laryngeal microvasculature and patterns of IPCL change. A1-3=Type I, B1-B3 =Type II, C1-C3=Type III, D1-D3=Type IV, E1-E3=Type Va, F1-F3=Type Vb and G1-G3=Type Vc. IPCL = Intraepithelial papillary capillary loops. Image from study by Ni et al [89].

In type I, the IPCLs are almost invisible and small diameter vessels can be seen at the surface. In type II, the IPCLs are still invisible but the vessels are slightly enlarged. Type III, the mucosa appears white and the surrounding vessels may or may not be seen depending on the thickness of the white patch. This is frequently seen with leukoplakia. In type IV, the IPCLs may be detected as small, scattered brown spots with a relatively regular arrangement.

Type V lesions are subdivided into Va, Vb and Vc depending on the distribution of IPCLs. In type Va the IPCLs are dilated with brownish, speckled features appearing in high density. In types Vb and Vc, the IPCLs are progressively destroyed with remnants appearing as thin branch like vessels (Vb) and the mucosal surface is replaced by necrotic tissue with irregularly scattered speckles on the mucosal surface (Vc).

In their series, Ni and colleagues reported types I-IV lesions to be benign, with type V representing malignant lesions [89].

Several studies have reported this classification system to be accurate in diagnosing laryngeal cancer with a sensitivity and specificity being reported at 94% and 89% respectively [78].

NBI has been found to be superior to most imaging modalities mentioned above. Piazza and colleagues [90] have reported NBI to be more sensitive than WLE leading to a diagnostic gain of 20%. In 2016, Ni and colleagues [73] found that although the sensitivities of NBI and autofluorescence endoscopy were similar, the specificity of NBI was significantly higher than both WLE and autofluorescence. Similar findings have been reported when NBI is compared to videostroboscopy (VS), demonstrating that although the sensitivity of detecting premalignant and malignant lesions were similar, NBI had a significantly higher specificity and positive predictive value compared to VS [91]. To date there are no studies comparing NBI with OCT, however NBI overcomes many of the limitations of OCT including broader ranges of application (e.g. office-based use, intra-operatively) and assessment of exophytic, ulcerated and papillomatous lesions.

NBI is increasingly being used worldwide with many imaging stacks now incorporating NBI or equivalent technology in their systems since 2017. This allows the examiner to assess a lesion with WLE and switch to NBI by pressing a button. The current applications of NBI include in office use in the workup of laryngeal lesions, intra-operatively in the resection of margins or to detect synchronous primaries and surveillance of disease recurrence.

The disadvantages of using NBI include presence of blood or mucus on the tissue, which may limit vision. Furthermore, a learning curve associated with interpretation of the images, which may lead to increased false positive results and lesions with a white patch such as leukoplakia or hyperkeratosis become difficult to interpret.

Given its high sensitivity, NBI using the Ni classification has been recommended by some authors to be used as an “optical biopsy” method preventing unnecessary biopsies under general anaesthesia [92,93]. However, this relies on the assumption that there is little variability between different observers, which remains an area of further research.
Furthermore, to date there are no Australian studies to assess the validity and reliability of NBI in the diagnosis of laryngeal cancer using the Ni classification.

Conclusion

In addition to conventional radiological investigations such as CT, MRI and PET scans, many novel optical diagnostic modalities have been developed to diagnose and treat early laryngeal cancer. NBI using the Ni classification is a promising modality with broad applications in the workup, management and surveillance of laryngeal cancer. The next chapter with discuss the aims and objectives of this thesis.

Chapter Three: Research Aims and Objectives

Aims

Current optical diagnostic modalities such as white light endoscopy lack the sensitivity to detect premalignant or early malignant lesions of the larynx. Narrowband imaging (NBI) is a promising modality used in the detection of pre-malignant and malignant lesions of the head and neck.

The main aim of this study is to examine the role of NBI in the diagnosis of laryngeal cancer and laryngeal leukoplakia. Furthermore, we aim to assess the accuracy of the Ni classification in predicting premalignant and malignant lesions of the larynx.

Objectives

There are three main objectives in this project to evaluate the role of NBI in different applications:

1. Determine the diagnostic accuracy of NBI using the Ni classification in the diagnosis of pre-malignant and malignant laryngeal lesions
    a. In this objective, we evaluate whether a Ni grade of V correlates with malignancy on histology and if there is a correlation between higher Ni grades and more advanced cancer. (Chapter 4)
2. Determine the reliability of NBI in differentiating between benign and malignant leukoplakia of the larynx
    a. Leukoplakia may obscure the tissue surface and an accurate pre-histologic assessment is difficult. This objective examines whether NBI can differentiate benign from malignant leukoplakia (Chapter 5)
3. Inter and Intra-Observer reliability of using NBI and the impact of operator experience
    a. Evaluate the role of NBI as an optical biopsy method. Although NBI using the Ni classification has been reported to be an accurate test for malignant lesions, it is still observer dependent with a learning curve. This objective evaluates the inter and intra-rater reliability of the Ni classification. It also assesses the impact of operator experience on interpreting NBI images (Chapter 6).

Chapter Four: The Value of Narrowband Imaging using the Ni Classification in the Diagnosis of Laryngeal Cancer (As Submitted)

The Value of Narrowband Imaging using the Ni Classification in the Diagnosis of Laryngeal Cancer

Short running title: Narrowband imaging using the Ni classification

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Narrowband Imaging, NBI, Laryngeal Cancer, Glottic cancer, Ni Classification,

Abstract

Introduction

Narrowband Imaging (NBI) is a special endoscopic optical enhancement setting allowing better visualisation of mucosal microvasculature compared to white light endoscopy. This contrast-enhanced image allows for better visualisation of laryngeal lesions compared to white light endoscopy. This study evaluates the validity of NBI using the Ni classification in the detection and differentiation of severe dysplasia (SD) and glottic squamous cell carcinoma (SCC).

Methods

Patients with suspicious vocal cord lesions underwent conventional white light endoscopy followed by clinically indicated biopsy. At the same time, NBI images were obtained and graded independently. Lesions were graded from I-V according to the Ni Classification and compared to histopathological findings.

Results

Fifty-two patients were included in this study (40 SCC and 12 SD). The sensitivity and specificity of NBI in diagnosing laryngeal cancer was 95.0% (CI, 83.9%-99.4%) and 83.3 % (CI, 51.6%-97.9%) respectively. The negative likelihood ratio was 0.06. Higher Ni grades correlated very strongly with more advanced disease based on a Spearman's correlation value of 0.83.

Conclusions

NBI using the Ni classification is a sensitive diagnostic tool for the detection and differentiation of early malignant and pre-malignant glottic lesions. As higher Ni classification correlates strongly with advanced disease, it serves as a useful modality in the diagnosis of laryngeal cancer.

Introduction

Laryngeal squamous cell carcinoma (SCC) is one of the most common types of head and neck cancer with a global incidence of over 170 000 patients per year, resulting in almost 95 000 deaths in 2018 [94]. The survival rate of laryngeal cancer has remained unchanged over the last 30 years despite advances in treatment [95,962].

Early detection, histopathological diagnosis and treatment significantly improve prognosis, reducing both patient morbidity and mortality [97].

Current workup for laryngeal cancer includes imaging such as computed tomography, magnetic resonance imaging and nasoendoscopy with white light.
However various studies have demonstrated that white light endoscopy (WLE) lacks the sensitivity to detect early, superficial neoplastic lesions (carcinoma in situ or T1 and T2) or pre-neoplastic lesions such as severe dysplasia [85,89,98]

Growth and progression of laryngeal SCC relies on neo-angiogenesis, a process where new blood vessels are formed from the surrounding pre-existing blood vessels. This “angiogenic switch” shifts the balance in favour of pro-angiogenic factors, allowing for the formation of new vessels to support the metabolic requirements of the tumour [99]. These new vessels lack the histological architecture and structural anatomy of native vessel and this difference in morphology can only be detected by WLE in later stages [86]. Earlier detection of this change plays a critical role in early diagnosis of laryngeal cancer leading to improved outcomes.

Narrowband imaging (NBI) is an optical imaging modality, which uses optical interference filters to spectrally narrow the bandwidth of light [30] The two wavelengths emitted from this filter include blue light (wavelength peak of 400-430nm) and green light (wavelength peak of 515-555 nm) corresponding to the absorption peaks of haemoglobin [17]. This enhances and better contrasts the mucosal microvasculature also known as intraepithelial papillary capillary loops (IPCL) [100,101]

By observing for changes in the morphology and architecture of IPCLs, NBI can play a key part in early detection of neoplasia and its role has been established in the detection of lesions in the oesophagus, pharynx and oral mucosa[83,102-104]

In 2011, Ni and colleagues [89] proposed a classification system based on the morphological changes of laryngeal IPCLs to differentiate between benign and malignant laryngeal lesions. Briefly, this system describes five patterns of change in the IPCL morphology. Types I-IV are usually associated with benign or even pre-neoplastic change. Type V is associated with neoplastic change and is further subdivided into Va, Vb and Vc [89] (Table 1). Previous studies have demonstrated this classification system to be more sensitive than WLE with many authors recommending NBI to be a routine part of assessment and workup for laryngeal lesions [78,80,105]

Classification	**Description**
Type I	Thin, oblique and arborescent vessels are interconnected and IPCLs are almost invisible
Type II	Diameter of oblique and arborescent vessels is enlarged, and IPCLs are almost invisible
Type III	IPCLs are obscured by white mucosa
Type IV	IPCLs can be recognised as small dots
Type Va	IPCLs appear as solid or hollow, with a brownish, speckled pattern with various shapes
Type Vb	IPCLs appear as irregular, tortuous, line-like shapes
Type Vc	IPCLs loops appear as brownish speckles or tortuous, line-like shapes with irregular distribution, scattered on the tumour surface

Table 1: Ni classification and corresponding description of IPCL morphology. Adapted from Ni et al [89]. IPCL = intraepithelial papillary capillary loops

The aim of this study is to evaluate the diagnostic accuracy of NBI using the Ni classification in detecting neoplastic or pre-neoplastic laryngeal lesions. Furthermore, we aim to assess if there is a correlation between higher Ni grades and tumour stage.

Methods

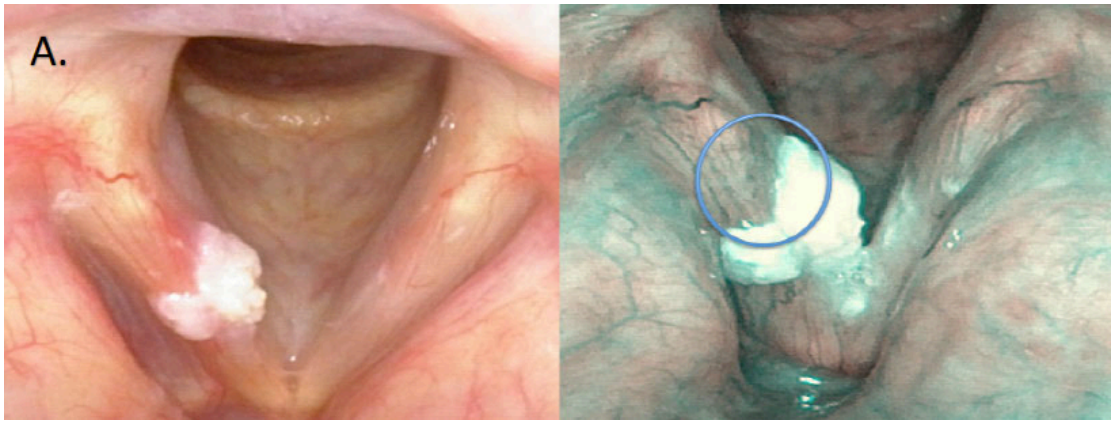
This study was approved by the institutional human research ethics committee (Ethics number 5424). From March 2018 to September 2019, patients presenting to the Head and Neck cancer clinic at Westmead Hospital (Sydney, Australia) were recruited if they had a clinically suspicious glottic lesion. These patients were referred to the tertiary head and neck clinic from general Otolaryngology clinics or by independent specialists. The reasons for referral were patients with chronic hoarseness or voice change with a lesion on the vocal cord identified either by imaging (computed tomography or magnetic resonance imaging) or WLE.

During the consultation, an in-office nasoendoscopy was performed. The nasal cavity was anaesthetised topically with Co-Phenylcaine (Lignocaine Hydrochloride 5%/Phenylephrine 0.5%) spray. Nasoendoscopy was performed with an ENF-VQ transnasal flexible fiberscope connected to an Evis Exera III CV 190 light source (Olympus Medical Systems, Tokyo, Japan) while patients were awake and seated. Any suspicious lesions of the vocal cords on WLE including leuko-erythroplakia, polypoid lesions, ulcerated lesions, exophytic and endophytic lesions were captured. The light was then switched to NBI mode where the lesions were assessed in real time and captured.

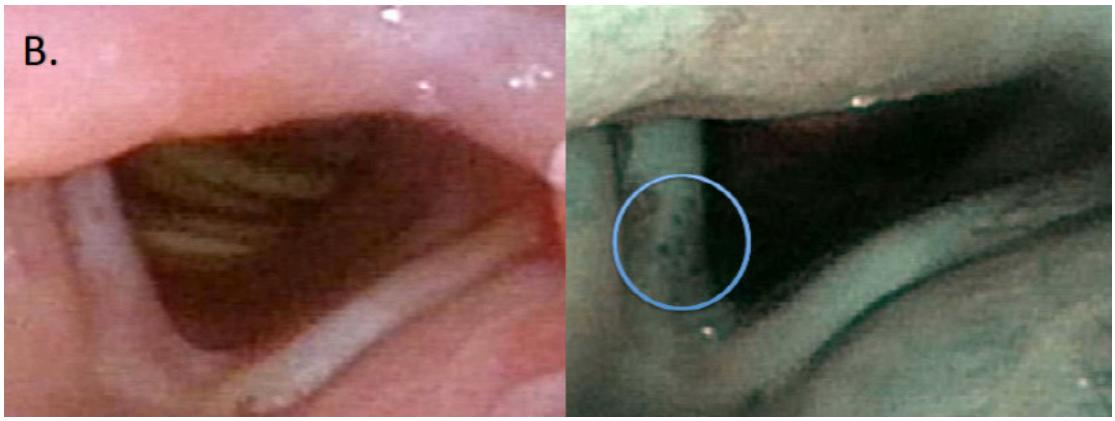
A clinically indicated biopsy was performed under general anaesthesia in the operating theatre. The biopsy specimens were fixed in formalin, embedded in paraffin and sent for histopathological examination. Slides were examined with haematoxylin and eosin staining by an experienced head and neck pathologist.
Lesions with a histopathological diagnosis of SCC were included and graded clinically based on tumour size as carcinoma in situ (Cis), T1, T1a, T1b, T2, T3 and T4 according to the American Joint Committee on Cancer grading of glottic cancer[31]. Lesions with severe dysplasia (SD) on histopathology were also included and considered pre-malignant. Following the histopathology results, the respective NBI images captured during in office nasoendoscopy were independently graded according to the Ni classification [89]. The lesions were graded by an experienced Otolaryngologist Head and Neck Surgeon (FR), who was blinded to the

histopathology. Any lesions considered neoplastic on NBI were graded V (including Va, Vb or Vc) (See Figure 1A-D). Premalignant lesions were graded I-IV.

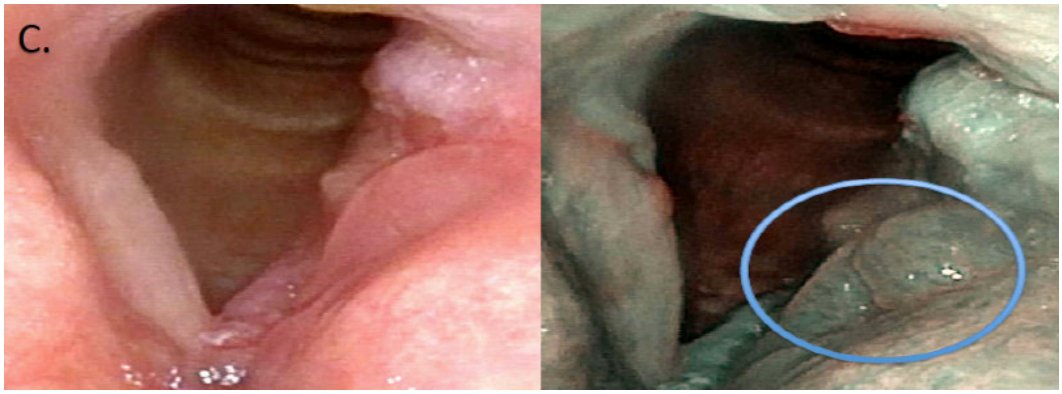
Figure 1. Comparison of WLE and NBI images with Ni classification IV-Vc. IPCL = intraepithelial papillary capillary loops; SD = Severe dysplasia; WLE = white light endoscopy; NBI = Narrowband imaging



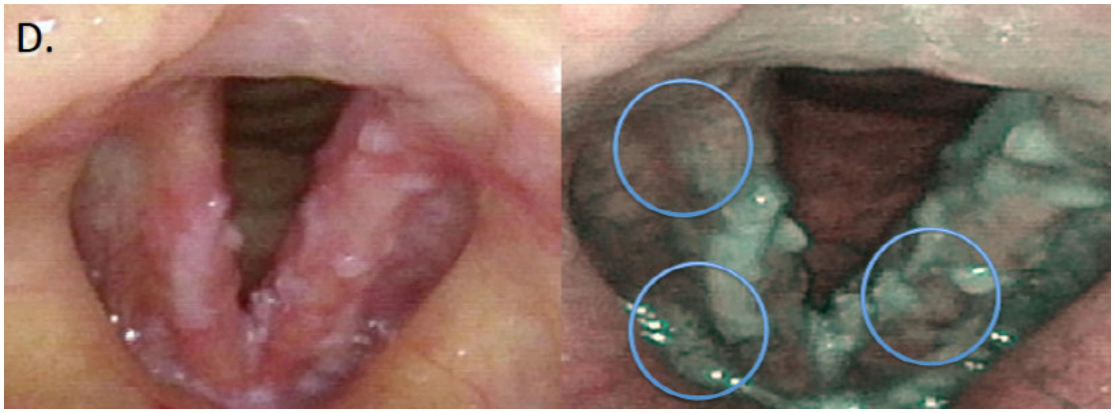
A) Type IV pattern; IPCL recognised as small dots as marked. Histopathology *demonstrated SD*



B) Type Va pattern; IPCL appear as solid brownish dots scattered on right vocal cord as marked. Histopathology demonstrated carcinoma in situ



C) Type Vb pattern; IPCL appear as irregular, line-like shapes as marked. Histopathology demonstrated T1a.



D) Type Vc pattern; IPCL appear as scattered brownish speckles on multiple sites. Histopathology demonstrated T1b.

Histopathological grades were then compared to their respective Ni classification. Based on this, the sensitivity and specificity of NBI using the Ni classification in detecting glottic cancers were calculated. Correlations between the Ni classification and grade of tumour were also measured.

Patients were excluded if they had an adverse reaction/allergy to co-phenylcaine, had previous laryngeal cancer, or were unable to tolerate nasoendoscopy.

The SPSS 26.0 statistical software package (SPSS, Inc., Chicago, IL, USA) was used for statistical analysis. Data was found to be non-parametric by Shapiro-Wilk test and

histogram analysis. Sensitivity, specificity, positive predictive value and negative predictive values were calculated using a two-by-two cross tabulation table. Correlation between the Ni classification and tumour grade was calculated using the Spearman's Rank-Order correlation test. A p-value <0.05 was considered statistically significant.

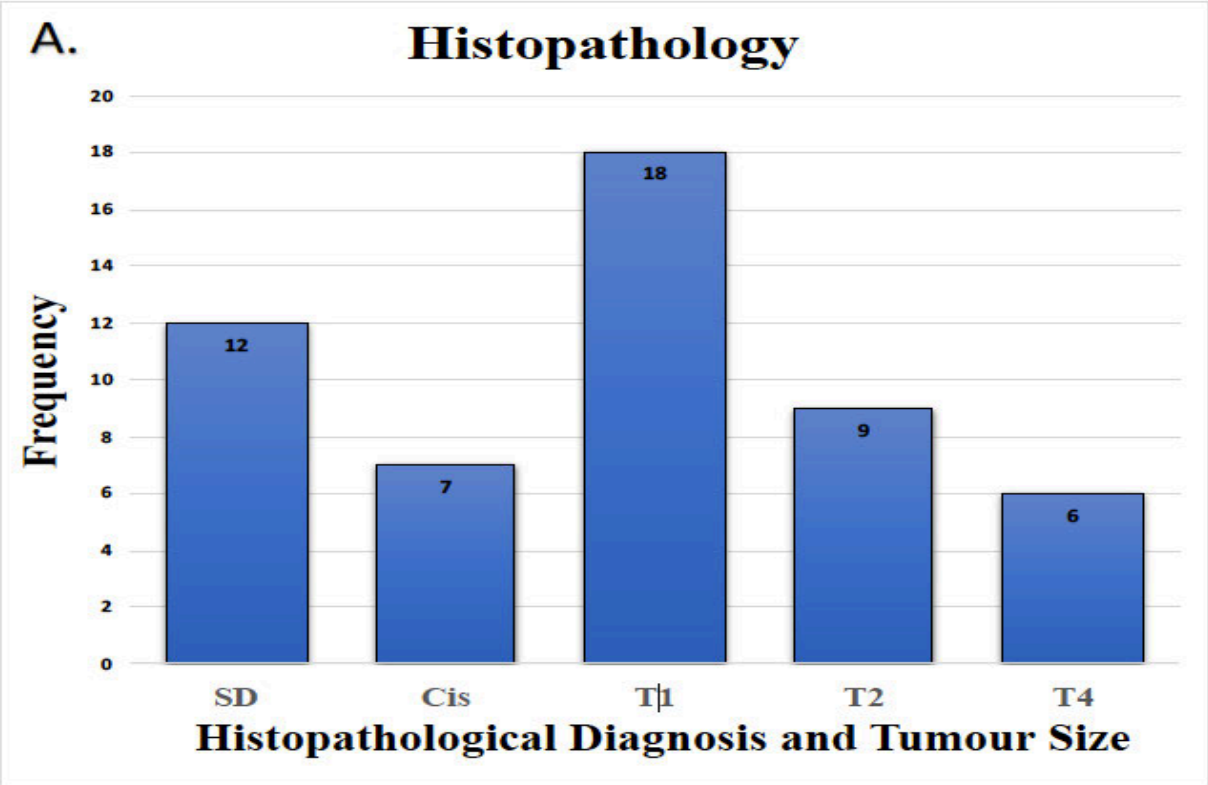
Results

From March 2018 to September 2019, fifty-two patients were included in this study with a male to female ratio of 3.5:1. The mean age was 67 (42-86).
All patients were able to tolerate an in-office nasoendoscopy for WLE and NBI assessment.

On histopathological examination 23.1% (n=12), 13.5% (n=7), 34.6% (n=18) and 17.3% (n=9) of patients were diagnosed with SD, Cis, T1 and T2 respectively (Figure 2a). Six patients who were biopsy proven SCC were staged T4 after CT examination demonstrated thyroid cartilage invasion.

Based on the Ni classification, 23.1% (n=12) of lesions were graded type IV while 77.0% (n=40) of patients were graded type V. Of the type V lesions, 32.7% (n=17) were graded Va, 32.7% (n=17) were graded Vb and 11.5% (n=6) were graded Vc (Figure 2b).

The sensitivity and specificity of NBI in diagnosing laryngeal cancer was 95.00% (CI, 83.94%-99.43%) and 83.31 % (CI, 51.65%-97.92%) respectively. The negative predictive value and the positive predictive values were 82.95% and 95.69% respectively (**Table 2**). Higher Ni grades correlated very strongly with more advanced disease as demonstrated by the Spearman's Rank-Order correlation value of 0.83.



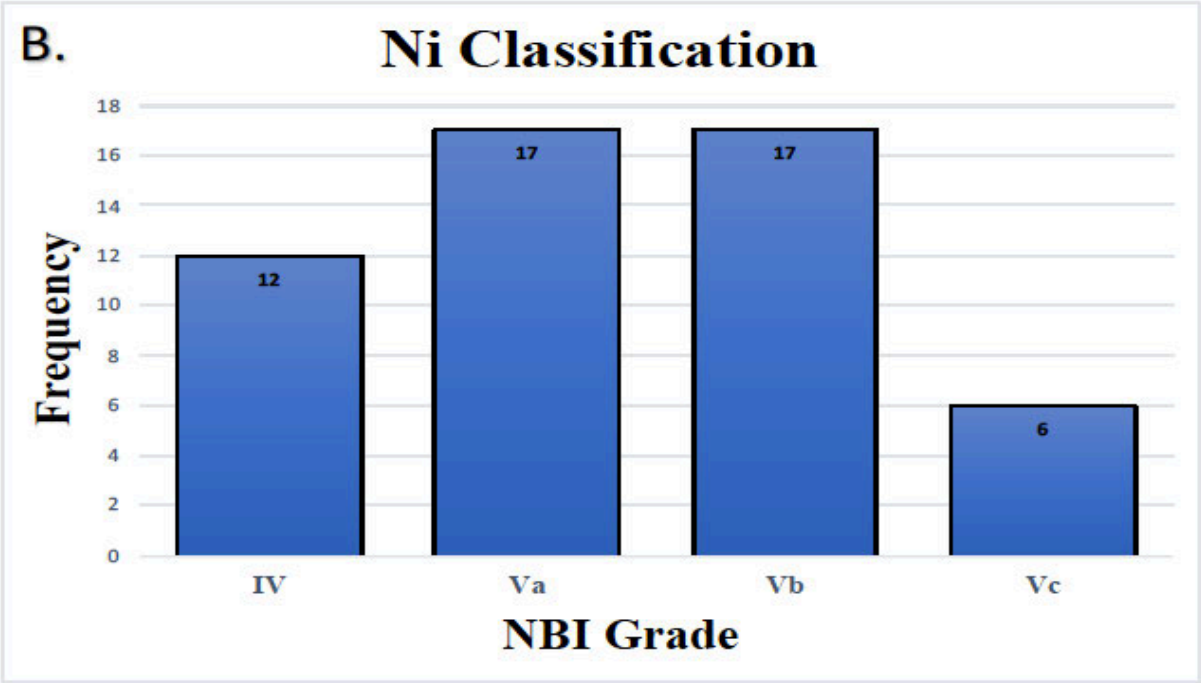


Figure 2: Distribution of lesions based on a) Tumour size following histopathological diagnosis b) NBI grade using the Ni Classification [89].

NBI = Narrowband Imaging; SD = Severe dysplasia; Cis = Carcinoma in situ

	Value	95% Confidence Interval
Sensitivity	95.00%	83.94% to 99.43%
Specificity	83.31%	51.65% to 97.92%
PPV	95.69%	85.99% to 98.77%
NPV	82.95%	55.38% to 95.02%
PLR	5.70	1.61 to 20.24
NLR	0.06	0.02 to 0.24

Table 2: Diagnostic Accuracy of Narrowband Imaging using the Ni Classification[89] NBI=narrow band imaging; PPV=positive predictive value; NPV=negative predictive value; PLR=positive likelihood ratio; NLR=negative likelihood ratio

There were two false negative results in this study where both patients were graded as type IV on NBI but were diagnosed as Cis and T2 on histopathology (Figure 2). Both patients had previous severe dysplasia in that site. In the two false positive results, both patients were graded as type Va but histopathology demonstrated severe dysplasia.

Discussion

This study demonstrates narrowband imaging to be a sensitive diagnostic tool in the work up and management of glottic cancer. The high sensitivity of 95.0% is comparable to sensitivities reported in many previous studies [85-87,89,106].

In 2017, Sun and colleagues [78] published a systematic review and meta-analysis assessing the diagnostic value of NBI in laryngeal cancer demonstrating a pooled sensitivity of 94%. Furthermore, they demonstrated a high negative likelihood ratio of 0.08 using a likelihood ratio scattergram analysis, similar to the negative likelihood ratio of 0.06 demonstrated in this study. A limitation of their systematic review and meta-analysis however, was the low number of included studies.

Several other studies that did not meet their inclusion criteria have reported a much lower sensitivity of NBI in detecting laryngeal cancer. Piazza and colleagues [90], assessed 279 patients with laryngeal cancer and reported a sensitivity of only 61% using flexible NBI alone. Although the sensitivity increased substantially to 98% when NBI was coupled with HDTV, most office based flexible video endoscopes do not have the capability of HDTV integration at the time of this writing. In a separate study, Shoffel-Havakuk and colleagues [107] compared NBI with WLE in diagnosing laryngeal cancer and reported a sensitivity of 58.62% using NBI. However, it is important to note that the sensitivities reported in this study were based on three experienced assessments of still NBI and WLE images, not histopathological diagnosis. Recently, Hosono and colleagues [108] further reported a sensitivity of 84.4% using NBI even with high definition magnifying endoscopy. Although this is a higher sensitivity relative to Piazza et al [90] and Shoffel-Havakuk et al[109], it is much lower than the pooled sensitivity reported by Sun et al [78]. The reasons for the low sensitivities remain unclear but may be due to regional or institutional differences in patient referral or presentation, quality of image processing and/or method of assessment.

More recent studies however, have demonstrated higher sensitivities of >90% when using NBI in laryngeal cancer. Yang and colleagues [110] reported a sensitivity of 91.2% in 23 malignant lesions. Sakthivel and colleagues [106] reported a sensitivity of 100% when using NBI with WLE. Rzepakowska and colleagues [91] demonstrated a sensitivity of 98.8% when using NBI in diagnosing precancerous and malignant laryngeal lesions.

The high sensitivities and low negative likelihood ratio in this study suggests that NBI is a reliable tool in differentiating between benign and malignant laryngeal lesions. By contrasting and enhancing the mucosal microvasculature, early changes in IPCL morphology can be detected, particularly when integrated with HDTV. This is evident in this study as 46/52 lesions were either pre-neoplastic change or early stage cancer. This can significantly affect patient outcomes especially since the local control with trans-oral laser microsurgery for early stage laryngeal cancer can be up to 85-100% [80,111]. Furthermore, the use of intraoperative NBI allows for greater precision of

resection margins greatly reducing second looks and their associated complications and costs.

Another finding in this study was the very strong correlation between Ni classification and primary size of tumour as suggested by a Spearman's rank-order correlation value of 0.83. Types Va and Vb accounted for almost all of the Cis-T2 lesions while all of the lesions that were T4 were graded Vc. Most severe dysplasia was graded as type IV and no lesions received a grade of I-III.
This is similar to previous studies suggesting type V on the Ni classification is strongly suggestive of neoplasia. In 2018, Mehlum and colleagues [105] reported that Ni type IV and V had a high diagnostic accuracy for detecting laryngeal cancer with a sensitivity of 89% and specificity of 82%. Rzepakowska and colleagues[91] compared laryngovideostroboscopy (LVS) and NBI in malignant laryngeal pathologies and demonstrated that LVS was sensitive (97.6%) in detecting malignant laryngeal pathologies. The LVS findings correlated strongly with NBI findings using the Ni classification with a spearman correlation value of 0.54.

In type IV and V of the Ni classification, the IPCLs begin to lose the oblique and longitudinal vascular pattern seen in types I-III and appear as “brown dots”[89]. These brown dots change morphology and become progressively fainter as the IPCLs are destroyed suggesting progression of the neoplasm. Many authors have also reported the appearance of “brown dots or spots” using NBI as a sign of neoplasia and could reflect perpendicular branching of new vessels seen in growing tumours[84,102,112].
Recently the European Laryngological Society proposed nomenclature dividing superficial vascular changes into longitudinal and perpendicular lesions. While longitudinal vessels may be benign, perpendicular changes of IPCL (including enlarged vessel loops, dot like vessels and worm like vessels) represent pre-cancerous and cancerous lesions [80,113].

Given the ability of NBI to detect early change in IPCL morphology using the Ni classification, many authors have suggested using NBI not only as an adjunct for the workup of laryngeal cancer but also for surveillance of disease, as an “optical biopsy” to prevent unnecessary biopsies requiring general anaesthesia and intra-operatively to

assist in resection margins [93,114,115] The findings of this study suggest NBI is a useful visualisation adjunct to white light endoscopy and clinical examination in monitoring known disease. However, despite the sensitivity of 95%, the confidence interval of 83.94% to 99.43% suggests that further studies are required to validate NBI before it can be recommended as an "optical biopsy".

This study is limited be the relatively small number of patients who were included. Although patients were recruited from a major tertiary head and neck clinic, the small number is reflective of a single institution study.

In this study only severe dysplasia, Cis and early laryngeal cancer were included, which may affect the specificity rate and introduce referral bias. This is due to the tertiary clinic patients were recruited from, which is a dedicated head and neck cancer clinic. In this clinic, only lesions that appeared suspicious on WLE were referred, allowing for assessment using an endoscope with NBI capabilities. Lesions that appeared benign or were biopsied under general anaesthesia from the general otolaryngology clinics were assessed using an endoscope without NBI capabilities and were thus excluded.

Although all patients tolerated nasoendoscopy well, a thorough assessment is needed for complete visualisation of the lesion for a reliable assessment. This becomes difficult if the lesion is obscured by mucus, blood or excessive saliva. Furthermore lesions with leukoplakia can become difficult to assess owing the "umbrella effect" obscuring adequate assessment of underlying IPCL changes[116].

Although some authors have mentioned the extra costs associated with purchasing the NBI filter, most video-endoscopy machines are fitted with NBI filters since 2017.

Many authors have also suggested a learning curve exists before reliable assessment can be made when using NBI [106,117,118]. Vilaseca and colleagues [119] suggested a learning curve of 200 examinations using NBI based on analysis of 480 patients. In this study, they analysed 480 biopsy proven cancers in the head and neck region with WLE and NBI and subdivided the sample in groups of 100 patients according to the experience of the examiner. They demonstrated that accuracy of detecting malignant lesions using NBI increases with experience and, plateaus after approximately 200 cases [119].

During this learning curve there may be an increased rate of false positive findings on NBI particularly in areas with active inflammation, mucositis, recurrent respiratory papillomatosis or previous radiotherapy [81,113]. However NBI has been shown to differentiate between post radiotherapy changes and neoplasm and is even recommended as a routine part of surveillance for cancer [120,121].

Further prospective studies are required to validate the role of NBI in surveillance of laryngeal cancer and its intraoperative use to assess suspicious lesions. Although the Ni classification has been demonstrated to be reliable in detecting laryngeal cancer, it is still operator dependent. More studies addressing the role of inter and intra-observer reliability in the detection of laryngeal cancer are needed to validate its role in both surveillance and use as an "optical biopsy" technique.

Conclusion

This study demonstrates NBI using the Ni classification to be a sensitive diagnostic tool in the detection of early malignant and pre-malignant glottic lesions. Given its high sensitivity and low negative likelihood ratio, it is recommended to be a routine part of work up for laryngeal cancer.

Chapter 5: The Validity of Narrowband Imaging in the Diagnosis of Laryngeal Leukoplakia: A Systematic Review and Meta-Analysis (As Submitted)

The Validity of Narrowband Imaging in the Diagnosis of Laryngeal Leukoplakia: A Systematic Review and Meta-Analysis

Short running title: Narrowband imaging in laryngeal leukoplakia

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Narrowband Imaging, NBI, Laryngeal Cancer, leukoplakia, Ni Classification,

Abstract

Introduction

Narrowband imaging has been validated to be an accurate imaging modality in the workup of laryngeal cancer. However, assessment of laryngeal leukoplakia is a limiting factor due to the white patch obscuring microvascular changes at the mucosa. This systematic review and meta-analysis evaluate the validity of NBI in differentiating between low-risk leukoplakia and high-risk leukoplakia.

Methods

A systematic review was performed using Medline, EMBASE, Scopus and Cochrane Database of Systematic Reviews (DSR) and Database of Abstracts of Reviews of Effects (DARE). Studies were screened and selected according to the Preferred Reporting Items for Systematic Reviews and Meta-Analysis statement. Studies evaluating the diagnostic accuracy of NBI in the assessment of laryngeal leukoplakia were included. Benign lesions, mild and moderate dysplasia were considered low-risk leukoplakia. Severe dysplasia, carcinoma and invasive cancer was considered high-risk leukoplakia. Pooled sensitivity, specificity and diagnostic odds ratio were calculated.

Results

Seven studies met the inclusion criteria assessing a total of 572 lesions with laryngeal leukoplakia. Six studies were eligible for meta-analysis using NBI in the workup of laryngeal leukoplakia with a pooled sensitivity and specificity of 85.4% (95% CI 76%-99.9%) and 94.9% (95% CI 91.1%-97.2%) respectively. Pooled diagnostic odds ratio was 99.2 (95% CI 38.28-257.18).

Conclusion

NBI is a useful imaging modality in differentiating between low-risk leukoplakia and high-risk laryngeal leukoplakia. The high sensitivity, specificity and diagnostic odds ratio makes NBI a useful tool in the workup of laryngeal leukoplakia. Further higher-powered studies are needed to establish precise NBI criterion and guide clinical recommendations.

Introduction

Leukoplakia is the presence of a white patch on mucosal tissues indicating increased keratin deposition on epithelial surfaces [79]. Histopathologically, leukoplakia can correlate to either normal epithelium, keratosis, hyperplasia, dysplasia or malignancy [79]. Leukoplakia involving the larynx remains a diagnostic challenge due to the low pre-histologic accuracy of conventional white light endoscopy in differentiating between benign, pre-malignant or malignant lesions [122]. Consequently, the diagnosis is confirmed histologically after a biopsy has been obtained under general anaesthesia.

Early diagnosis and management of pre-malignant or malignant laryngeal lesions are essential for prognosis and survival. However, survival of glottic cancer has remained unchanged in many countries [2,96]. Recently the World Health Organisation (WHO) updated the histological classification of laryngeal pre-malignant lesions to a simplified three tier system including low grade dysplasia, high grade dysplasia and carcinoma [35].

In addition to a simplified histological classification system, various other modalities have been developed to improve the pre-histologic diagnostic accuracy of laryngeal leukoplakia including autofluorescence, optical coherence tomography and contact endoscopy [39]. However, these are limited by the presence of leukoplakia, which lead to increased false negative rates [64].

Narrowband imaging (NBI) is optical imaging modality, which has been validated to have high sensitivity and specificity in diagnosing pre-malignant and early malignant laryngeal lesions [78]. This technology uses optical interference filters to spectrally narrow the bandwidth of light to two wavelengths, which preferentially penetrate the superficial and deep layers of the laryngeal mucosa and enhance the mucosal microvasculature [30]. This allows early detection of pre-malignant or malignant lesions by detecting changes in the mucosal microvasculature also known as intraepithelial papillary capillary loops (IPCLs) [30] (Figure 1).

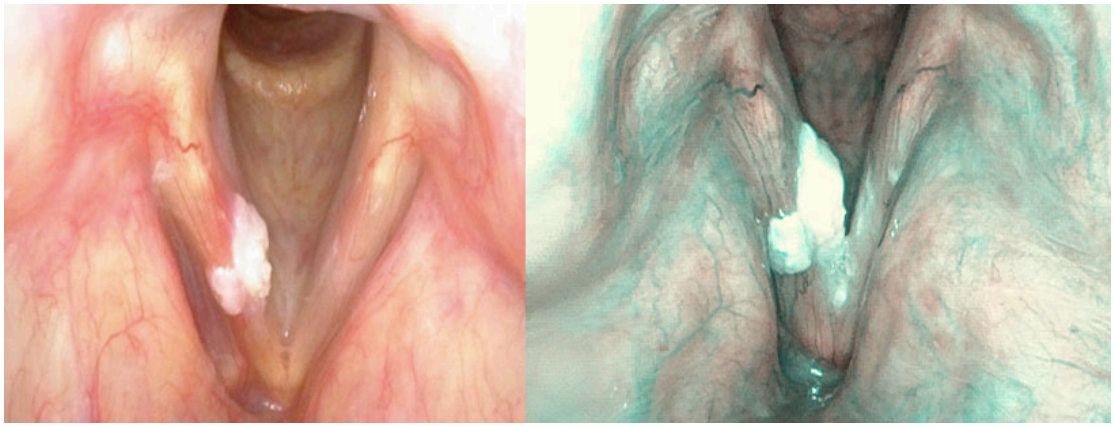


Figure 1: Comparison between WLE and NBI in assessing laryngeal leukoplakia

In 2011, Ni and colleagues [89] proposed a classification system based on the morphological changes of laryngeal IPCLs to differentiate between benign, pre-malignant and malignant laryngeal lesions. The "Ni 2011" classification has been shown to have high sensitivity in detecting microvascular mucosal changes and predicting glottic cancer [105]. The European Laryngological Society (ELS) also proposed a simplified two-tier classification system based on the presence of IPCLs on laryngeal mucosa [80]. Briefly, they divided superficial vascular changes into longitudinal and perpendicular changes depending on the direction of vessel changes in relation to the antero-posterior axis of the vocal cord. Longitudinal vascular changes are vessels running parallel to the mucosal surface, either along the longitudinal axis of the vocal fold or in the transverse direction and are more likely to be benign. Perpendicular changes are seen when vessels from deeper aspects of mucosa extend in a perpendicular direction toward the mucosal surface and are more likely to represent malignancy, pre-malignancy or recurrent respiratory papillomatosis [80].

One limitation of NBI using both the Ni classification and the ELS classification, is the presence of leukoplakia and hyperkeratosis which can obscure the mucosal surface and preclude adequate assessment of the IPCLs [81,85,87,106].
However, various authors have demonstrated that NBI is still able to differentiate benign from malignant laryngeal leukoplakia with high accuracy by observing for IPCL changes at the peripheries of leukoplakia [93]. Rzepakowska and colleagues

assessed 91 laryngeal lesions with leukoplakia and reported high sensitivities and specificities of 100% and 97.4% respectively [123]. Huang and colleagues [116] also demonstrated that NBI can differentiate between benign and malignant laryngeal leukoplakia, however the accuracy reported was much lower.

The aim of this systematic review is to assess the ability of NBI to differentiate between low risk and high-risk laryngeal leukoplakia as defined by the authors of this study. Low risk is defined by a histological diagnosis of normal epithelium, simple hyperplasia, mild dysplasia and moderate dysplasia. High risk is defined by a histological diagnosis of severe/or high-grade dysplasia, carcinoma (Cis) and squamous cell carcinoma (SCC).

Where possible, we aimed to evaluate the pooled diagnostic accuracy of NBI, including sensitivity (Se), specificity (Sp), positive predictive value (PPV), negative predictive values (NPV) and diagnostic odds ratio (DOR).

Materials and Methods

Eligibility Criteria

The inclusion criteria for this systematic review were: 1) studies describing the diagnostic accuracy (including Se, Sp, PPV, NPV or DOR) of NBI using IPCL changes in patients with leukoplakia of the larynx seen on WLE; 2) studies where the true positive (TP), true negative (TN), false positive (FP) and false negative (FN) could be extracted in a 2x2 table. 3) studies where the histopathological results could be extracted and used as the gold standard comparator 4) studies involving adults >18years with no language restrictions and no limit on historical range and 5) studies where full text articles could be reviewed.

The exclusion criteria were: 1) studies including patients with previous laryngeal cancer or previous radiotherapy to the larynx; 2) case reports, conference abstracts, reviews, theses; 3) studies where data on diagnostic accuracy of NBI cannot be compared to histopathology and 4) studies assessing advanced laryngeal cancer only including T3 and T4.

Search Strategy

A literature search was performed on Medline, EMBASE, Scopus and Cochrane Database of Systematic Reviews (DSR) and Database of Abstracts of Reviews of Effects (DARE) on 15 December 2019. The search strategy was formulated using the PICOS (population, intervention, comparison, outcome and study design) format. The population targeted were patients with benign, pre-malignant or malignant laryngeal lesions who had leukoplakia on WLE. Detection of abnormal IPCL using either the Ni classification or the ELS classification was the intervention with the histopathological diagnosis as the gold standard comparator.

The outcomes measured were sensitivity, specificity, positive and negative predictive values, positive and negative likelihood ratio and diagnostic odds ratio of NBI. A comprehensive search strategy for each database is provided in Appendix 1.

Study Selection

Studies were selected according to the Preferred Reporting Items for Systematic Reviews and Meta-Analysis (PRISMA) statement [124]. Combined results from the databases were screened by keyword, title, and abstract after duplicates were removed. Articles for full text review were then selected and the bibliographies were searched for additional articles meeting the eligibility criteria. Two authors (SA, KV) independently screened and appraised all articles for eligibility and disagreements were resolved by a senior author (FR).

Data Extraction

Data was extracted independently by two reviewers (SA, KV) according to a pre-defined data extraction table. Details of each study extracted were author names, publication year, country of origin, study type, number of patients enrolled, lesion assessment criteria under WLE, results of NBI and classification used (Ni 2011, Ni 2019 or ELS), histopathology grade used (WHO 2005 or WHO 2017), parameters of accuracy (Se, Sp, PPV, NPV, TP, FP, TN, FN) and level of evidence according to the NHMRC guidelines[125]. Repeat studies derived from the same datasets were excluded and discrepancies were resolved by FR.

In studies using the Ni 2011 classification on NBI, grades I-IV were considered benign or low risk and grades Va, Vb and Vc were considered malignant or high risk

according the original description by Ni and colleagues [89]. Perpendicular changes or presence of "brown spots" or "brown dots" scattered at the peripheries of leukoplakia or within the white patch were considered malignant if the ELS classification was used. One study by Ni and colleagues[122] assessed IPCL changes based on a new classification system designed for laryngeal leukoplakia. In this classification system, IPCLs underlying the leukoplakia and at the peripheries were classified into 6 different types (Table 1). Types I-III were considered benign and type IV-VI were considered malignant.

Histologically, lesions were considered low risk if they were diagnosed as normal epithelium, hyperkeratosis, inflammation, mild dysplasia or moderate dysplasia. High grade dysplasia/severe dysplasia, carcinoma (Cis) or squamous cell carcinoma were considered high risk.

Risk of bias assessment

Risk of bias was assessed using the Quality Assessment tool for Diagnostic Accuracy Studies (QUADAS-2) [126]. This tool comprises 4 domains including patient selection, index test, reference standard, and flow and timing. Risk of bias in each section is ranked as high risk, low risk or unclear. Two authors (SA, KV) independently completed QUADAS-2. QUADAS-2 assessment was performed using RevMan (Review Manager (RevMan) [Computer program]. Version 5.3. Copenhagen: The Nordic Cochrane Centre, The Cochrane Collaboration, 2014.)

Data analysis

A bivariate meta-analysis using a random effects model was used to calculate summarized pooled estimates of Se, Sp, and a diagnostic odds ratio (OR), using tissue histopathology as the gold standard comparison. A hierarchical summary receiver-operating characteristics curve was plotted, with summary point and 95% confidence intervals represented and the area under the curve calculated.
Statistical analysis was performed using R version 3.6.2 (St. Louis, Missouri, USA) and Stata version 16.1 (StataCorp LLC, Texas).

CLASSIFICATION	Type	Description
Ni 2011	**Type I**	IPCLs are almost invisible, and small diameter oblique and arborescent vessels can be seen.
	Type II	IPCLs are also almost invisible, with enlarged oblique and arborescent vessels
	Type III	Mucosa is white and IPCLs cannot be seen; the oblique and arborescent vessels are obscured but may be seen indistinctly if white patch is thin
	Type IV	Mucosal IPCLs are visible with a relatively regular arrangement and low density. Capillary terminals are bifurcated or slightly dilated, and IPCLs appear as scattered, small, dark brown spots; the oblique and arborescent vessels are usually not visible.
	Type V	Va - IPCLs are significantly dilated and of relatively high density, and appear to be solid or to have hollow, brownish, speckled features and various shapes. Vb - IPCL itself is destroyed, with its remnants presenting in a snake-, earthworm-, tadpole- or branch-like shape, and the vessels are dilated, elongated and 'woven' in appearance. Vc, - Lesion surface is covered with necrotic tissue, and the IPCLs present as brownish speckles or tortuous shapes of uneven density which are irregularly scattered on the tumour surface.
ELS	**Longitudinal**	Enlarged and/or ectatic, local blood vessels which may appear dilated and tortuous. Longitudinal vascular change becomes visible further branching of vessels and anastomoses between them. Vascular changes in the transverse direction can be observed as a singular ''indicator'', ''finger'' or ''feeding'' vessel
	Perpendicular	IPCLs are visible with symmetrical arranged dot-like loops representing the narrow-angled turning point and tip of the loop under the still translucent epithelium arising from deeper layers of the vocal folds. In the carcinogenic process, vascular loops become more spiralling
NI 2019	**Type I**	There are no IPCLs, but white plaque can be observed on the vocal cord, with obliquely running vessels and branching vessels indistinctly present under the white plaque.
	Type II	There are white patches on the vocal cord, but neither IPCLs nor obliquely running vessels or branching vessels can be found.
	Type III	IPCLs can be seen at the surface of the vocal cord mucosa where the epithelium is not covered by the leukoplakia, showing small brown spots with a relatively regular arrangement and without clear boundaries. No obliquely running vessels or branching vessel can be observed.
	Type IV	IPCLs can be observed on the vocal cord, showing large brown spots and embedded at the surface of white plaque.
	Type V	IPCLs on the vocal cord can be seen, showing large brown spots, which appear at the surface of the vocal cord mucosa outside the leukoplakia with obvious boundaries.
	Type VI	IPCLs are visible at the surface of the vocal cord, characterized by large brown spots or twisted earthworm-like vessels distributed at the surface of the leukoplakia and also at the sur- face of the vocal cord epithelium outside the leukoplakia.

Table 1: Ni 2011, European Laryngological Society & Ni 2019 Classification systems

Adapted from Ni & Colleagues 2011[11], Arens & Colleagues[13] & Ni and Colleagues 2019 [2]

Results

Study Selection

The combined search strategy returned a total of 587 studies. Figure 2 illustrates the study selection process. Seven studies were identified following bibliographic screening during the full text review process and a total of 535 articles were screened (Figure 2). Sixteen studies underwent full text review with 7 studies meeting the inclusion criteria for data extraction. Five studies were in the English language and 2 studies were translated to English from Traditional Chinese [127,128].

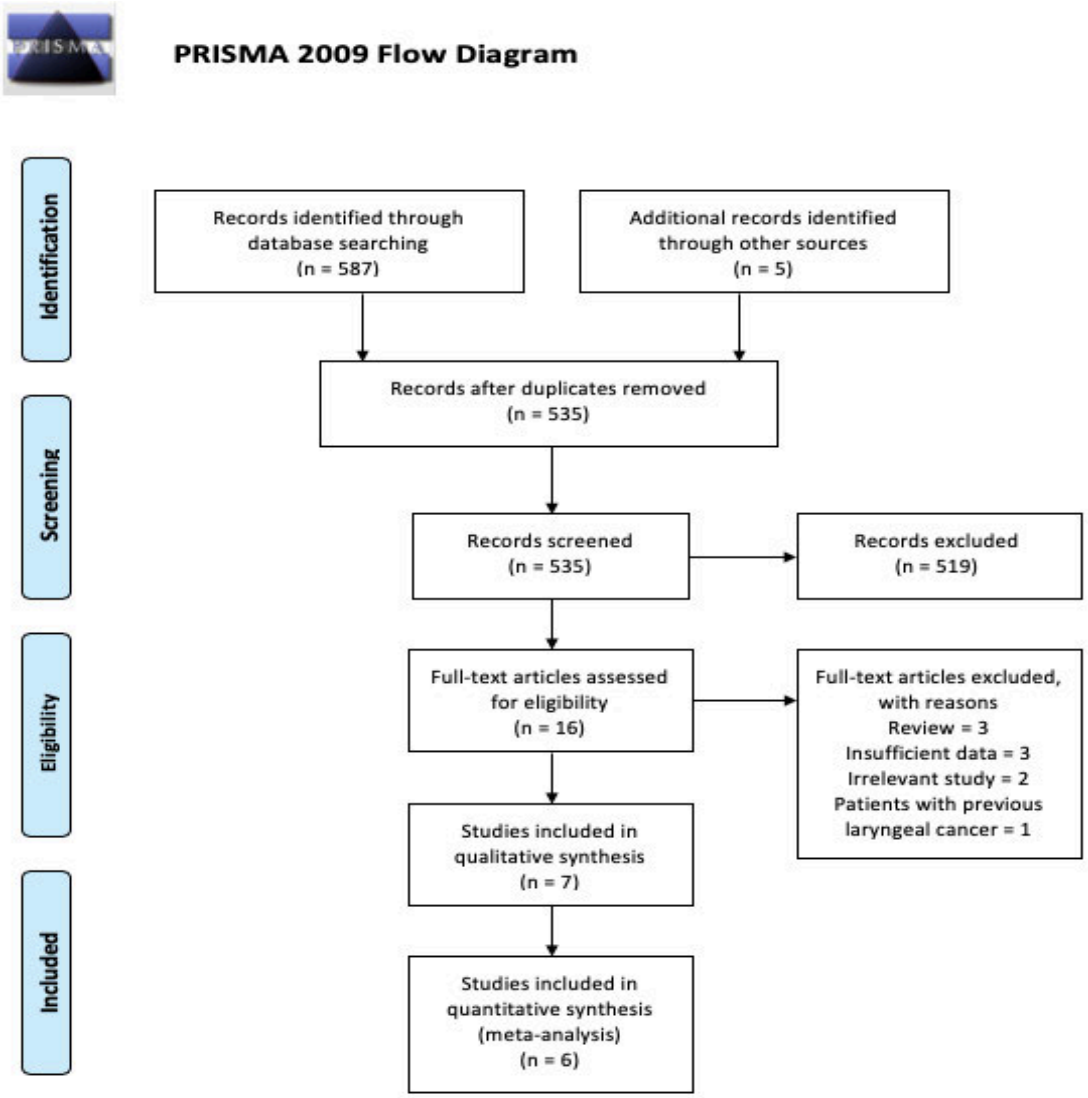


Figure 2: PRISMA flow diagram of literature search results

Study Characteristics

Four studies were prospective cohort studies. In three studies [116,122,128], it was not clarified whether the cohort study design was prospective or retrospective.

A total of 474 patients with 586 lesions were included in the systematic review with a pooled male to female ratio of 6.8:1. There were 398 benign lesions and 188 malignant lesions assessed in total. Four studies classified IPCL changes according the Ni 2011 classification alone [123,127-129]. Two studies classified IPCL changes according to both Ni 2011 and ELS classification system [93,116]}. One study by Ni and colleagues [122]assessed IPCL changes based on a new classification system (Ni 2019) designed for laryngeal leukoplakia as described above. A summary of study characteristics is provided in Table 2**.**

Table 2: Summary of demographics of included studies

Study	Country	Number of Participants	Number of lesions	Benign:Malignant Lesions	Classification	WHO classification
Guo 2018	China	62	84	63:21	Ni 2011	NR
Huang 2017	China	57	78	49:29	Ni 2011 and ELS	NR
Klimza 2017	Poland	41	47	36:11	Ni 2011	2005
Ni 2019	China	100	120	97:23	Ni 2019	2017
Rzepakowska 2018	Poland	62	91	77:14	Ni 2011	NR
Stanikova 2017	Czech Republic	63	63	37:26	Ni 2011 and ELS	NR
Zhu 2019	China	89	103	39:64	Ni 2011	2005

Description of Studies

A summary of included studies is provided in Table 3. The study by Guo and colleagues examined 62 patients with 84 lesions of vocal cord leukoplakia. Lesions under NBI were classified according to the Ni 2011 classification. Following raw data extraction, the Se and Sp of NBI was 95.24% and 92.06% respectively.

STUDY	Sensitivity %	Specificity %	DOR	PPV %	NPV %	PLR	NLR	TP:FP	TN:FN
Guo 2018	95.24	92.06	232	80	98.31	12	0.05	20:5	58:1
Huang 2017	86.21	97.96	300	96.15	92.31	42.24	0.14	25:1	48:4
Klimza 2017	63.64	100	121.67	100	90	99.99	0.36	7:0	36:4
Ni 2019	82.6	92.8	61.07	73.1	95.7	11.45	0.19	19:7	90:4
Rzepakowska 2018	100	97.4	875.8	87.5	100	38.5	0	14:2	75:0
Stanikova 2017	78.57	88.57	28.42	84.62	83.78	6.87	0.24	22:4	31:6
Zhu 2019	54.69	89.74	10.56	89.74	54.69	5.33	0.50	35:4	35:29

Table 3: Summary of Included Studies

Huang and colleagues [116] examined 57 patients with 78 lesions detected on WLE. Lesions on NBI were classified according to the Ni 2011 classification and ELS classification of IPCL change. The Se and Sp of NBI following data extraction were 86.21% and 97.96% respectively. The diagnostic accuracy for detecting dysplastic or malignant lesions were higher than detecting benign leukoplakia (95.56% vs. 69.70% respectively).

Klimza and colleagues [129] prospectively reviewed 41 patients with 47 lesions of laryngeal leukoplakia. IPCL changes were determined according to the Ni 2011 classification. In this study, types I and II were considered benign, type IV was considered suspicious and type V and above was considered malignant. Lesions considered suspicious or malignant underwent more aggressive full thickness biopsies at the site of leukoplakia. After raw data extraction and application of eligibility criteria in this systematic review, the Se and Sp was determined at 63.6% and 100% respectively.

Ni and colleagues [122] recruited 100 patients with 120 lesions diagnosed with leukoplakia using WLE. Patients with obvious benign lesions (e.g. polyp, Reinke's oedema, papilloma) or malignant lesions (e.g. ulcerative or cauliflower-like tumours) were excluded. Lesions were classified according to the Ni 2019 classification of IPCL morphology at the mucosal surface as described above (Table 1). This was the only study in this review to compare the diagnostic accuracy of WLE with NBI against the histopathology. The Se and Sp of WLE were 60.9% and 72.2%

respectively. The Se and Sp of NBI in comparison was 82.6% and 92.8% respectively. Although the Se of NBI was higher than WLE, this difference was not statistically significant.

Rzepakowska and colleagues [123] prospectively analysed 62 patients with 91 lesions of vocal cord leukoplakia. Patients were enrolled if they had a vocal cord leukoplakia and were undergoing microsurgery. Preoperative NBI images were obtained and graded according the Ni 2011 classification. Lesions graded I, II and IV were considered benign and lesions graded as Va, Vb and Vc were considered malignant. Following data extraction, the Se and Sp of NBI in detecting malignant lesions was 100% and 97.4% respectively.

Stanikova and colleagues [93] assessed 63 patients with vocal cord leukoplakia detected during WLE. Patients presented with hoarseness or chronic laryngitis lasting more than 3 weeks. NBI images were obtained simultaneously during WLE examination and lesions classified according to Ni 2011 classification and ELS classification of perpendicular vascular changes. The authors considered hyperkeratosis, mild/moderate and severe dysplasia as benign and Cis and SCC as malignant and reported a Se and Sp of 88% and 89.5% respectively. However, using the criteria applied to this systematic review, the Se and Sp after raw extraction were 78.6% and 88.6% respectively.

Zhu and colleagues [128] assessed 89 patients with103 laryngeal leukoplakia. Lesions in NBI were classified according to the Ni 2011 classification and divided into benign, suspicious and malignant groups. Lesions were considered benign if the mucosa surrounding the white plaque appeared normal and accompanied by Ni type III and/or IV and suspicious for malignancy if the mucosa surrounding the white plaque was rough, irregular and erythematous with IPCL III and/or IV. Lesions were malignant if the surrounding mucosa was accompanied by type Va, Vb or Vc ICPLs. The pre-operative Ni classification determined the type of resection, with benign lesions receiving submucosal vocal cord resection, suspicious lesions receiving subvocal ligament resection and malignant groups receiving resection of leukoplakia including the vocalis muscle. For benign and suspicious lesions, a frozen section was sent and if

cancerous lesions were suspected, more extensive resection was performed at the same time or staged for a later time. Although there was a significant correlation between NBI classification and malignant degree of classification (Spearman correlation = 0.725), the Se and Sp were 42% and 89.74% following raw data extraction and application of eligibility criteria used in this review.

Risk of Bias Assessment

Of all the included studies, 2 studies met all 7 items [93,123]. Among the other 5 studies [116,122,127-129], it was unclear whether pathologists were blinded to the NBI findings and the IPCL classifications. In all studies the NBI assessment was performed before biopsy and histopathological assessment followed afterwards. All studies clearly specified pre-defined IPCL classification, adhering to the Ni 2011, Ni 2019 classification or the ELS classification. In the study by Klimza and colleagues [129], despite the consecutive sampling of patients with leukoplakia, no patients were diagnosed with Cis or invasive cancer. Figure 3 outlines the quality assessment based on the QUADAS-2.

Meta-Analysis

Six studies were included in the meta-analysis. There was considerable heterogeneity when all studies were included (Higgins $I^2 = 79.7\%$, $p < .001$). Following exclusion of study by Zhu and colleagues [130], Higgins' I^2 was 27.8% (Cochrane Q (5) = 6.92, $p = 0.227$) indicating that there was weak heterogeneity.

A meta-analysis of the studies demonstrated a summary Se of NBI of 85.4% (95% CI 76%-99.9%) and a summary Sp of 94.9% (95% CI 91.1%-97.2%) (Figure 5 and Figure 4A & 4B).

Summary diagnostic odds ratio was 99.2 (95% CI 38.28-257.18) (Figure 4C). Hierarchical receiver operating characteristic curve plotting sensitivity against specificity is demonstrated an AUC of 0.94.

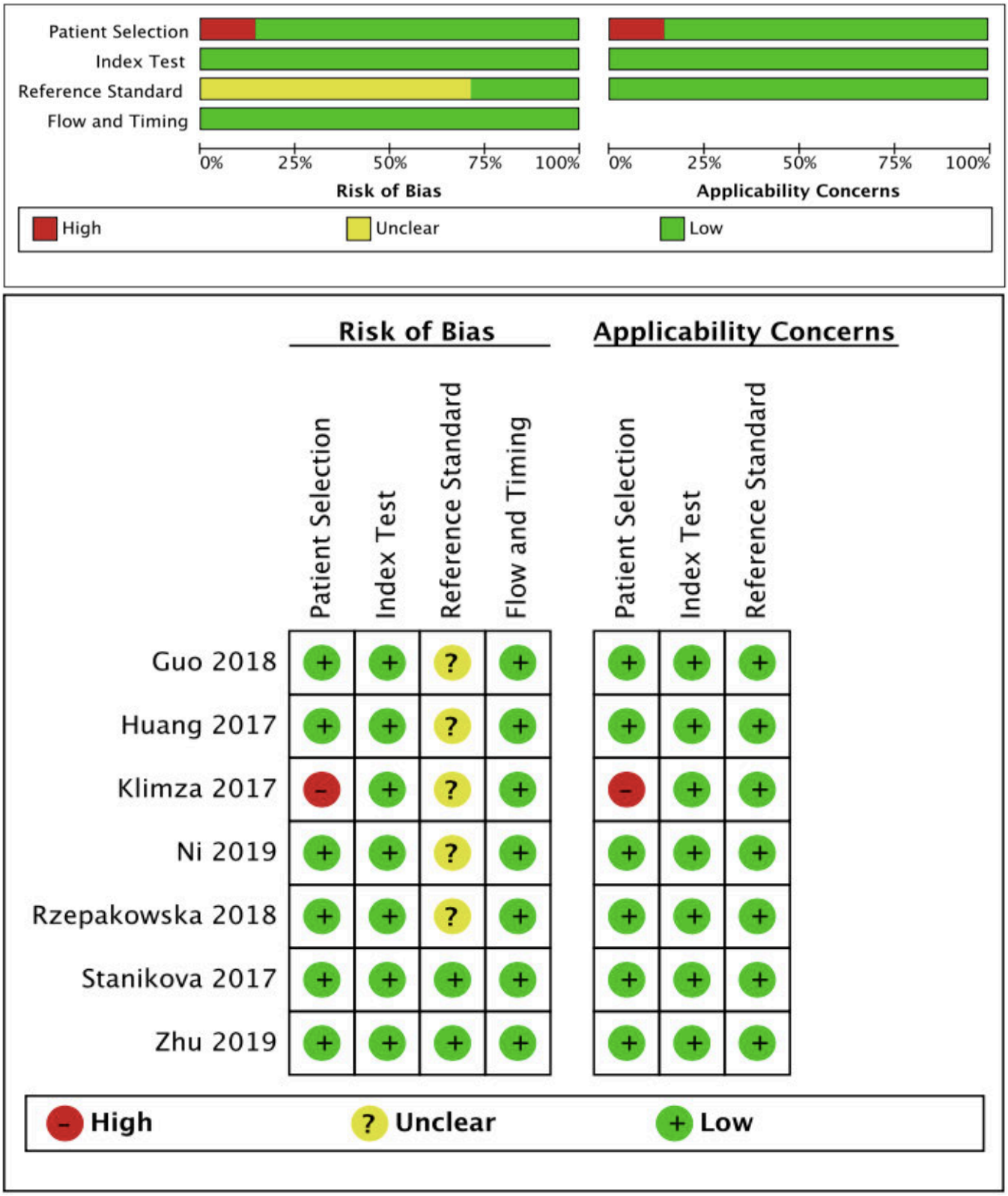
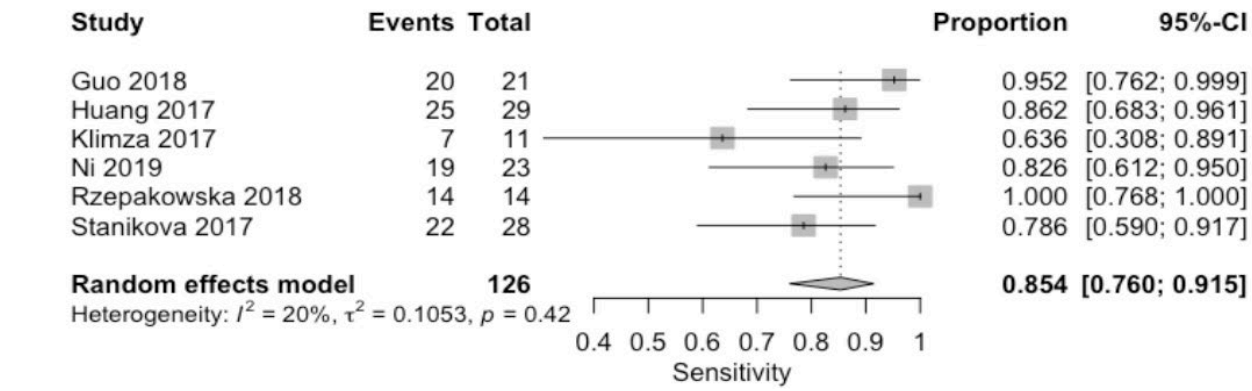
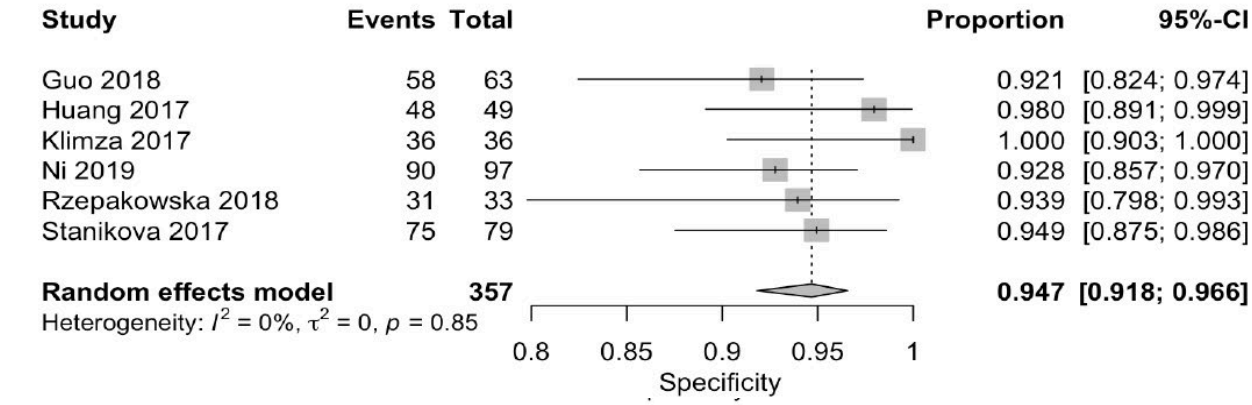


Figure 3: Quality assessment according to the Quality Assessment of Diagnostic Accuracy Studies–2

A)



B)



C)

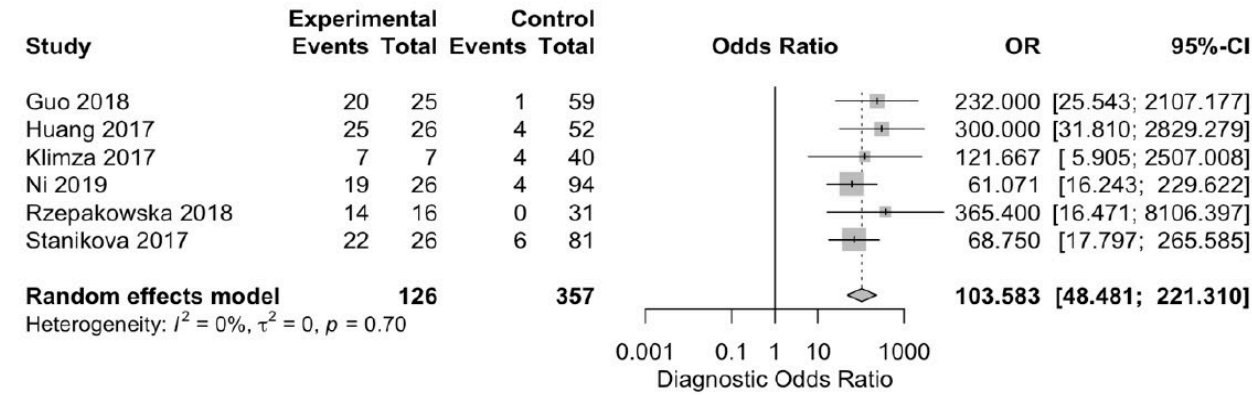


Figure 4) Forest plots of a) sensitivities b) specificities and c) diagnostic odds ratio of individual studies.

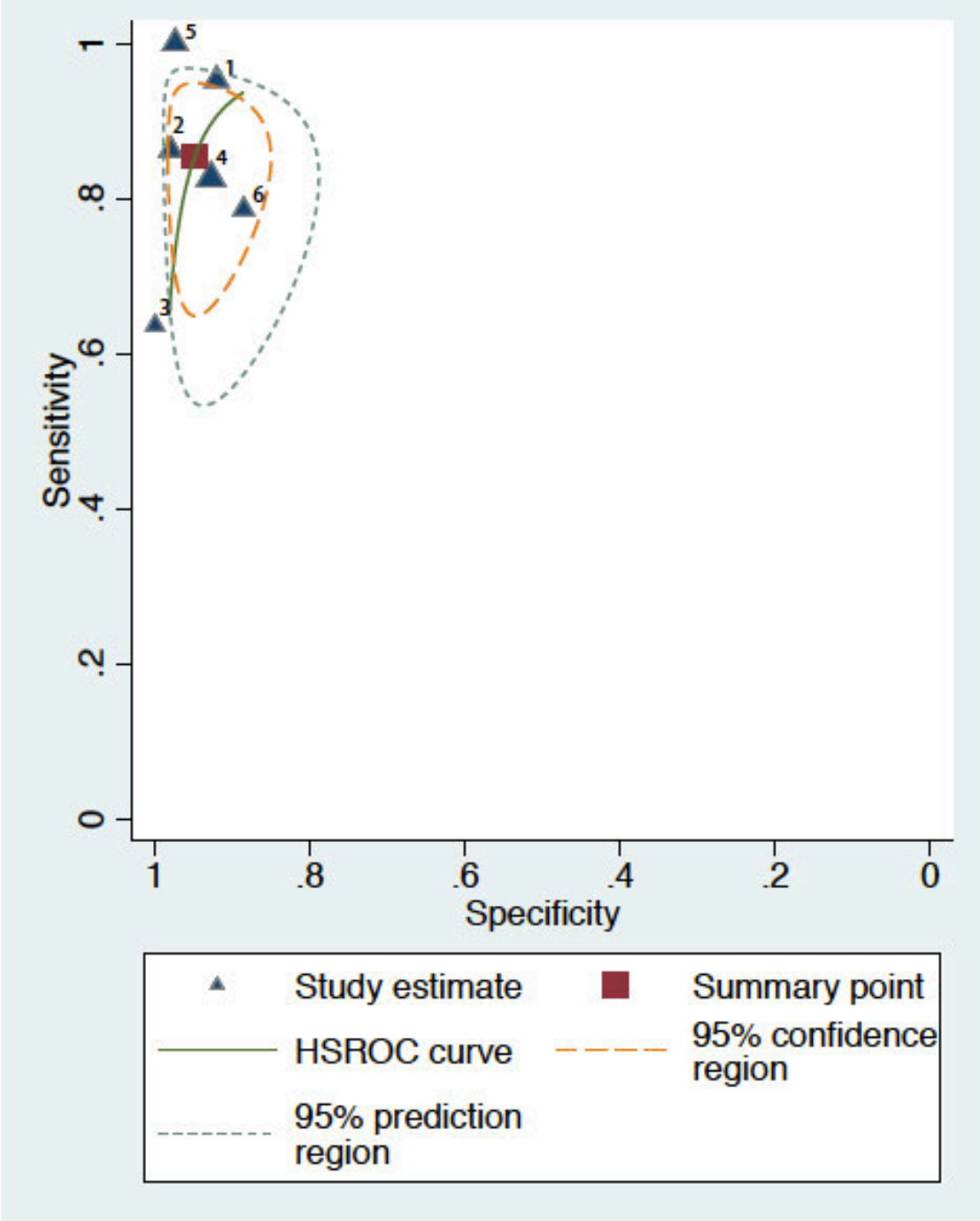


Figure 5. Hierarchical receiver operating characteristic curve (HSROC) using data from 6 studies assessing diagnostic utility of narrow band imaging in laryngeal leukoplakia. 1: Guo 2018; 2: Huang 2017; 3: Klimza 2017; 4: Ni 2019; 5: Rzepakowska 2018; 6: Stanikova 2017

Discussion

This systematic review analysed the diagnostic accuracy of NBI in differentiating between low risk and high-risk laryngeal leukoplakia. The diagnostic value of NBI in detecting pre-malignant or early malignant laryngeal lesions overall has been previously established [78]. However, the presence of leukoplakia was a limiting factor in many studies due to the "white patch" precluding adequate assessment of IPCLs to establish a pre-histological diagnosis [81,86,87,90,101].

The studies included in this systematic review demonstrate that NBI is a reliable optical imaging modality in the assessment of laryngeal leukoplakia with a high summary Se of 85.4%, summary Sp of 94.9% and a diagnostic odds ratio of 99.21.

Guo and colleagues [127] compared the accuracy of NBI using the Ni classification in differentiating between grades of dysplasia and carcinoma . They demonstrated that leukoplakia with higher Ni grades (i.e. type IV and V) correlated positively with the type and degree of pathological differentiation. Raw data extracted from their study also demonstrated a high Se and Sp of differentiating between low risk and high-risk laryngeal leukoplakia (Table 3).

Huang and colleagues [116] reported similar correlations between Ni grade and degree of pathological differentiation. The overall Se was Sp were also similar at 86.21% and 97.96% respectively.

The study by Klimza and colleagues [129] divided patients with vocal cord leukoplakia into "normal" and "suspicious" groups depending on the pre-histological Ni classification. Ni grades IV and V correlated strongly to moderate and severe dysplasia. However, in their cohort despite the consecutive sampling of patients with leukoplakia, no patients were diagnosed with Cis or invasive cancer, which may introduce bias in their sample.

Ni and colleagues [122] examined the greatest number of lesions and were the only study to compare the accuracy of NBI with WLE. Under WLE, two experienced endoscopists, who were blinded to the histopathology determined if lesions were benign or suspect for malignancy based on the appearance of the leukoplakia. Leukoplakia appearing as a thick white plaque with a rough surface and congested vocal cord mucosa were deemed suspect for malignancy. Under NBI, they graded IPCL morphology based on a new classification system tailored for laryngeal

leukoplakia. In this classification system (Ni 2019), types IV-VI were considered malignant and were determined by the presence of large brown spots embedded at the surface of leukoplakia (type IV), outside the leukoplakia with clear boundaries (type V) or both (type VI). These criteria correspond strongly to type V of the original Ni classification [89] where IPCLs may also appear as solid or hollow brown spots of various shapes. For this reason, this study was included in the systematic review and meta-analysis.
The Se and Sp reported were 82.6% and 92.8%, which were higher than WLE (Se 60.9% and Sp of 72.2%). The introduction of a tailored Ni classification to laryngeal leukoplakia may indeed overcome some limitations of the original classification and is an area of further research.

Rzekapowska and colleagues [123] reported very high accuracy of NBI in laryngeal leukoplakia with a Se of 100% and Sp of 97.4%. However, among the 91 lesions examined, only 14 lesions were high risk (severe dysplasia, Cis and SCC).
Stanikova and colleagues [93] divided patients in two groups depending on the malignant Ni 2011 grade (Type V) and presence of perpendicular changes on ELS classification. They demonstrated a Se and Sp of 88% and 89.5% in differentiating benign (defined in their study as hyperkeratosis or mild/moderate/severe dysplasia) from malignant lesions (defined in their study as carcinoma or invasive SCC). However according the criteria used in our systematic review, the Se was slightly lower at 78.6% in differentiating between low risk and high-risk leukoplakia.
Zhu and colleagues [128] assessed IPCL changes around the peripheries of the white patch using Ni 2011 classification. This study demonstrated the lower sensitivity after data was extracted owing to a different study design where lesions were judged suspicious or malignant based on a combination of WLE and NBI appearances.

There were some variations among the definition of true positives based on both the Ni 2011 classification and the reference histopathological result. In some studies, severe dysplasia was considered test positive for malignancy, whereas in others it was considered test negative. This may explain the differences in sensitivity and specificity reported in this systematic review after the histological criteria from this review was applied raw data extracted from included studies. Given the malignant

potential of severe dysplasia and the close histopathological relation to Cis, severe dysplasia was considered high risk in this review.

Vocal cord leukoplakia is difficult to diagnose and manage due to the inability to distinguish benign from premalignant and malignant lesions with WLE. The high accuracy of NBI may help guide management both pre-operatively with flexible endoscopy or intra-operatively during biopsy or resection. Piazza and colleagues [90] have demonstrated that intra-operative use of NBI during suspension microlaryngoscopy provides a diagnostic gain of 9.2 % and can upstage the disease in just up to 10% of cases. This may also apply during assessment of vocal cord leukoplakia as demonstrated by Zhu and colleagues, who performed more extensive resections following frozen sections [131]. Although some authors have suggested NBI to be used as an "optical biopsy" method [93] preventing excessive biopsies under general anaesthesia, it may still lack the predictive power to supplant histological assessment. Rather, we recommend NBI to be used as an adjunct to standard examination including WLE. Further studies with long term follow up and higher sensitivity is needed before NBI can be utilised as an optical biopsy modality alone. Furthermore, there are several other considerations that are highlighted in the literature. Interpretation of NBI and the Ni classification requires a learning curve before a reliable assessment can be made [106,117,118]. Vilaseca and colleagues [119] suggested a learning curve of 200 examinations using NBI based on analysis of 480 patients. During this learning curve there may be an increased rate of false positive findings on NBI particularly in areas with active inflammation, mucositis, recurrent respiratory papillomatosis or previous radiotherapy [81,113]. In some lesions, blood, mucous or excessive hyperkeratosis may also obscure assessment of the mucosal surface and the IPCL morphology.

This review is limited by the small number of included studies. Although Du and colleagues [132] examined 91 patients with vocal cord leukoplakia using NBI, adequate data could not be extracted and this study was excluded from this review. However, they reported NBI to be superior to WLE in observing small suspicious lesions with higher accuracy in differentiating benign from malignant vocal cord leukoplakia. Also, all of the included studies assessed the lesions with WLE first then switched

NBI, which may potentially introduce bias influencing the grading using either Ni classification or ELS classification. Only Ni and colleagues [122] directly compared NBI to WLE and found NBI to be more accurate than WLE. The histological grading of leukoplakia varied among studies with some studies using the 2005 WHO classification [133] and others using the updated 2017 classification system [35], with possible effects on the overall diagnosis, especially with differentiating severe dysplasia from Cis. For this reason, severe dysplasia and Cis were considered high risk, especially given the risk of malignant transformation of severe dysplasia can be as high as 18-30% [79,134]. In the studies by Huang *et al* and Stanikova et al, both Ni 2011 and ELS classification were used to differentiate benign and malignant lesions. This may introduce misclassification bias given that Ni 2011 type IV (which is considered benign), may also contain IPCLs corresponding to perpendicular change using the ELS classification. This systematic review and meta-analysis defined type IV of the Ni 2011 classification as low risk or test negative as described initially by the authors [89]. Similarly, although type III of the Ni 2019 [122] classification system was considered benign according to the authors, the presence of IPCLs may add heterogeneity to the results. However, given that the IPCL morphology was different between benign type III lesions and malignant types IV-VI, this systematic review and meta-analysis defined type III as low risk or test negative. Although the similarities between Ni 2011 and Ni 2019 have been mentioned above, the two classifications could not be directly translated potentially adding heterogeneity to the results.

Given the applications of NBI in laryngeal lesions are expanding, further prospective studies are needed to assess its role as an optical biopsy method and efficacy in surveillance of disease, particularly with pre-malignant and malignant lesions. Although the Ni 2011 classification [89] of laryngeal lesions has been well established, the Ni 2019 classification tailored for vocal cord leukoplakia requires further studies to validate its accuracy.

Conclusion

In conclusion, this systematic review supports the use of NBI in differentiating between low-risk and high-risk laryngeal leukoplakia. In contrast to previous studies suggesting the white patch limits adequate assessment, IPCLs at the periphery or within the white patch allows for reliable assessment using NBI. The high sensitivity, specificity and diagnostic odds ratio makes NBI a useful imaging modality in both pre-operative workup and surveillance of disease. Further high-powered prospective studies are required to validate its utility in other settings such as intra-operative use or as an optical biopsy method before accurate clinical guidelines can be established.

Chapter Six: The Inter-Observer and Intra-Observer Reliability of Narrowband Imaging in the Optical Biopsy of Laryngeal Lesions

Abstract

Introduction

Narrowband Imaging (NBI) is a special endoscopic optical enhancement tool, which allows for better visualisation of mucosal microvasculature compared to white light endoscopy. NBI has been suggested as an optical biopsy tool due to its high sensitivity and negative predictive values in detecting laryngeal cancer. However, reliability of NBI is dependent on the interpretation of the operator. This study compares the inter-observer and intra-observer reliability of NBI in detecting laryngeal lesions and evaluates the effect of observer experience in reliability.

Methods

Patients with a clinically suspicious laryngeal lesion underwent flexible nasoendoscopy with white light endoscopy (WLE) followed by NBI. Both WLE images and NBI images were graded by two experienced observers and two non-experienced observers. Images were presented in 3 formats (WLE, NBI and WLE+NBI). Observers were asked to classify the lesion as either benign or malignant and make an optical diagnosis. Lesions were graded according to the Ni classification and European Laryngological Society of determining perpendicular intraepithelial papillary capillary loop (IPCL) change. Inter and intra-observer agreement were analysed using Fleiss and Cohen's kappa values respectively.

Results

One hundred and eighty images of laryngeal lesions from sixty patients were collected. The diagnostic accuracy of differentiating benign from malignant lesions and making an optical diagnosis was higher among experienced observers compared to non-experienced observers when viewed with NBI only (differentiating benign from malignant: 76.67% vs. 56.67%, p=0.02; Optical biopsy: 58.3% vs. 40%, p = 0.04). NBI with WLE demonstrated increased interobserver reliability compared to WLE alone in differentiating benign from malignant lesions ($\kappa = 0.61$ [Substantial agreement] vs. 0.53 [Moderate agreement]) and making an optical diagnosis ($\kappa = 0.51$ [Moderate agreement] vs. 0.36 [Fair agreement]). Agreement was higher in detecting "brown dots" as a sign of perpendicular change than the Ni classification.

There were no differences in intra-observer agreement between the three modalities across all groups.

Conclusion

The addition of NBI to WLE improves the inter-observer agreement but does not improve diagnostic accuracy, particularly for experienced observers, in differentiating benign from malignant laryngeal lesions and making an optical diagnosis. Application of ELS criteria of perpendicular change was more reliable than using the Ni classification in improving inter-observer reliability. Further large scale, multi-institutional studies are required to compare the ELS criteria to Ni classification and evaluate the effect of a training program on improving the reliability of using NBI in laryngeal lesions.

Introduction

Current diagnostic work up for laryngeal cancer includes history and examination, white light nasoendoscopy and radiological studies such as computed tomography, magnetic resonance imaging and positron emission tomography [37] However, these modalities lack the sensitivity to detect early stage neoplastic disease with the diagnostic accuracy of history and examination alone being reported as low as 5% [40]. White light endoscopy (WLE) with a flexible nasoendoscope has been recommended as the modality of choice in the workup and surveillance of laryngeal cancer [40] and is often used in an office-based setting. However, it still lacks the sensitivity to detect early, superficial neoplastic lesions of the larynx [78,98] affecting morbidity and mortality [97].

Narrowband imaging has recently been utilised in the detection of laryngeal lesions, particularly due to its high sensitivity in detecting early neoplastic change [26]. By narrowing the bandwidth of light to two wavelengths (blue light corresponding to wavelength peak of 400-430nm and green light corresponding to a wavelength peak of 515-555 nm) the absorption peaks of haemoglobin are optimally captured allowing for better contrast and visualization of laryngeal microvasculature also known as intraepithelial papillary capillary loops (IPCL)[30,39,98]

Morphological changes of IPCL may be a sign of neoangiogenesis and is a hallmark of neoplastic growth and progression. These characteristic changes have been classified to differentiate between benign and neoplastic changes in various regions including the oesophagus and the oral cavity [102,135] In the larynx, the Ni classification [26] system of IPCL change has been proposed and validated to be a reliable system owing to its sensitivity in detecting early laryngeal cancer [78,88,90,105,106]. Briefly, this system describes five patterns of change in IPCL morphology. Types I-IV are usually associated with benign or even pre-neoplastic change. Type V is associated with neoplastic change and is further subdivided into Va, Vb and Vc [89].
Recently the European Laryngological Society also proposed descriptive guidelines for differentiating between benign and neoplastic change based on the direction of microvasculature changes observed [80]. In lesions where longitudinal vascular changes are observed, the lesions are more likely to be benign.

Perpendicular vascular changes are observed in malignant or pre-malignant lesions where IPCL changes are three-dimensional. These changes have been described as appearing like "brown dots" during examination using NBI. Many authors have also reported the appearance of "brown dots or spots" alone in NBI as a sign of neoplasia reflecting perpendicular branching of new vessels seen in growing tumours[84,102,112].

Given the ability of NBI to detect early change in IPCL morphology, many authors have suggested using NBI not only as an adjunct for workup or laryngeal cancer but also for surveillance of disease, intra-operatively to assist in resection margins or as an "optical biopsy" to prevent unnecessary biopsies requiring general anaesthesia [93,114,115]. However NBI is observer dependent and a learning curve has been suggested before a reliable assessment can be made, particularly in using the Ni classification [109,112,118]. Previous studies have demonstrated that NBI improves inter and intra-observer reliability when used intraoperatively using rigid laryngoscopes [112]. Furthermore, the use of the flexible nasoendoscope improves the detection of neoplastic lesions using NBI compared to white light alone [109].

The aim of this study is to assess the intra and interobserver reliability of NBI compared with WLE in both benign and neoplastic laryngeal lesions using the Ni classification. This study further evaluates the sensitivity of "brown dots" as a sign of neoplastic change and the role of operator experience in the assessment of lesions using NBI.

Methods

This study was approved by the institutional human research ethics committee (Ethics number 5424). From March 2017 to January 2020, patients presenting to the Department of Otolaryngology Head and Neck Surgery at Westmead Hospital (Sydney, Australia) were recruited if they had a clinically suspicious laryngeal lesion. During the consultation, an in-office nasoendoscopy was performed. The nasal cavity was anaesthetised topically with Co-Phenylcaine (Lignocaine Hydrochloride 5%/Phenylephrine 0.5%) spray. Nasoendoscopy was performed with an ENF-VQ transnasal flexible fiberscope connected to an Evis Exera III CV 190 light source (Olympus Medical Systems, Tokyo, Japan) while patients were awake and seated.

Laryngeal lesions were first captured with WLE then again in the NBI mode. Both WLE and NBI images were captured as close as possible so the images appeared identical. Lesions that appeared benign (e.g. polyp, keratosis, Reinke's oedema) or suspicious for neoplasm were captured.

An image database was compiled using Microsoft Powerpoint (Microsoft Inc., Redmond, WA). Images were arranged in a random order and displayed in 3 formats (slide with WLE alone, NBI alone and WLE and NBI combined) **Figure 1 A-C**.

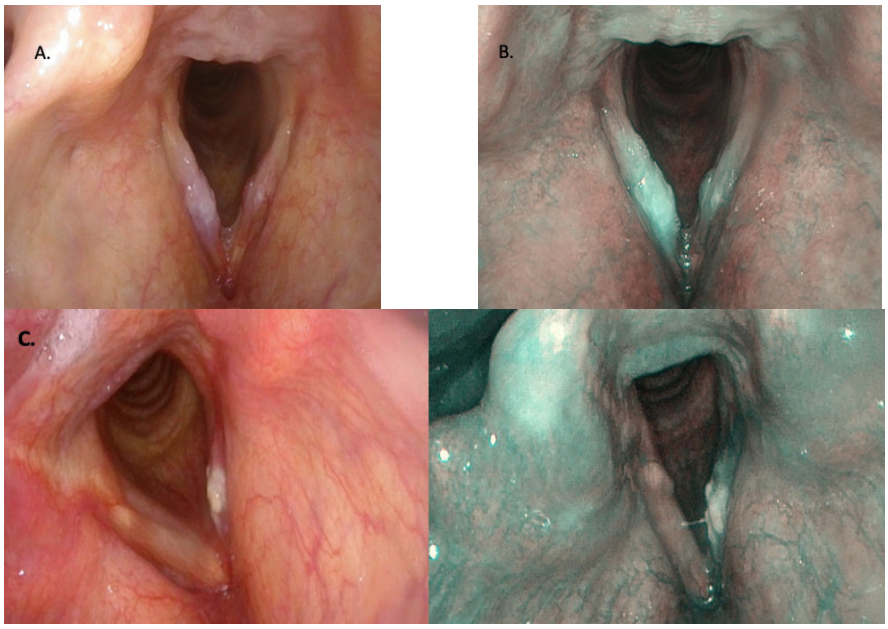


Figure 1: Images were presented in three formats; a) WLE alone, b) NBI alone, c) WLE+NBI presented together. Each image was presented in Microsoft PowerPoint in a random order. WLE = White light endoscopy, NBI = Narrowband Imaging.

In slides presenting WLE alone and WLE+NBI, observers were asked to answer from two multiple choice questions; Question 1 (*The images demonstrate:* a. benign Lesion(s) or b. Malignant Lesion(s)) and Question 2 (*What is the likely histopathology**?*** a. Nodule/cyst/polyp, papillomatosis, benign keratosis, b. Dysplasia (Mild/Moderate), c. Malignant (severe dysplasia/carcinoma , invasive squamous cell carcinoma), d. Unsure).

Slides where an NBI image only was presented, two additional questions were asked: Question 3 (*Are you able to see brown dots?* a. Yes, b. No) and Question 4 (2. *What is*

the likely Ni Classification of this lesion? a. Type I-IV, b. Type Va c. Type Vb, d. Type Vc). The Ni classification was present the entire time for reference.

To assess inter observer reliability; 4 observers were chosen to assess the images and answer the respective questions. The observers were blinded to the histopathological diagnosis. Two experienced observers had over 10 year of experience in otolaryngology and over 3 years in the use of NBI. Two non-experienced observers were junior doctors in training with limited experience in NBI.

To assess for intra-observer reliability, observers were asked to answer the same questions after a 2-week interval.

Statistical analysis

The SPSS 26.0 statistical software package (SPSS, Inc., Chicago, IL, USA) was used for statistical analysis. Data was found to be non-parametric by Shapiro-Wilk test and histogram analysis. Inter and intra-observer reliability was assessed using Fleiss multi-rater kappa value and Cohen's kappa values respectively. As described by Landis and Koch [136], kappa values were interpreted from 0-1 where 0 is defined as no agreement and 1 is defined as perfect agreement. A comprehensive list of interpretation of kappa values is listed in Table 1.

κ Value	**Interpretation**
<0	Poor agreement
0.00	As expected by chance
0.01–0.20	Slight agreement
0.21–0.40	Fair agreement
0.41–0.60	Moderate agreement
0.61–0.80	Substantial agreement
0.81–0.99	Almost perfect agreement
1.00	Perfect agreement

Table 1: Interpretation of j Values According to Landis and Koch [136]

Results

A total of 180 images from 60 patients with laryngeal lesions were assessed. The mean age was 68.6 years with a male to female ratio of 6.3:1. The lesions assessed consisted of 87 (48.3%) benign lesions including nodules, cysts, keratosis,

inflammation, 18 (10%) lesions with low risk pre-malignant lesions including mild and moderate dysplasia, and 75 (41.6%) with severe dysplasia, Cis or SCC (Table 2).

Summary of Laryngeal lesions assessed	
Benign Lesions (polyps, nodules, cysts, Reinke's oedema, laryngitis)	87
Dysplasia (Mild, moderate)	18
Malignant (severe, Cis, SCC)	75
Total	180

Table 2: Summary of histopathological diagnosis of laryngeal lesions

Optical Diagnosis

When viewing images in NBI mode alone there was a statistically significant difference between experienced observers and non-experienced observers in differentiating benign from malignant lesions (76%vs.56%, p=0.02).
Although a higher percentage of experienced observers were able to correctly differentiate between benign and malignant lesions in WLE and WLE+NBI, this difference was not statistically significant (WLE: 75%vs60%, p=0.08. WLE+NBI; 71%vs65%, p=0.434) (Table 3).

Optical diagnostic accuracy was higher among experienced observers compared to non-experienced observers when viewing images in NBI mode (58% vs. 40%, p = 0.04). Although the optical diagnostic accuracy was higher among experienced observers when viewing image with WLE and WLE+NBI, the difference was not statistically significant (Table 4).

Imaging Modality	**Observer Group**	**Observer Correctly Differentiated Benign vs. Malignant (%)**	**p value**
WLE	Experienced	75.00	p = 0.08
	Non-experienced	60.00	
NBI	Experienced	76.67	**p = 0.02**
	Non-Experienced	56.67	
WLE+NBI	Experienced	71.67	p = 0.43
	Non-experienced	65.00	

Table 3: Percentage of Observers differentiating benign from malignant lesions across all imaging modalities.

Imaging Modality	Observer Group	Histopathology Correctly Identified (%)	p value
WLE	Experienced	61.67	0.10
	Non-Experienced	46.67	
NBI	Experienced	58.33	***p=0.04***
	Non-Experienced	40.00	
WLE+NBI	Experienced	63.33	p=0.14
	Non-Experienced	50.00	

Table 4: Accuracy of Optical Diagnosis Between Experienced and Non-Experienced Observers Across All Imaging Modalities.

Inter-Observer Agreement

There was moderate agreement between experienced observers when differentiating between benign and malignant lesions with a κ of 0.53 when images were viewed with WLE. Among non-experienced observers, the agreement in differentiating benign from malignant lesions was Fair, however with a lower κ value of 0.40 (Figure 2). Agreement between experienced and non-experienced observers was Moderate with a κ of 0.51.

Using the NBI mode, substantial agreement was found among experienced observers in differentiating benign from malignant lesions with a κ of 0.61. Among non-experienced, agreement remained Moderate (κ=0.44). Agreement between experienced and non-experienced observers was Moderate with a κ = 0.51. When images were viewed with WLE+NBI the agreement was Substantial among Experienced (κ=0.76) observers and among Experienced vs. non-Experienced observers (κ=0.71). When evaluating optical diagnosis, agreement among experienced and non-experienced observers was κ=0.36, 0.46 and 0.513 when images were viewed with WLE, NBI and WLE+NBI respectively. This suggests that using NBI as an adjunct to WLE increases interobserver reliability.

Agreement was lowest among all groups in using the Ni classification with a κ value of 0.32 (Fair agreement) among experienced observers, and a κ = 0.20 (Slight agreement) among non-experienced observers. Agreement between experienced and non-experienced observers in using the Ni classification was Fair with κ=0.30. However, agreement was much higher in detecting "brown dots" among both experienced and non-experienced observers with a κ value of 0.63 (Substantial Agreement) and 0.51 (Moderate agreement) respectively. Agreement between experienced and non-experienced observers in detecting brown dots was moderate with a κ value of 0.56.

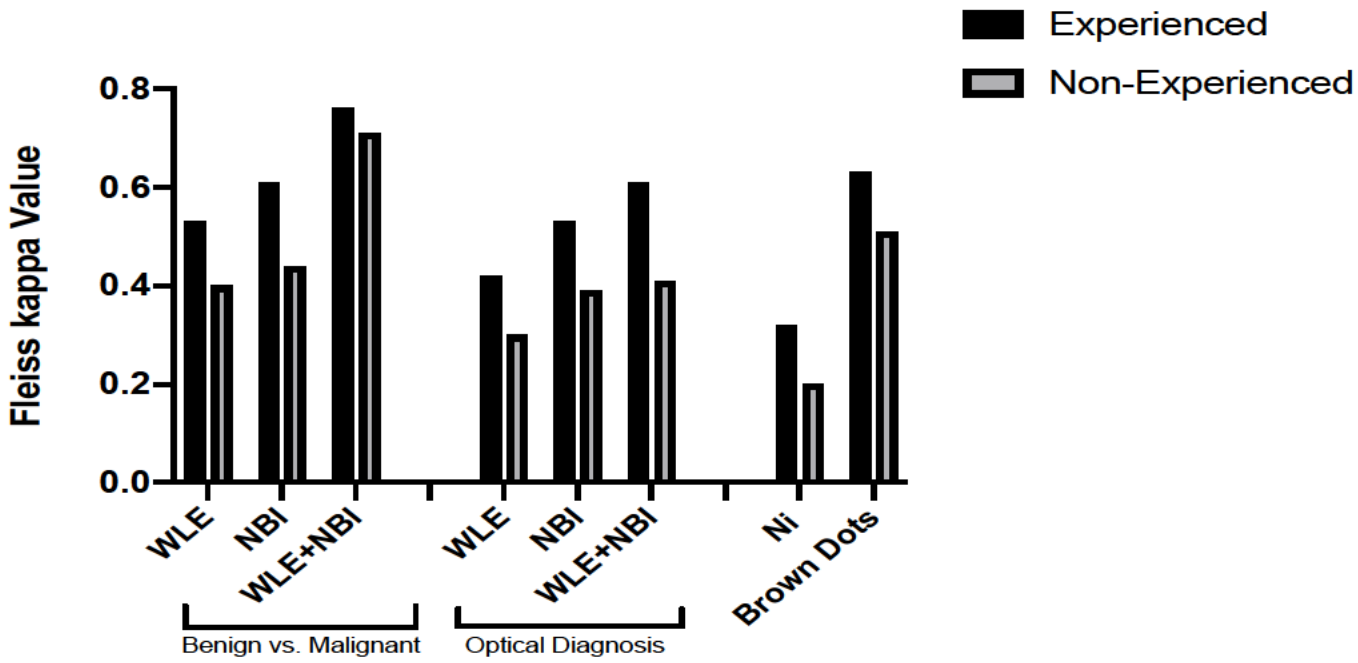


Figure 2: Inter-observer agreement for differentiating benign from malignant lesions, making an optical diagnosis, grading Ni classification and detecting presence of brown dots based on WLE alone, NBI alone and WLE+NBI. Agreement is presented between experienced and non-experienced observers. WLE = White Light Endoscopy, NBI = Narrowband Imaging.

Intra-Observer Agreement

The Cohen's Kappa values for each group is displayed in Table 5. Overall, the intra-observer agreement in differentiating benign from malignant lesions using WLE was moderate with κ=0.56. Although NBI and WLE+NBI improved intra-observer agreement in all groups based on κ values, agreement levels were still Moderate. Intra-observer agreement in optical diagnosis overall was highest at κ=0.50 when images were viewed with WLE+NBI.

Interestingly, the overall intra-observer agreement was using the Ni classification substantial with κ=0.71 and almost perfect in detecting brown dots with a κ= 0.82.

Imaging Modality	**Observer Group**	**Benign vs. Malignant (k)**	**Optical Diagnosis (k)**	**Ni**	**Brown Dots**
WLE	Experienced	0.68 Substantial	0.56 Substantial	-	-
	Non-Experienced	0.44 Moderate	0.30 Fair	-	-
	Overall	0.56 Moderate	0.48 Moderate	-	-
NBI	Experienced	0.64 Substantial	0.58 Moderate	0.72 Substantial	0.83 Almost Perfect
	Non-Experienced	0.42 Moderate	0.32 Fair	0.70 Substantial	0.81 Almost Perfect
	Overall	0.54 Moderate	0.45 Moderate	0.71 Substantial	0.82 Almost Perfect
WLE+NBI	Experienced	0.67 Substantial	0.62 Substantial	-	-
	Non-Experienced	0.43 Moderate	0.38 Fair	-	-
	Overall	0.55 Moderate	0.50 Moderate	-	-

Table 5: Intra-observer agreement analysed with Cohen's K between experience and non-experienced observers with different imaging modalities. Agreement was analysed for differentiating benign from malignant lesions, making an accurate optical diagnosis, grading IPCL using Ni classification and detecting presence of brown dots.

Discussion

The applications of NBI in the workup of laryngeal cancer are increasing owing to its high diagnostic accuracy using the Ni classification and ELS classification of perpendicular change [78,105]. However, there is a learning curve associated with interpreting NBI findings as reported by many authors [117,119]. Furthermore, interpretation of NBI is observer dependent, and may affect reliability of the findings particularly when using the Ni classification with 7 subtypes (Types I, II, III, IV, Va, Vb, Vc).

The findings of this study suggest that the diagnostic accuracy of NBI is affected by operator experience. This is evidenced by a significantly lower accuracy in using NBI to differentiate between benign and malignant lesions and make an optical diagnosis between experienced and non-experienced observers. Although the diagnostic accuracy of WLE and WLE+NBI in differentiating between benign and malignant lesions and making an optical diagnosis was higher in experienced observers, this difference was not statistically significant. This finding is consistent with previous studies where the diagnostic accuracy of laryngeal lesions under WLE and NBI was lower in the less experienced group compared to experienced laryngologists [131]. Use of the Ni classification may be complex and the differences between the 7 subtypes may be subtle and overlapping (e.g. malignant leukoplakia can be diagnosed as type III and type V). This may affect the reliability of interpreting findings particularly to the untrained eye. However, an NBI training program may improve the diagnostic accuracy and agreement among non-experienced observers to the level of experienced laryngologist [131]. The ELS have recently proposed a more simplistic alternative to the complex Ni classification by proposing a system, which incorporates a dichotomous distinction between benign and malignant lesions based on direction of change in superficial vessels [80]. This is supported by this study as observed by higher κ values in identifying brown dots by both experienced and non-experienced observers compared to the Ni classification.

Interestingly the accuracy of differentiating benign and malignant lesions and making an optical diagnosis using NBI was relatively low. This may be due to a high number of benign lesions included in this study. A study by Dippold and colleagues[137]

assessing the role of NBI in detecting benign laryngeal lesions found similar accuracies ranging from 48% to 61% in detecting benign lesions such as vocal cord polyps and cysts.

The inter-observer agreement was higher among experienced observers across all imaging modalities in differentiating benign from malignant lesions and making an optical diagnosis. Use of NBI improved agreement from Moderate to Substantial among experienced observers only. This is consistent with previous studies where NBI improved inter-observer agreement compared to WLE in both experienced and non-experienced observers [112,118].

In this study NBI also improved agreement rates in making an optical diagnosis among experienced observers compared with WLE.
However, contrary to findings in the previous literature, use of NBI had little effect on the reliability among non-experienced observers in this study. This may be due to a number of reasons including the complexity of the Ni classification, low number of observers in this study and quality of the images assessed. Although the images were of high resolution, they were not coupled with high definition capabilities as opposed to the study by Zwakenberg and colleagues [112].

There were no differences in intra-observer agreement after the addition of NBI across all observers. Results from previous studies are conflicting with some studies demonstrating an improved result with the addition of NBI [118] and some studies reporting similar intra-observer agreement among some of their observers [118]. The intra-observer agreement in the Ni classification was substantial across all groups and almost perfect for identifying brown dots. This suggests that the ELS classification displays higher inter and intra-observer reliability compared to the Ni classification across experienced and in-experienced observers. However, as opposed to the Ni classification, which has been validated in laryngeal cancer, further studies are required to determine the diagnostic accuracy of the ELS classification against the gold standard histopathological diagnosis.

A limitation of this study is that lesions were viewed as still images as opposed to video format, which may have influenced the response of the observers. Furthermore, in clinical practice the benefit of a clinical history and examination may improve the accuracy and pre-histological diagnosis among both groups. However, by blinding the observers to the histological diagnosis, less confounders may affect the observer's decision and image interpretation. This study assessed the WLE and NBI images of patients who were already undergoing biopsy under general anaesthesia potentially introducing selection bias. However, as histopathology is the gold standard reference test and is obtained under general anaesthesia, this is unavoidable in this study.
Further studies purely assessing observer reliability without histological data may provide a more representative sample of patients who present to the outpatient Otolaryngology clinics.
Although 4 observers assessed the images, a multi-institutional study with a larger sample and multiple observers may improve the power of results and more accurately determine if NBI increases reliability among observers and be used confidently for optical diagnosis.

Conclusion

In conclusion, the findings of this study suggest that the addition of NBI to WLE improves the inter-observer agreement but does not improve diagnostic accuracy, particularly for experienced observers, in differentiating benign from malignant laryngeal lesions and making an optical diagnosis. Application of ELS criteria of perpendicular change was more reliable than using the Ni classification in improving inter-observer reliability. Further large scale, multi-institutional studies are required to compare the ELS criteria to Ni classification and evaluate the effect of a training program on improving the reliability of using NBI in laryngeal lesions.

Chapter Seven:

Summary and Discussion

Summary of Studies and Key findings

The aim of this thesis was to evaluate the role of NBI in the workup of laryngeal cancer. The first two studies assessed the diagnostic accuracy of NBI in malignant lesions of the larynx and laryngeal leukoplakia. Both studies demonstrated high diagnostic accuracy of NBI using the Ni classification. The third study assessed the inter and intra-observer reliability of interpreting NBI images compared with WLE and its use as adjunct to WLE. This study demonstrated that although the use of NBI improved agreement between different observers, there was little agreement in using the Ni classification. However, there was strong agreement in identification of the IPCLs appearing as brown dots, which has been recently adapted by the ELS and proposed as an alternative classification system.

NBI in the diagnosis of malignant laryngeal lesions

The sensitivity and specificity of NBI in the diagnosis of laryngeal cancer in this study was 95.0% (CI, 83.9%-99.4%) and 83.3 % (CI, 51.6%-97.9%) respectively. This was consistent with much of the previous published literature with two systematic review reporting results comparable to this study [78,105].
However, both systematic reviews were limited by the low number of included studies and recommended further studies to validate their findings. Additionally, several large cohort studies that did not meet their inclusion criteria reported much lower sensitivities and ranging from 58-84% [90,108]. This may have been attributed to institutional or regional variations in patient referral/presentation, quality of image processing and/or method of assessment.

At the time of this writing, there is only one Australian study analysing the diagnostic accuracy of NBI by comparing it with autofluorescence in head and neck cancer [104]. Although the authors reported a high sensitivity of NBI in HNSCC, only 22/73 lesions involved the larynx [104]. Thus, the rationale for the first study was to assess the diagnostic accuracy of NBI in laryngeal cancer in a larger Australian cohort. This study also found a strong correlation between higher Ni grades and pathological stage of tumour suggesting NBI is useful intraoperatively to clinical staging of tumour. A large prospective cohort study found that use of NBI intraoperatively coupled with

HDTV upstaged tumours in up to 10% of causes by delineating superficial surgical margins more clearly than WLE [90].

NBI in differentiating benign from malignant laryngeal leukoplakia

Laryngeal leukoplakia is a diagnostic challenge owing to the low accuracy of WLE in differentiating benign from malignant laryngeal leukoplakia [131]. In the largest comprehensive review of 2188 biopsies of laryngeal leukoplakia, Isenberg and colleagues [79] demonstrated that 53% of biopsies lesions demonstrated no dysplasia and a further 33% demonstrated low/moderate dysplasia.

Leukoplakia has often been reported to be a limiting factor in NBI assessment due to the "umbrella effect" obscuring IPCLs [81,85,87,106]. However other studies suggested NBI to an accurate imaging modality and can be used to assess IPCL change at the periphery of the white patch or in some cases on the mucosal surface if the leukoplakia is translucent [123]. The systematic review of 586 lesions and meta-analysis of 483 lesions demonstrated a high sensitivity and specificity of 85.4% (95% CI 76%-99.9%) and 94.9% (95% CI 91.1%-97.2%) respectively. The pooled diagnostic odds ratio was 99.2 (95% CI 38.28-257.18). These results support the use of NBI in differentiating between low-risk and high-risk laryngeal leukoplakia. Together with WLE, it serves as a useful adjunct in the workup of laryngeal leukoplakia and may reduce the number of second looks by serving as an "optical frozen section".

NBI improves inter-rater reliability of laryngeal lesions

Given the diagnostic accuracy of NBI using the Ni classification, a natural progression is to utilise NBI as an optical biopsy method. However, few studies have assessed the reliability of NBI between different observers particularly with varying levels of experience.

The third study demonstrated that the addition of NBI improved inter-observer reliability in differentiating between benign and malignant laryngeal lesions as well as making an optical diagnosis. Levels of agreement increased proportionately when lesions were assessed with NBI alone and in combination to WLE. Experienced observers displayed higher levels agreement across all imaging modalities compared to non-experienced observers and this was significant when images were viewed in

NBI only (Differentiating benign from malignant: 76.67% vs. 56.67%, p=0.02; Optical biopsy: 58.3% vs. 40%, p = 0.04). However, accuracy of NBI was no different to WLE in experienced observers and slightly lower in inexperienced observers. This may be due to the low number of observers who graded lesions and further studies with more observers are required.

Interestingly, agreement in using Ni classification was low for both experienced and non-experienced observer. However, agreement was substantially high among all groups in the detection of malignant IPCL change (appearing as "brown dots"). Recently the ELS have proposed a dichotomous classification system based on longitudinal and perpendicular change in laryngeal microvasculature [80]. In this system, longitudinal change is likely to be benign and perpendicular change appearing as brown dots represent malignant change. Although perpendicular ICPL change is very similar to type V of the Ni classification, the authors have suggested a less complex classification system may improve agreement among observers [80]. The findings of the third study support this statement. Further studies are required to validate the accuracy of the ELS classification.

Clinical Implications

Based on the studies from this thesis, NBI may be applied in many clinical settings. Most commonly, it is a useful adjunct to WLE to be used pre-operatively in an office-based setting with a flexible or rigid laryngoscopy. NBI may also be coupled with HDTV and used intra-operatively to stage disease, assess for synchronous primaries and assist in resection of disease with clear surgical margins. Garofolo and colleagues [138] divided 82 patients undergoing TLM for early glottic cancer into two groups based on whether intraoperative NBI was used as an adjunct to WLE. They reported a positive superficial margin rate of 3.6% in the NBI group compared with 23.7% in patients without intraoperative NBI [138].

The sensitivity of NBI in differentiating benign from malignant disease is maintained after radiotherapy, making it a useful modality for surveillance of disease. Zabrosky and colleagues [121] determined the sensitivity and specificity of NBI in detecting recurrent disease in patients who have underwent radiotherapy and/or chemotherapy to be 92% and 76% respectively.

However, in all settings, a learning curve may initially increase the false positive rates as evidenced in the third study. Many authors have thus recommended a training program, particularly for junior practitioners to increase the reliability of interpreting NBI images [118,131]. Previous studies have demonstrated the accuracy of NBI increased with operator experience and plateaus after approximately 200 examinations [119]. Although NBI is a useful adjunct to WLE in detecting pre-malignant and early malignant laryngeal lesions, the authors believe it provides little value over WLE in advanced laryngeal cancer.

Study Limitations and Directions For Future Research

A limitation of all studies was the low number of included patients. The reasons for this are multifactorial including the single institution recruitment, low incidence of laryngeal cancer in Australia and the time constraints placed in the completion of this thesis. In our institution, only one endoscopy stack had NBI capabilities limiting patient recruitment. However, the first and third studies represented the largest Australian cohort to date. Further prospective, multi-institutional studies are needed to add power and validate the findings presented in this thesis.

Another possible limitation is the NBI system used was not coupled with HDTV. Although images obtained from flexible nasoendoscopy were high resolution allowing accurate assessment of IPCL, use of NBI coupled HDTV may have enhanced pictures and increased utility of NBI to be used intra-operatively.

Although most endoscopy systems include NBI capabilities since 2017, there is a cost associated with installing NBI. Similar optical modalities such as iSCAN (Pentax Medical Company, Montvale, NJ) and SPIES (Karl Storz, Tuttlingen, Germany) have been developed with the similar principles but are yet to be validated. Recently, Stanikova and colleagues compared both NBI and SPIES system in detection of superficial neoangiogenesis and found similar diagnostic accuracy between the two modalities [139].

Conclusion

Overall, NBI is a reliable optical imaging modality with high diagnostic accuracy in the detection of pre-malignant and malignant laryngeal lesions. In addition, it is a useful tool to differentiate between low risk and high-risk laryngeal leukoplakia by assessing mucosal microvasculature either at the peripheries or underlying the within the white patch. NBI in addition to WLE increased inter-observer reliability in differentiating between benign and malignant lesions and making an optical diagnosis. However, the findings of this study suggested that it did not increase the diagnostic accuracy compared to WLE alone. Furthermore, a learning curve is required in interpreting the findings, particularly the Ni classification. The recent dichotomous classification by the ELS may provide an alternative method in assessing NBI images, however its diagnostic accuracy needs to be validated with high power prospective studies.

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Appendix A

Search Strategy for Chapter 3

Patient/Population	Intervention	Comparison	Outcome
cancer* or precancer* or pre-cancer* or malignan* or premalignan* or pre-malignan* or neoplas* or tumo* or carcinoma* or squamous cell carcinoma OR SCC or Leukoplakia or leucoplakia or Leukoplakic or Leukokeratos* or dysplas* or low-grade dysplasia or low grade dysplasia or moderate dysplasia or severe dysplasia or high grade dysplasia or high-grade dysplasia AND laryn* or vocal cord or vocal fold or	Narrow band imag* or Narrowband imag* or Narrow-band imag* or NBI		

glottic or glottis or vocal ligaments or Rima Glottidis OR supraglottis OR supra-glottis OR subglottis OR sub-glottis or anterior commissure OR posterior commissure			

Appendix B

HREC Committee Secretariat:

A/Prof Clement Loy
Medical Graduate – Neurologist

Mrs Patricia Fa
Clinical Trials Pharmacist

HREC Committee Members:

Ms Narelle Bell
Lawyer

Ms Jay Bowen
Catholic Chaplain

Prof Angus Dawson
Professor of Bioethics

Mr John Fisher
Lawyer

Mr John McLeod
Layman

Mr Sean Mungovan
Physiotherapist

Mrs Janette Parry
Laywoman

Dr Christopher Ryan
Medical Graduate - Psychiatrist

Mrs Katherine Schaffarczyk
Nurse Educator

Mr John Shaw
Layman

Our ref: (5424) **QA**
Date: 2 March 2018

A/Prof Faruque Riffat
Department Of Otolaryngology
Westmead Hospital

Dear A/Prof Riffat

Quality Assurance Project: Narrow Band Imaging in the histopathological diagnosis of laryngeal lesions

Your request to undertake the above protocol as a Quality Assurance project was reviewed by the Westmead Scientific Advisory QA Committee and the Secretary of the WSLHD Human Research Ethics Committee. We are satisfied your proposal meets the criteria for quality assurance and it is therefore approved.

Please send a copy of the results of the study to the Research Office when they become available.

Yours sincerely

[RED ACTION]

Mrs Pat Fa
Secretary
WSLHD Human Research Ethics Committee