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Main Article

Comparison of contact endoscopy and frozen section histopathology in the intra-operative diagnosis of laryngeal pathology

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Abstract

Andrea *et al.* were the first to use contact endoscopy in the diagnosis of laryngeal disease, in 1995. This method enables *in vivo* microscopy of laryngeal mucosa.

In the present study, comparison of contact endoscopy with frozen section histopathology was performed in 142 patients with various diseases of the larynx. Paraffin section histopathology diagnosed 70 benign lesions, 23 precancerous lesions and 49 malignant lesions. Frozen section histopathology showed a sensitivity of 89.8 per cent, a specificity of 98.9 per cent and an accuracy of 95.7 per cent ($\chi^2 = 1.5$; $p = 0.18$). Frozen histopathology diagnosed 45 malignant lesions, including one false positive and five false negative results. Contact endoscopy yielded a sensitivity of 79.59 per cent, a specificity of 100 per cent and an accuracy of 92.95 per cent ($\chi^2 = 8.1$; $p = 0.002$). All malignant lesions diagnosed by contact endoscopy were confirmed by histopathology; contact endoscopy failed to recognise malignant lesions in 10 patients.

Contact endoscopy is preferable to frozen section histopathology as it is noninvasive, provides information on microscopic diagnosis and laryngeal lesion margins, and enables visualisation of the laryngeal mucosa microvasculature. The use of contact endoscopy along with frozen section histopathology improves diagnostic accuracy and allows for operative (or other) therapy to continue according to the results obtained.

Key words: Contact Endoscopy; Frozen Sections; Larynx Neoplasms; Pathology

Introduction

The high prevalence of laryngeal carcinoma imposes the need for an early, reliable and noninvasive diagnostic method to enable primary and secondary prevention. Frozen section histopathological analysis is currently a widely accepted intra-operative diagnostic method characterised by a high rate of accuracy (98 per cent) in the diagnosis of head and neck pathology by exclusion of particular diseases.[1–3] However, frozen section is inferior to paraffin section, and errors may occur. Biopsy compromises the laryngeal tissue integrity, leading to permanent voice modification, which may cause considerable problems in individuals with speech-based occupations.

Andrea *et al.* were the first to use contact endoscopy in the diagnosis of laryngeal disease, in 1995.[4,5] Contact endoscopy enables visualisation of laryngeal mucosa pathology without requiring tissue biopsy. The method is noninvasive, and the cells are analysed *in vivo*, thus avoiding cell damage and deformity during preparation. In addition, this method allows visualisation of the laryngeal mucosa microvasculature, which may prove helpful in the initial diagnosis of early laryngeal lesions.

We compared frozen section histopathology and contact endoscopy, in order to investigate their advantages and shortcomings in the intra-operative diagnosis of laryngeal pathology.

Material and methods

The study included 142 patients with various laryngeal diseases which required direct microlaryngoscopy. There were 101 (71 per cent) male and 41 (29 per cent) female patients, with an age range of 19–81 years. Laryngeal pathology included: laryngeal polyps, cysts and Reinke's oedema ($n = 61$); chronic laryngitis ($n = 12$); leukoplakia ($n = 10$); papilloma ($n = 1$); suspected laryngeal tumour recurrence ($n = 8$); and laryngeal tumour ($n = 50$).

When performing direct microlaryngoscopy, the entire larynx was examined by use of a rigid endoscope; the laryngeal lesion was then stained by pressing a cotton swab soaked in 1 per cent methylene blue against the lesion for three minutes. Contact

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endoscopy was performed by pressing the instrument against the lesion, which was then analysed by a pathologist directly through the endoscope or via a video monitor connected to the endoscope. Upon completion of endoscopic examination, a biopsy specimen was obtained for frozen section and paraffin section histopathology. The findings obtained by paraffin section histopathological analysis were compared with those obtained by contact endoscopy and frozen section histopathological analysis. A contact endoscope (Karl Storz 8715 AA, Tuttlingen, Germany) of 0°, ×60 and ×150 magnification was employed. Staining of the larynx with 1 per cent methylene blue has no adverse effects and disappears within a few hours.

Results

The results of paraffin section and frozen section histopathology and of contact endoscopy are presented in Table I.

Paraffin section histopathology diagnosed 70 benign lesions, 23 precancerous lesions and 49 malignant lesions. Malignant lesions were diagnosed in 43 of 50 patients with a clinical picture of laryngeal tumour, in two of 12 patients with chronic laryngitis, in one of 10 patients with leukoplakia, in two of eight patients with suspected post-operative tumour recurrence and in one of 61 patients with polypoid lesions of the larynx.

Frozen section histopathology diagnosed 45 malignant lesions, including one false positive and five false negative results. Frozen section histopathology yielded a sensitivity of 89.8 per cent, a specificity of 98.9 per cent and an accuracy of 95.7 per cent ($\chi^2 = 1.5$, $p = 0.18$).

All malignant lesions diagnosed by contact endoscopy were verified by histopathology. In 10 patients, contact endoscopy failed to identify malignant lesions diagnosed by histopathology. Inconsistent data on the grade of dysplasia were recorded in six patients. Contact endoscopy showed a sensitivity of 79.59 per cent, a specificity of 100 per cent and an accuracy of 92.95 per cent ($\chi^2 = 8.1$, $p = 0.002$).

As frozen section testing differentiates only between malignant and benign lesions, the findings obtained by contact endoscopy were also categorised into these two groups for comparison. Comparison of individual findings obtained by contact endoscopy and by histopathology yielded an 88.73 per cent accuracy.

Contact endoscopy enables *in vivo* identification of the stratified squamous and laminated columnar epithelial margins. Expansion of squamous epithelium from the vocal fold edges to the areas of columnar epithelium can be clearly visualised by contact endoscopy. Hyperkeratosis (i.e. deposits of anuclear cells on the epithelial surface) is clearly observed on contact endoscopy. Leukoplakia or hyperkeratosis may be present as an isolated lesion or in association with a certain grade of dysplasia. By moving the endoscope along the leukoplakia lesion of the laryngeal mucosa, the epithelial cells underlying these deposits of dead cells can also be visualised. The grade of dysplasia is indicated by the impaired nucleus/cytoplasm ratio, nuclear hyperchromasia, and variation in the number and appearance of the nucleoli.

In chronic laryngitis, epithelial cells are of a homogeneous appearance with enhanced but regular microvasculature, and the examination may be hampered by the abundance of inflammatory cells. Adult papilloma is characterised by koilocytosis, the diagnosis being facilitated by axial microvasculature visualisation.

Contact endoscopy indicated the diagnosis of carcinoma in 39 patients and produced false negative findings in 10 patients. The diagnosis of carcinoma can be definitely made in the presence of: marked heterogeneity of the cell population; impaired nucleus/cytoplasm ratio; nuclei varying in size, shape and staining intensity; hyperchromatism; intranuclear inclusions; prominent nucleoli and abundant divisions; and angioneogenesis. On contact endoscopy, grade III dysplasia was diagnosed in three of five patients and carcinoma in only two of five patients with carcinoma in situ.

TABLE I

COMPARISON OF FINDINGS OF PARAFFIN SECTION AND FROZEN SECTION HISTOPATHOLOGY, AND CONTACT ENDOSCOPY*

Finding	Histopathology (*n* (%))		Contact endoscopy (*n* (%))
	Paraffin section	Frozen section	
Benign	70 (49)	97 (68)	75 (53)
Hyperplasia	5 (3.5)	–	8 (5.5)
Dysplasia I	5 (3.5)	–	–
Dysplasia II	7 (4.5)	–	6 (3)
Dysplasia III	5 (3.5)	–	13 (10.5)
Papilloma	1 (1)	–	1 (1)
Ca in situ	5 (3.5)	–	–
SCC	44 (31.5)	45 (32)	39 (27)

*For 142 patients. Ca = carcinoma; SCC = squamous cell carcinoma

Discussion

In our study, frozen section histopathological analysis produced five false negative results, mostly related to insufficient or inadequate biopsy material.[3–5] If biopsy material is too small, it may 'melt away' on preparation and thus be useless for testing. Furthermore, frozen section biopsy samples only part of the lesion, and therefore may miss a malignant process which does not involve the entire lesion. Post-operative and post-radiotherapy patients pose a specific problem because the presence of granulation tissue, abundant inflammatory infiltrate and post-irradiation cell changes make diagnosis more difficult. In the present study, the false positive result was obtained in a patient who had undergone post-operative radiotherapy (the histopathological finding was grade III dysplasia). Rapid freezing may cause the intracellular water crystals to distort the shape of the nucleus and cytoplasm, producing a false impression of alteration. In addition, frozen section is thicker than paraffin section, thus impairing

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image resolution. According to literature reports, the rate of false positive results ranges from 0.5 to 1 per cent.[2,3]

In the present study, histopathological analysis confirmed the presence of a malignant process diagnosed by contact endoscopy in all cases, i.e. there were no false positive results. This was attributed to the high criteria set for the diagnosis of laryngeal lesion malignancy by contact endoscopy, which yielded a specificity of 100 per cent but a sensitivity of only 79.59 per cent. Setting lower criteria for the diagnosis of malignancy would increase the sensitivity while decreasing the specificity of the method. As this diagnostic method is expected to provide intra-operative data on the type of laryngeal pathology, thus considerably influencing the subsequent operative course, the criteria for malignancy were set high in order to minimise the use of unnecessary operative procedures.

There were 16 false negative results, attributable to multiple causes. It may be quite difficult to establish the grade of dysplastic laryngeal lesion by contact endoscopy.[5–9] In histopathological analysis, the diagnosis of dysplasia grade is based on the epithelial layer involved by cell alteration, whereas in contact endoscopy the pathologist has to determine the epithelial layer and dysplasia grade based on cellular appearance. In addition, the stain must penetrate throughout the epithelial thickness in order to enable visualisation of grade I or II dysplasia. The presence of secretion in the laryngeal mucosa decreases stain penetration, while the secretion itself precludes any direct contact between the endoscope and the mucosa epithelium. In our study, laryngeal mucosa was stained by pressing a cotton swab soaked in 1 per cent methylene blue against the mucosa for three minutes, thus achieving full-thickness epithelial staining. The method was quite simple, as it only required the staining area to dry once thoroughly before methylene blue application. The depth of stain penetration was determined by subsequent histopathological sections without additional staining.[5] One per cent methylene blue stains the cytoplasm light blue and the nucleus dark blue. Cells are visualised from the surface to the deeper layers by altering magnification. At greater magnification (from $\times 100$ to $\times 150$), clear image resolution can only be achieved at the edges of the vocal folds or the lesion. Therefore, most lesions are diagnosed at a medium magnification ($\times 60$).

The eight patients who had undergone post-operative radiotherapy posed a special diagnostic challenge, due to the abundance of inflammatory cells hampering the examination. Laryngeal papilloma was diagnosed in one patient. This diagnosis is considerably facilitated by the appearance of regular axial microvasculature.

Carcinoma in situ is characterised by heterogeneity of the cell population; however, angiogenesis is not present because the tumour process does not cross the basement membrane. It is therefore difficult to differentiate carcinoma in situ from invasive carcinoma. For this reason, all malignant findings in this study were described as squamous cell carcinoma. The finding of angioneogenesis definitely indicates carcinoma, whereas its absence does not exclude the possibility of invasive carcinoma, i.e. the diagnosis of carcinoma in situ cannot be made with certainty. Large laryngeal tumours with abundant necrosis will bleed readily when touched, thus impairing tissue staining, while the necrotic content makes identification of the cell population even more difficult.

For all these reasons, false negative results may occur, as it is not always easy to achieve appropriate study conditions.

The majority of authors report the reliability of contact endoscopy as being within 75 to 88 per cent.[7,9] Contact endoscopy enables visualisation of tumour margins, dysplasia and normal epithelium, thus offering the possibility of more precise removal of laryngeal lesions *in toto*. Along with *in vivo* studies, contact endoscopy can also be used to analyse the excised segment of the lesion, and to ensure whether the lesion has been completely extirpated.

Laryngeal mucosa microvasculature can be visualised by contact endoscopy even without previous staining. Visualisation of angioneogenesis provides clear insight into the malignant nature of the laryngeal lesion. Subepithelial tumours of the larynx can also be visualised using this method.

Contact endoscopy is useful in the diagnosis of various diseases of the oral and nasal cavity,[10–14] while the use of computer image processing allows clearer visualisation of the endoscopic image.[15] Contact endoscopy can be used in combination with autofluorescence (compact endoscopy).[16]

- **The high prevalence of laryngeal carcinoma imposes the need for an early, reliable and noninvasive diagnostic method to enable primary and secondary prevention**
- **Contact endoscopy provides an insight into the type of laryngeal mucosa pathology, without requiring tissue biopsy**
- **The method is noninvasive and the cells are analysed *in vivo*, thus avoiding cell damage and deformity during preparation**
- **In this study of 142 patients, all malignant lesions diagnosed by contact endoscopy were confirmed by histopathology; contact endoscopy failed to recognise malignant lesions in 10 patients**

Contact endoscopy has a number of advantages over frozen section histopathology. Firstly, it is noninvasive and can be safely repeated on several occasions. Secondly, when performed pre-operatively it provides an insight into the microscopic margins of the laryngeal mucosa lesion. Thirdly, it can be used intra-operatively to ensure that the lesion has been completely removed.

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Fourthly, contact endoscopy allows visualisation of the microvasculature, which can assist the initial diagnosis of early laryngeal lesions.

As contact endoscopy supplies intra-operative information on the type of laryngeal pathology, thus significantly influencing the subsequent operative course, the criteria for malignancy are set high in order to minimise the use of unnecessary operative procedures. A false negative result will only delay optimal operative or other therapeutic intervention until histopathology results are obtained, whereas an operative procedure performed on the basis of a false positive result may imply major and irreversible error.

Diagnostic accuracy is greatly improved by the combined use of contact endoscopy and frozen section histopathology. The operative procedure (or other therapeutic approach) can be more safely continued on the basis of the findings thus obtained.

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