Imprint Cytology in Laryngeal and Pharyngeal Tumours

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Accepted for publication 10 August 2006

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Objective: The aim of this study was to evaluate the role of cytology in providing a reliable diagnosis upon which the clinician can base further investigative or treatment strategies in patients with laryngeal and pharyngeal tumours.

Methods: Imprint cytology diagnoses from 174 patients were correlated with the histological result of a corresponding biopsy.

Results: We found that the imprint cytology proved to be a useful, quick and reliable method with complete diagnostic accuracy, sensitivity, specificity, positive predictive value and negative predictive values of 97%, 96%, 100%, 100% and 92% respectively.

Conclusion: Imprint cytology allows diagnostic statements in a shorter time than is possible with histological sections and proves a useful adjunct in evaluating laryngeal and pharyngeal lesions. The validity of the method depends on the care with which the specimen is sampled and on the experience of the investigator.

Keywords: imprint cytology, cytodiagnosis, laryngeal neoplasms, pharyngeal neoplasms, diagnostic accuracy, sensitivity, specificity, positive and negative predictive, pathology oral, diagnosis carcinoma

Introduction

Malignancies localized in the pharynx or larynx are far too often diagnosed in an advanced stage, resulting in a relatively poor subsequent prognosis. To achieve a better survival rate, these malignancies should be diagnosed at an earlier stage. The aim of cytological diagnostic procedure is early recognition of intraepithelial lesions of the upper respiratory tract whenever it is possible to cure the disease or prevent malignant transformation.

Morrison *et al.*[1] described cytological examination of the pharynx by the smear technique in 1949. In 1951 Friedmann[2] studied exfoliative cytology as an aid in the diagnosis of tumours of the upper respiratory tract. Since then this simple technique, based on a method of collecting material from surface of lesions, has been established in differential diagnosis and follow-up of premalignant and malignant laryngeal and pharyngeal tumours.[3–6]

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Of all cytological techniques imprint cytology is the closest to pathology and has been found to be useful, quick, reliable and popular in the diagnosis of solid tumours.[7–12] In the upper respiratory tract, imprint cytology has been used only for the investigation of some aspects of Reinke's oedema.[13] We introduced touch imprint cytology to test its reliability and accuracy in preoperative management in patients with pharyngeal and laryngeal tumours, as well as the possible application of this method in diagnosis during operation.

As gold standard the histological result of a corresponding biopsy was used.

Study population

The retrospective study encompasses a 35-month period during which 174 patients with a laryngeal and pharyngeal lump, who reported for the first time in the Department of Otorhinolaryngology, were examined by pharyngo-laryngoscopy and tumour biopsy. Imprint cytology was examined at the Department of Clinical Cytology. Tumour biopsy was examined at the Department of Pathology and Forensic Medicine.

Cytopathology 2007, **18**, 40–43

The diagnostic quality reported here was reached after a prior training period of 12 months.

Methods

The same surgeon who examined fresh biopsies prepared imprint cytology. Each specimen, of average size 1–3 mm, was touched and gently rolled onto two glass slides with fine tissue forceps. The biopsy specimen was fixed in formalin solution, processed and reported after 6 days. The imprint slides were stained using Papanicolaou and May-Grünwald–Giemsa methods and reported the next day. The clinicians' decision about surgery depended on the final histology report. False negative diagnoses had no effect on the management of the patient.

The cytological diagnoses were correlated with the histological diagnoses in 174 cases, which were used as the gold standard. In evaluating the clinical efficiency of the method, we included unsatisfactory results as negative diagnoses.

The diagnostic value of imprint cytology (sensitivity, specificity, accuracy, positive and negative predictive values) was calculated according to standard formulas.[14]

Cytopathological examination resulted in the following diagnoses: (i) unsatisfactory; (ii) negative for malignancy; (iii) atypical – compatible with dysplasia; (iv) suspicious for malignancy; and (v) positive for malignancy. Localization of tumour biopsies and imprints is shown in Table 1. The histological classification was performed according to the World Health Organization classification.

Unsatisfactory cases were samples consisting of few squamous cells, blood and mucus. Cases negative for malignancy included patterns consistent with benign, hyperkeratotic and inflammatory processes. Atypical cases had dyskaryotic cellular changes compatible with dysplasia. Suspected positive cytology results showed only a few cells with malignant morphological features. Cases positive for well-differentiated, keratinizing squamous cell carcinoma, based on the identification of abnormal squamous cells with malignant nuclear criteria, included enlargement, dense hyperchromasia, angularity and dense cytoplasmic orangeophilia.

Poorly differentiated squamous carcinoma cells were often arranged in thick groups rather than as dissociated cells. The nuclei were enlarged, and chromatin was coarsely granular.

In moderately and poorly differentiated tumours, keratinization was rare and absent.

A detailed description of cellular components was done in all cases. Histological typing of the lesion was presented when possible.

Table 1. Localization of tumour biopsies and touch imprints ($n = 174$)

	Cytological diagnoses				
Localization	Negative	Atypical	Suspicious	Malignant	Total
Larynx	51	3	1	83	138
Pharynx	5	–	1	30	36
Total	56	3	2	113	174

Results

Imprint cytology was compared to histology of subsequent biopsy (Table 2).

The sensitivity, specificity, positive predictive value and negative predictive value of imprint smear results were 96%, 100%, 100% and 92% respectively. There were no false positive imprint results, and there were five false-negative cases (3%). The inadequate rate was 2.3%.

In these statistical analyses, the atypical cases were included with the negative cases and the suspicious ones were included in the positive group.

Discussion

More than 90% of all laryngeal and pharyngeal cancers, collectively known as head and neck cancers, are squamous cell carcinomas with varying degrees of differentiation (Figure 1). Apart from squamous cell and undifferentiated carcinomas, other common types include lymphoma and adenoid cystic carcinoma (Figure 2), both more common in oral and pharyngeal sites than in the larynx.[15] Non-squamous histology, including sarcomas, is quite rare. Primary involvement

Table 2. Correlation of imprint smear results with the histopathological diagnosis ($n = 174$)

	Histopathology		
Imprint cytology	Negative	Malignant	Total
Unsatisfactory	3	1	4
Negative	49	3	52
Atypical	2	1	3
Suspicious	–	2	2
Malignant	–	113	113
Total	54	120	174

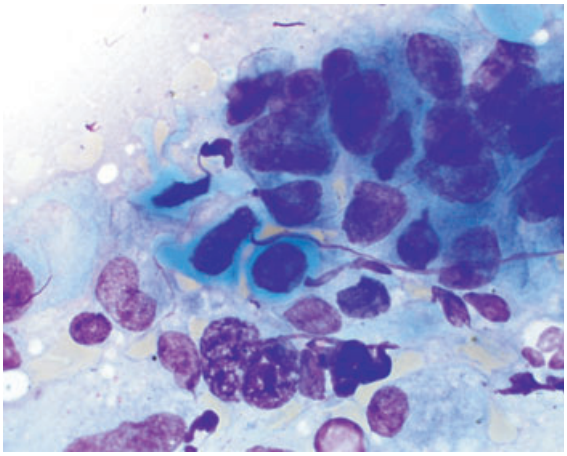

Figure 1. Keratinizing squamous carcinoma has hypercromatic nuclei with angular contours (May-Grünwald–Giemsa stain, ×1000).

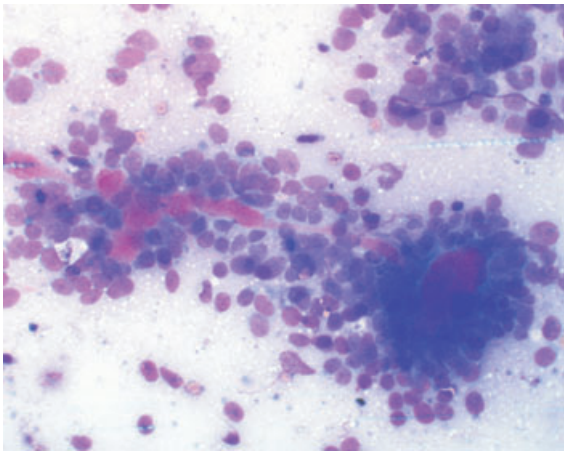

Figure 2. Adenoid cystic carcinoma. Rosette-shaped clusters of basaloid cells, with pink central mass (May-Grünwald–Giemsa stain, ×400).

of the larynx by mesenchymal tumours has been reported in case reports. Metastasis to the larynx from neoplasia elsewhere is even rarer.[16]

In our study malignant tumours were histologically squamous in origin in 96% (111/120) cases, with 61% (84/138) laryngeal carcinomas. The four other malignant tumours included adenoid cystic carcinoma (Figure 2), invasive papillary carcinoma, nasopharyngeal carcinoma (Figure 3) and metastases of malignant melanoma.

Among 59/174 cases with a histological diagnosis of a benign tumour there were three unsatisfactory imprints. Correct cytological diagnoses were made in 51 cases, and of those 49 were true negative and two

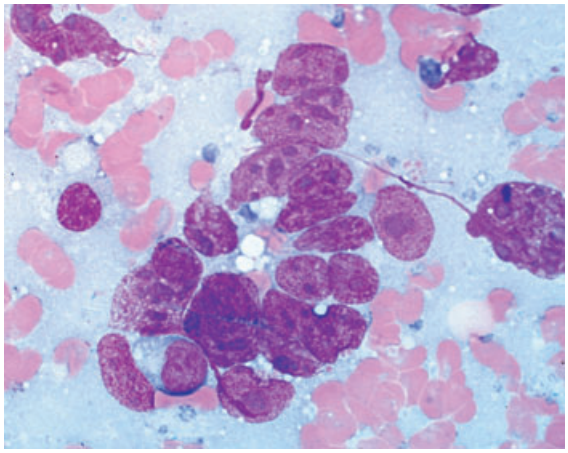

Figure 3. Nasopharyngeal carcinoma. Cluster of atypical and pleomorphic cells with prominent nucleoli. Lymphocytes, often commingled with epithelial cells (May-Grünwald–Giemsa stain, ×1000).

atypical imprints (Table 2). In two imprints mild dysplasia was diagnosed by cytology. There were no false-positive imprints.

In histologically malignant tumours (115/174), concordance with imprint cytology was found in 113 true positive and two suspected cases (Table 2). There were also five (3%) false-negative imprints, of which three were true negatives, one was atypical and one was an unsatisfactory imprint (Table 2). False negative cytology occurred in three laryngeal imprints and one hypopharyngeal imprint. In two cases of laryngeal imprints only extensive keratinization was found on cytology (Figure 4). The diagnostic problem and pitfall that could result in false-negative cytological diagnoses included highly differentiated squamous cell carcinoma with extensive keratinization, underlying malignant neoplasm with intact surface epithelium.[4] In these cases, the imprint was obviously performed with the 'wrong side' of the small biopsy. So, it was necessary to produce imprints from all surfaces of the biopsy specimen.

The hypopharyngeal imprint, from an undifferentiated nasopharyngeal carcinoma diagnosed on histology, was misinterpreted as inflammatory due to a reactive lymphoid background with scattered naked tumour nuclei. One of these five false-negative results showed only dyskaryotic cellular changes compatible with dysplasia without clearly malignant features. In our study false negative diagnoses had no effect on the management of the patients. All patients had a clinically malignant tumour, so there was no postponed appropriate therapeutic treatment.

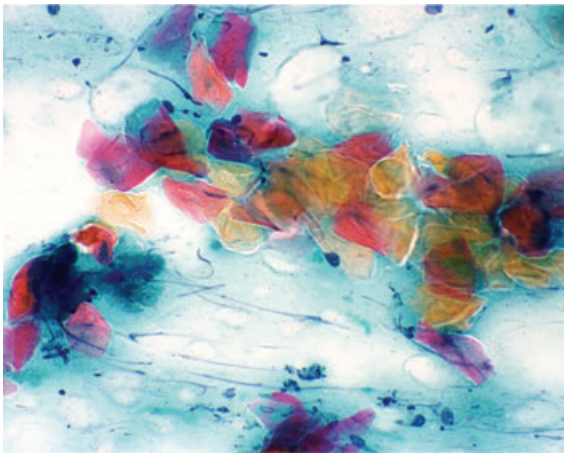

Figure 4. Extensive keratinization as cause of false-negative cytological diagnoses (Papanicolaou stain, ×400).

In three out of four unsatisfactory imprints only a few squamous cells and no malignant cells were found. In one imprint, a histologically and clinically malignant tumour, only blood and mucus were found. In cases of unsatisfactory or negative imprints on cytology in a clinically obvious malignant tumour, a repeated imprint was needed.

The sensitivity, specificity, positive predictive value, negative predictive value and diagnostic accuracy were 96%, 100%, 100%, 92% and 97% respectively. These diagnostic results were reached after a prior training period of 12 months. Diagnostic accuracy increased with experience and with higher numbers of imprint cytology, whether prepared by the surgeon or by the cytopathologists.

To reduce the rate of false negative cytology a close cooperation between clinicians and cytopathologists is needed, as well as a representative sample, careful imprint examination and an experienced investigator.

Some authors may be against the use of imprint cytology in management of patients with laryngeal and pharyngeal tumours because of the existing histological diagnosis. But in our experience imprint cytology of laryngeal and pharyngeal tumours is a reliable diagnostic method which enables faster pre-operative management in patients with malignant pharyngeal and laryngeal tumours.

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