Pathol. Oncol. Res. (2017) 23:519–523
DOI 10.1007/s12253-016-0143-9

ORIGINAL ARTICLE

# Role of Frozen Biopsy in Glottic Premalignant Lesions

**Young Ju Jin**[1] · **Woo-Jin Jeong**[1] · **Jin Ho Paik**[2] · **Soon-Hyun Ahn**[1]

Received: 24 August 2016 / Accepted: 26 October 2016 / Published online: 5 November 2016


**Abstract** Frozen biopsies are frequently used for decision making during surgery. This study aimed to evaluate the efficacy of frozen biopsy for guiding decision making before laser excision of glottic premalignant lesions. One hundred patients with 119 laser excisions were included in this study and reviewed retrospectively. After frozen biopsy, type I or II cordectomy was performed and the frozen result and final pathology of the excisional specimen were compared. The positive predictive value of frozen biopsy when the diagnosis is benign or malignant was relatively high (80.8 and 88.9 %, respectively) but the positive predictive value of a dysplasia or carcinoma *in situ* result was quite low (18.2 and 16.7 %). Under-diagnosis was frequent for dysplasia or carcinoma *in situ* (69.7 and 83.3 %). In particular, for lesions with suspicious features, lesions with dysplasia or carcinoma *in situ* had a much higher rate of under-diagnosis (81.8 and 100 %). Frozen biopsy was not reliable because the overall coincidence rate between final pathology and frozen biopsy was 63 %. Although a frozen biopsy result of a benign or malignant result was reliable, a dysplasia or carcinoma *in situ* result on frozen biopsy had a high risk of being an under-diagnosis.

**Keywords** Glottis premalignant lesion · Glottis cancer · Laser excision · Cordectomy · Frozen biopsy

✉ Soon-Hyun Ahn
ahnsh30@snu.ac.kr

[1] Department of Otorhinolaryngology-Head and Neck Surgery, Seoul National University College of Medicine, Seoul National University Bundang Hospital, 173-82 Gumiro, Bundang-gu, Gyeonggi-do 13620, South Korea

[2] Department of Pathology, Seoul National University College of Medicine, Seoul National University Bundang Hospital, 173-82 Gumiro, Bundang-gu 13620, Gyeonggi-Do, South Korea

## Introduction

Lesions in the glottis that are suspicious for cancer or premalignant lesions require laryngomicrosurgery to acquire the biopsy specimen. Most surgeons prefer to resect the lesion completely in initial surgery,[1] but as the pathology is uncertain, repeated surgery in cases where the lesion is diagnosed as carcinoma or carcinoma *in situ* is recommended by some surgeons. [2] Concerns about the voice quality after laser cordectomy is always an important factor in decisions about treatment. Although recent papers suggest type I or II cordectomy [3] results in a comparable voice with radiation therapy,[4] some patients hesitate agree to a cordectomy and request a biopsy first and a decision thereafter. In these situations, a frozen biopsy in the operation room can be an option for aiding the surgical decision about degree of cordectomy or observation. The purpose of this study was to evaluate the accuracy of frozen biopsy for glottis premalignant lesions, to determine whether a frozen biopsy can give us valuable information during surgery.

## Materials and Methods

Patients who received laryngomicrosurgery with laser excision from June 2004 to November 2014 were included retrospectively. The patients had a vocal fold lesion suspicious for glottis cancer or a premalignant lesion and they agreed to total excision of the lesion using a laser. The frozen biopsy was performed before starting surgery. A type I cordectomy to preserve the vocal ligament was tried first but in cases with invasion to the ligament, a type II cordectomy was performed. The patients who did a biopsy only for diagnosis or for lesions involving other than the glottis were excluded. The study

**Table 1** Frozen reports from patients

| Classification | Frozen report | Number |
|---|---|---|
| Benign | | |
| | Benign | 13 |
| | Acanthotic squamous epithelium | 2 |
| | Chronic granulomatous disease | 2 |
| | Hyperkeratosis | 3 |
| | Keratosis | 6 |
| | Parakeratosis | 2 |
| | Inflamed tissue | 1 |
| Subtotal | | 29 |
| Dysplasia | | |
| | Dysplastic epithelium | 7 |
| | Atypical cell | 7 |
| | Dyskeratosis | 1 |
| | Mild dysplasia | 11 |
| | Moderate dysplasia | 7 |
| | Severe dysplasia | 8 |
| | Carcinoma *in situ* | 7 |
| Subtotal | | 48 |
| Carcinoma | | |
| | Squamous cell carcinoma | 55 |
| Total | | 132 |

*Multiple frozen biopsy result from one operation is included; Two biopsy specimen in 7 procedures and 3 biopsy specimen in 3 procedures were included

protocol was approved by the institutional ethical committee (B-1608-358-101). The preoperative laryngoscopic image was reviewed by one senior author (SH Ahn) who classified the lesion into three types. The first group included vocal cord lesions with highly suspicious features showing a mass-like erythroleukoplakia lesion suspicious as carcinoma clinically. The second group was leukoplakia with an elevated lesion that was classified as a moderately suspicious lesion. Thin and plane leukoplakia was classified as the low suspicious group. When there were multiple frozen results, the worst result was selected as the frozen result of that patient. The over-diagnosis, under-diagnosis, and correct diagnosis rate were calculated for each patient. The size of the frozen specimen was also considered as an influencing factor in the diagnosis. The statistical analysis was performed using SPSS for Windows (IBM Corp., Armonk, NY, USA).

## Results

Hundred patients (91 men and 9 women) met the inclusion criteria. A total of 119 procedures were done to these patients, and up to four repeated surgeries were performed in one patient due to recurrent lesions. The average age at the first operation was 61.2 years, range 32–92 years. The follow-up period was 44.8 months on average and ranged from 10 days to 128.3 months.

In most cases, the frozen biopsy was performed before starting the laser excision and the most suspicious area was selected for biopsy. However, in 15 (12.6 %) procedures, multiple frozen biopsies were done. Various terminologies that were used in the pathologic report of the frozen biopsy are listed in Table 1. The results were further classified as benign, dysplasia, and carcinoma, and carcinoma *in situ*.

Table 2 shows the under- or over-diagnosis according to the frozen biopsy result. The positive predictive value was 80.8 and 88.9 % for benign and carcinoma. However, this positive

**Table 2** Correctness of diagnosis according to the laryngoscopic features

| | | Benign | Dysplasia* | Carcinoma *in situ* | Carcinoma | Total |
|---|---|---|---|---|---|---|
| Highly suspicious | Frozen biopsy | 0 | 2 (9.5 %) | 2 (9.5 %) | 17 (81.0 %) | 21 |
| | Change in diagnosis (%) | - | Cancer 2 (100 %) | Cancer 2 (100 %) | *In situ* 1 (5.9 %) | |
| Moderately suspicious | Frozen biopsy | 15 (22.4 %) | 22 (32.8 %) | 2 (3.0 %) | 28 (41.8 %) | 67 |
| | Change in diagnosis (%) | Dysplasia 3 (20.0 %) | Benign 3<br>*In situ* 7<br>Cancer 11 (95.5 %) | Cancer 2 (100 %) | *In situ* 3 (10.7 %) | |
| Low suspicious | Frozen biopsy | 11 (35.5 %) | 9 (29.0 %) | 2 (6.5 %) | 9 (29.0 %) | 31 |
| | Change in diagnosis (%) | *In situ* 1<br>Cancer 1 (18.2 %) | Benign 1<br>*In situ* 1<br>Cancer 2 (44.4 %) | Cancer 1 (50.0 %) | *In situ* 3 (33.3 %) | |
| Total | | 26 | 33 | 6 | 54 | 119 |
| | Positive predictive value | 80.8 % | 18.2 % | 16.7 % | 88.9 % | |

*Chi-square test: $p = 0.005$ (likelihood ratio)

**Table 3** Correctness according to the size of the frozen biopsy specimen

| Size of biopsy | Total | Over-diagnosis | Correct diagnosis | Under-diagnosis |
|---|---|---|---|---|
| <2 mm | 36 | 6 (16.7 %) | 16 (44.4 %) | 14 (38.8 %) |
| ≥2 mm | 83 | 5 (6.0 %) | 59 (71.1 %) | 19 (22.9 %) |
| p-value | | 0.071 | 0.007 | 0.080 |
| Total | 119 | 11 (9.2 %) | 75 (63.0 %) | 33 (27.7 %) |

predictive value falls to 18.2 and 16.7 % in the dysplasia or carcinoma *in situ* results. Among 33 dysplasia results on frozen biopsy, 23 cases were diagnosed as carcinoma *in situ* or invasive carcinoma and the under-diagnosis rate was 69.7 %. Five out of 6 cases with carcinoma *in situ* result were diagnosed as invasive carcinoma at the final biopsy. The accuracy of frozen biopsy was analyzed according to the preoperative laryngoscopic features also. As expected, the lesions with highly suspicious features were diagnosed as carcinoma in all except one case, including all dysplasia or carcinoma *in situ* results on frozen biopsy. The rate of change in diagnosis was similar after a benign, carcinoma *in situ*, or carcinoma result on frozen biopsy. However, the dysplasia result showed a significantly different rate of change in diagnosis. In the moderately suspicious lesions, 21 (95.5 %) among 22 with dysplasia on frozen biopsy had a different diagnosis after excisional biopsy and 18 (81.8 %) were under-diagnosed. In the low suspicion group, the change in diagnosis occurred in 4 (44.4 %) out of 9 cases, including 3 (33.3 %) with an under-diagnosis.

Groups of frozen specimens with a size of 2 mm or greater had a significantly higher rate of correct diagnosis when compared with results from specimens less than 2 mm (71.1 vs. 44.4 %, $p = 0.007$, Table 3).

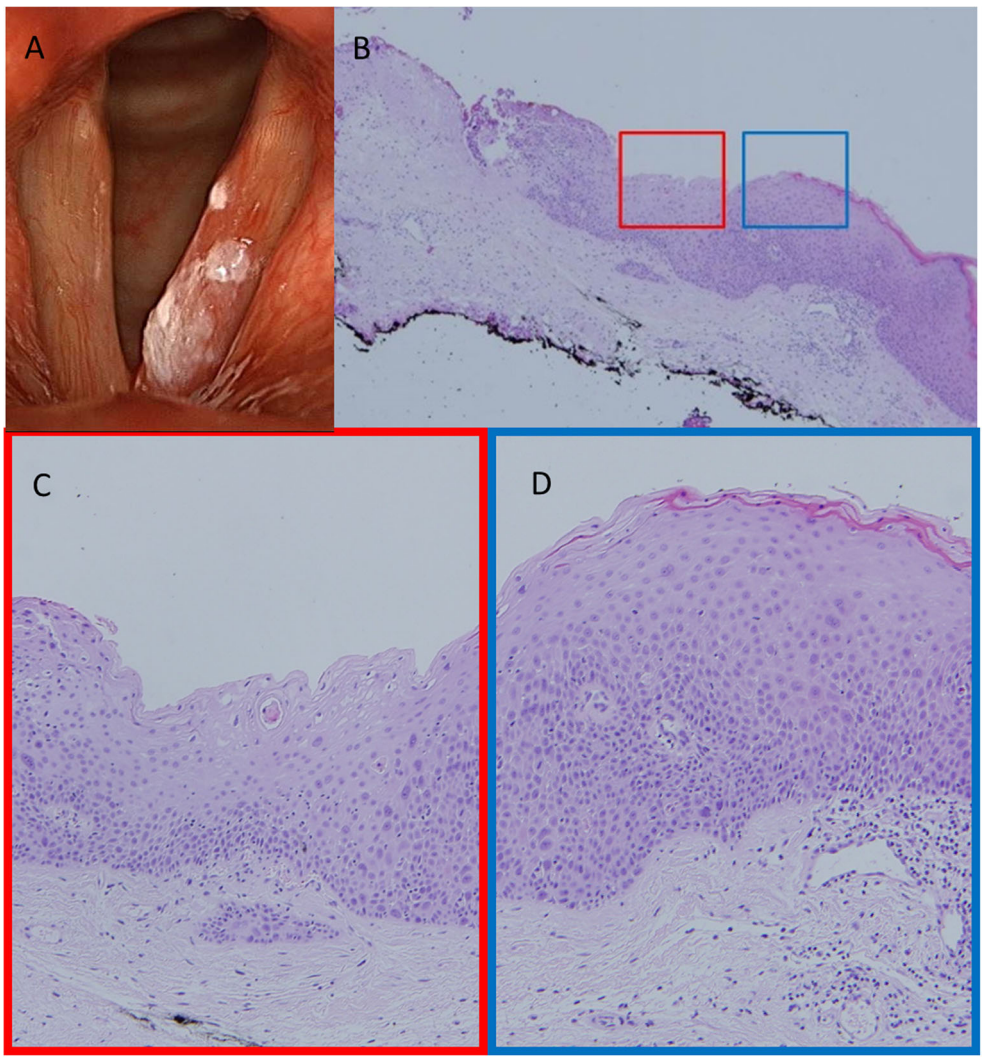


**Fig. 1** Mixed pathology in one lesion. (**a**). Laryngoscopic finding shows slightly elevated leukoplakia lesion (Moderately suspicious). (**b**). Final pathologic examination showing mixed feature including normal epithelial portion (Red box) and carcinoma portion (Blue box), (Hematoxylin & Eosin stain, X 40), (**c**). Pathologic examination showing normal epithelium (Red box, Hematoxylin & Eosin stain, X 200), (**d**). Pathologic examination predicting carcinoma with elevated cellular atypia and stromal infiltration (Hematoxylin & Eosin stain, X 200)

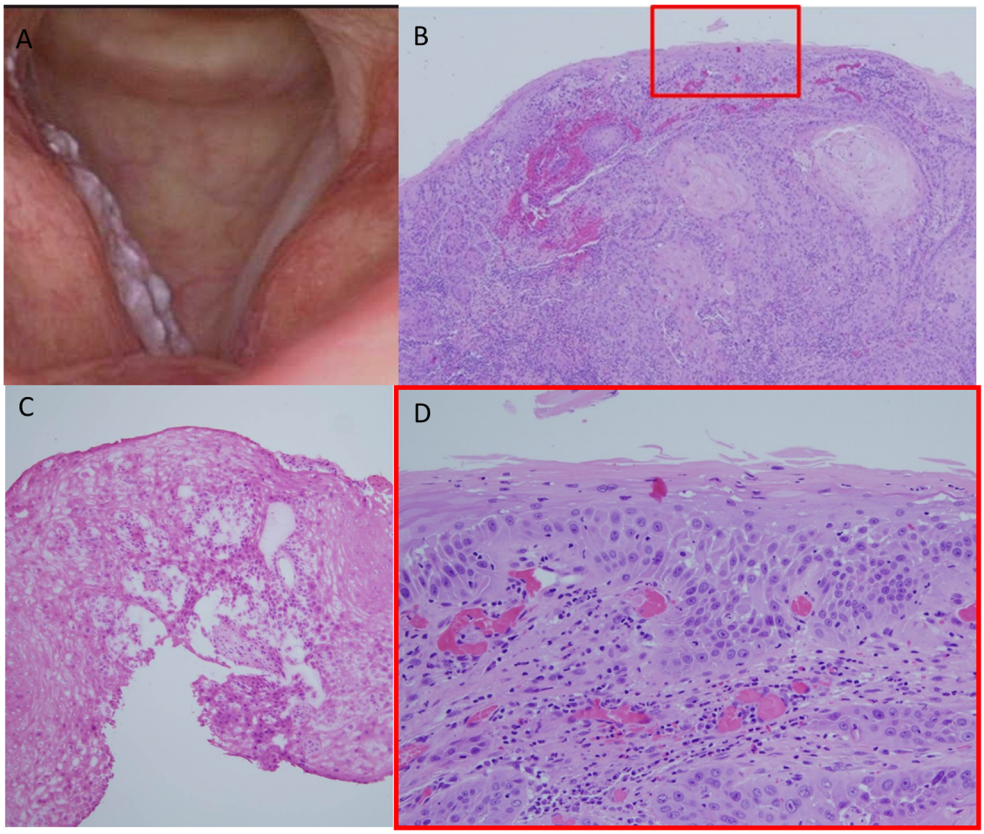


**Fig. 2** Superficially performed frozen biopsy. (**a**). Laryngoscopic finding shows a mass forming erythroleukoplakia lesion (Highly suspicious). (**b**). Final pathologic examination showing totally infiltrated cancer cell except epithelium (Hematoxylin & Eosin stain, X 40), (**c**). Frozen section's result showing absence of cancer cell (Hematoxylin & Eosin stain, X 100), (**d**). Infiltrated cancer cell at the whole layer of vocal fold except epithelium (Hematoxylin & Eosin stain, X200)

To evaluate the adequacy of this treatment strategy, the recurrence of disease was evaluated. Among 100 patients, 23 were diagnosed as benign after the first surgery and during follow up one patient developed carcinoma after 17 months. The dysplasia group including carcinoma *in situ* ($n = 21$) and the carcinoma group ($n = 56$) showed no difference in relapse free survival as calculated by Kaplan Myer analysis, with a 2-year recurrence rate of 11.2 and 13.3 %, respectively. One patient in the dysplasia group had recurrence in the supraglottis and was treated by concurrent chemoradiotherapy, and one patient in the cancer group required radiation therapy due to subglottic recurrence during follow-up, but there was no death from laryngeal carcinoma.

The comparison of frozen specimens and excisional specimens showed two reasons for discrepancy in the diagnosis of dysplasia. The differences between frozen and excisional biopsy were caused by either a different pathology was mixed into the excisional biopsy specimen (Fig. 1) or a superficially performed frozen biopsy (Fig. 2).

## Discussion

In general, the rate of transformation to malignancy is known to be significantly different between mild/moderate dysplasia and severe dysplasia/carcinoma *in situ*. The rate is reported as 10.6 and 30.4 %, respectively, according to a systematic review.[5] Due to this, some authors recommend a different treatment according to the degree of dysplasia.[1, 2, 6] However, this strategy needs repeated surgery because the degree of dysplasia can only be diagnosed after excisional biopsy. If a frozen biopsy that can be performed in an operation room can suggest the degree of dysplasia or cancer, we can decide on the appropriate procedure in one surgery. However, the accuracy of frozen biopsy in laryngeal leukoplakia is not certain.

A recent paper compared contact endoscopy and frozen biopsy results and in that series, the sensitivity and specificity of frozen biopsy in diagnosing cancer was 89.8 and 98.9 %, respectively.[7] However, the frozen result was classified dichotomically as benign or malignant without mentioning dysplasia in that study. Therefore, we do not know the diagnostic accuracy of frozen biopsy in dysplasia. If we consider the sensitivity and specificity for the diagnosis of malignancy only, our series showed 69.5 % sensitivity and 88.0 % specificity when we consider the carcinoma result only. If we take into consideration both carcinoma *in situ* and carcinoma as a diagnosis of malignancy, the sensitivity and specificity become 70.6 and 100 %, respectively. The sensitivity for benign was also quite high (84.0 %) and comparable with the literature. When considering the positive predictive value, the benign and malignancy results are 80.8 and 88.9 %, respectively,

and look reliable. However, the positive predictive value for dysplasia or carcinoma *in situ* was only 18.2 % and 16.7 %, respectively, and most cases were underdiagnosed (69.7 % for dysplasia and 83.3 % for carcinoma *in situ*). When considered along with the degree of clinical suspicion, dysplasia on frozen biopsy in the highly or moderately suspicious group was found to be carcinoma *in situ* or invasive carcinoma in 100 and 81.8 % of cases, respectively. In addition, all of the carcinomas *in situ* in these two groups were diagnosed as invasive carcinoma on excisional biopsy. The rate of under-diagnosis was significantly lower in the low suspicious group, 33.3 % of dysplasia cases were diagnosed as carcinoma *in situ* or invasive carcinoma. Although the size of the frozen specimen significantly influenced the rate of correct diagnosis, it is not always possible to get enough tissue for frozen biopsy as some leukoplakia cases of the glottis are very thin and small. Also, the pathologic comparison of the excision biopsy specimen showed that various degrees of dysplasia and cancer can coexist in one specimen. With this result, it seems that obtaining a reliable diagnosis of dysplasia on frozen biopsy may be difficult.

According to our data, the overall positive predictive value for a correct diagnosis of frozen biopsy was 63.0 % and it will be difficult to make a clinical decision based on the diagnosis from a frozen biopsy only. Therefore, as a recent paper suggests, complete excision may be the best approach to diagnosis and treatment.[8] However, if the patient does not agree to the risk of change in voice, we can consider a frozen biopsy before deciding on laser excision. When doing this, a benign or malignant diagnosis on frozen biopsy had a relatively high positive predictive value (80.8 and 88.9 %) but dysplasia in a frozen biopsy showed a low positive predictive value and a high probability of malignancy in the excisional specimen. Therefore, when a frozen biopsy result is dysplasia, especially with suspicious clinical features of malignancy, it should be treated as a malignant result.

**Compliance with Ethical Standards**

**Funding** No funding declared.

**Conflict of Interest** The Authors declare that they have no conflict of interest.

**Ethical Approval** The study protocol was approved by the institutional ethical committee (B-1608-358-101) and the formal consent was waived.

**Author Contribution** YJ Jin involved in data analysis and interpretation, drafting. WJ Jeong, JH Paik involved in conception, design of study, revision of draft, SH Ahn involved in conception, design, analysis of data and interpretation, draft and revision.

## References

1. Fleskens SA, van der Laak JA, Slootweg PJ, Takes RP (2010) Management of laryngeal premalignant lesions in the Netherlands. Laryngoscope 120(7):1326–1335. doi:10.1002/lary.20888
2. Dispenza F, De Stefano A, Marchese D, Martines F, Dispenza C (2012) Management of laryngeal precancerous lesions. Auris Nasus Larynx 39(3):280–283. doi:10.1016/j.anl.2011.08.002
3. Remacle M, Eckel HE, Antonelli A, Brasnu D, Chevalier D, Friedrich G, Olofsson J, Rudert HH, Thumfart W, de Vincentiis M, Wustrow TP (2000) Endoscopic cordectomy. A proposal for a classification by the Working Committee. Eur Laryngol Soc Eur Arch Oto-Rhino-Laryngol : Off J Eur Fed Oto-Rhino-Laryngol Soc 257(4):227–231
4. Tomifuji M, Araki K, Niwa K, Miyagawa Y, Mizokami D, Kitagawa Y, Yamashita T, Matsunobu T, Shiotani A (2013) Comparison of voice quality after laser cordectomy with that after radiotherapy or chemoradiotherapy for early glottic carcinoma. ORL; J Oto-Rhino-Laryngol Relat Spec 75(1):18–26. doi:10.1159/000346934
5. Weller MD, Nankivell PC, McConkey C, Paleri V, Mehanna HM (2010) The risk and interval to malignancy of patients with laryngeal dysplasia; a systematic review of case series and meta-analysis. Clin Otolaryngol : Off J ENT-UK; Off J Netherlands Soc Oto-Rhino-Laryngol Cervico-Fac Surg 35(5):364–372. doi:10.1111/j.1749-4486.2010.02181.x
6. Cosway B, Paleri V (2015) Laryngeal dysplasia: an evidence-based flowchart to guide management and follow up. J Laryngol Otol 129(6):598–599. doi:10.1017/S0022215115000833
7. Cikojevic D, Gluncic I, Pesutic-Pisac V (2008) Comparison of contact endoscopy and frozen section histopathology in the intraoperative diagnosis of laryngeal pathology. J Laryngol Otol 122(8):836–839. doi:10.1017/S0022215107000539
8. Mehanna H, Paleri V, Robson A, Wight R, Helliwell T (2010) Consensus statement by otorhinolaryngologists and pathologists on the diagnosis and management of laryngeal dysplasia. Clin Otolaryngol : Off J ENT-UK; Off J Netherlands Soc Oto-Rhino-Laryngol Cervico-Fac Surg 35(3):170–176. doi:10.1111/j.1749-4486.2010.02119.x