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# Percutaneous Ultrasound-Guided Fine-Needle Aspiration Cytology and Core-Needle Biopsy for Laryngeal and Hypopharyngeal Masses

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**Objective:** To evaluate the feasibility and diagnostic performance of ultrasound (US)-guided fine-needle aspiration cytology and core-needle biopsy (US-FNAC/CNB) for the diagnosis of laryngo-hypopharyngeal masses.

**Materials and Methods:** This was a single-center prospective case series. From January 2018 to June 2019, we initially enrolled 40 patients with highly suspicious laryngo-hypopharyngeal masses on laryngoscopic examinations. Of these, 28 patients with the mass involving or abutting the pre-epiglottic, paraglottic, pyriform sinus, and/or subglottic regions were finally included. These patients underwent US examinations with/without subsequent US-FNAC/CNB under local anesthesia for evaluation of the laryngo-hypopharyngeal mass.

**Results:** Of the 28 patients who underwent US examinations, a laryngo-hypopharyngeal mass was identified in 26 patients (92.9%). US-FNAC/CNB was performed successfully in 25 of these patients (96.2%), while the procedure failed to target the mass in 1 patient (3.8%). The performance of US caused minor subclinical hematoma in 2 patients (7.7%), but no major complications occurred. US-FNAC/CNB yielded conclusive results in 24 (96.0%) out of the 25 patients with a successful procedure, including 23 patients with squamous cell carcinoma (SCC) and 1 patient with a benign mass. In one patient with atypical cells in US-FNAC, additional direct laryngoscopic biopsy (DLB) was required to confirm SCC. Among the 26 patients who received US-FNAC/CNB, the time from first visit to pathological diagnosis was 7.8 days. For 24 patients finally diagnosed with SCC, the time from first visit to the initiation of treatment was 25.2 days. The mean costs associated with US-FNAC/CNB was \$272 under the Korean National Health Insurance Service System.

**Conclusion:** US-FNAC/CNB for a laryngo-hypopharyngeal mass is technically feasible in selected patients, providing good diagnostic performance. This technique could be used as a first-line diagnostic modality by adopting appropriate indications to avoid general anesthesia and DLB-related complications.

Keywords: Larynx; Hypopharynx; Laryngoscopy; Biopsy; Ultrasonography

# **INTRODUCTION**

Direct laryngoscopic biopsy (DLB) under general anesthesia is the gold standard for pathological

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This is an Open Access article distributed under the terms of the Creative Commons Attribution Non-Commercial License (https://creativecommons.org/licenses/by-nc/4.0) which permits unrestricted non-commercial use, distribution, and reproduction in any medium, provided the original work is properly cited. diagnosis in patients with a suspicious laryngeal or hypopharyngeal masses (1, 2). However, in some patients with major comorbidities involving cardiac, respiratory, or neuromuscular systems, general anesthesia would not be possible or may aggravate their underlying diseases (1, 2). Furthermore, arrangement for an operating room and evaluation for general anesthesia may delay the timing of DLB, resulting in delayed treatment for laryngeal and hypopharyngeal cancers. DLB may also be associated with exposure failure, tooth injury, and non-diagnostic results due to superficial biopsy.

Conventionally, ultrasound (US) examination has a limited capacity for the evaluation of the larynx and pharynx because these organs are covered with laryngeal framework



structures (3). Recently, the feasibility of US examination to evaluate the integrity of reconstructed pharynx and for pharyngeal fistula detection in laryngectomized patients was shown (3). Moreover, a few studies reported the usefulness of US examination and US-guided fine-needle aspiration cytology (FNAC) or core-needle biopsy (CNB) to evaluate laryngo-hypopharyngeal masses in patients with an intact larynx (2, 4-6). A study of 72 patients with cT1 and cT2 glottic cancers reported 57% US-detected glottic lesions (5). Another study of 34 cases with cT2-cT4 laryngo-hypopharyngeal cancers yielded 92.5% sensitivity and 100% specificity following successful percutaneous USguided tru-cut biopsy (2).

Although a few studies have suggested the feasibility of US examination and US-guided diagnostic procedures for the evaluation of laryngo-hypopharyngeal masses, most of those were case reports or retrospective studies, and the available data are not sufficient to popularize this procedure in actual clinical practice (4-7). In addition, there is marked uncertainty about which patients are eligible for laryngohypopharyngeal US examination and subsequent US-guided FNAC/CNB (US-FNAC/CNB).

The purpose of this study was to evaluate the feasibility and diagnostic performance of US-FNAC/CNB, with consideration of patient selection, for the diagnosis of laryngo-hypopharyngeal masses.

## **MATERIALS AND METHODS**

## Possible Accessible Sites with Consideration of US Propagation in Tissue

Based on the characteristics of US propagation in tissues, particularly in the bones, cartilages, and air, we assumed that a laryngo-hypopharyngeal mass involving or abutting the following sub-sites would be detectable by US even when it has no extra-laryngeal extension: the pre-epiglottic region, which can be depicted via thyrohyoid membrane; the paraglottic and pyriform sinus regions, which medially abut the thyroid cartilage and can be depicted through the unossified thyroid cartilage; the subglottic region that can be depicted via the cricothyroid membrane.

### Patients

This was a single-center prospective case series. The Institutional Review Board of our institution approved the study protocol (201810019) and written informed consent was obtained from all patients.

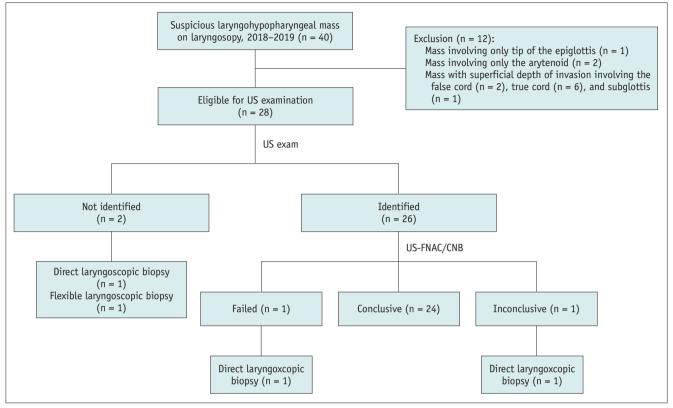
Figure 1 shows the study Standards for Reporting of Diagnostic Accuracy Studies diagram. From January 2018 to June 2019, 40 patients with untreated laryngohypopharyngeal masses that were highly suspected for malignancy on laryngoscopic examinations were initially enrolled. Of these, 12 patients (30.0%) were excluded from the study as their masses did not involve or abut the laryngeal/hypopharyngeal sub-sites that could be accessible by US or were too superficial for detection by US. These included patients who had a mass involving only the tip of the epiglottis (n = 1) and only the arytenoid (n = 2), and who had a mass with superficial depth of invasion involving the false cord (n = 2), true cord (n = 6), or subglottis (n = 1). Finally, 28 patients with non-superficial laryngohypopharyngeal masses on laryngoscopic examinations, possibly involving or abutting the pre-epiglottic, paraglottic, pyriform sinus, and/or subglottic regions, were included in the study.

#### **US Examination and US-FNAC/CNB**

All US examinations and US-FNAC/CNB procedures were performed in the outpatient department by a head and neck surgeon with 12 years of experience in interventional procedures for the head and neck region. The HS 70A US device (Samsung Medison) with a high-frequency, linear, 3-12-MHz transducer was used for all procedures. For the procedure, patients were placed in the supine position with the neck extended and the head rotated if required. First, US examination of the entire neck, including the larynx, pharynx, and lymph nodes, was performed. If a laryngohypopharyngeal mass was identified by US, subsequent US-FNAC or US-CNB was performed for pathological diagnosis. If a concurrent suspicious neck lymph node was identified by US, US-FNAC, or US-CNB for lymph node was also performed. To access the larynx and hypopharynx during FNAC or CNB, one of the following approaches was used, depending on the location of the mass: the thyrohyoid (through the thyrohyoid membrane), cricothyroid (through the cricothyroid membrane), lateral (through the lateral end of the thyroid cartilage), or trans-cartilaginous (penetrating the thyroid cartilage) approach. These four approaches were used for the supraglottic and pre-epiglottic regions, subglottic region, pyriform sinus, and glottic and paraglottic regions, respectively.

US-FNAC was performed using a non-aspiration capillary technique with a 1.5-inch 25-gauge needle (Profi Needle, Shinchang Medical), in two passes. However, suction





**Fig. 1. The Standards for Reporting of Diagnostic Accuracy Studies diagram for patient enrollment.** US = ultrasound, US-FNAC/CNB = US-guided fine-needle aspiration cytology/core-needle biopsy

aspiration technique was used when scanty cellular material was expected in the first pass. Local anesthesia was not used for the skin; however, 2 mL of 4% lidocaine was instilled into the trachea and larynx via a cricothyroid puncture, as a topical anesthesia, to prevent the cough and swallowing reflexes during the procedure (8-10). Depending on the tumor location, a long-axis or oblique-axis method was used.

US-CNB was performed using a disposable 18-gauge, double-action, spring-activated needle with a 1.1-cm excursion length (TSK Ace-cut, Create Medic). For local anesthesia, 1% lidocaine mixed with 1:100000 epinephrine was injected along the path of biopsy. Furthermore, 2 mL of 4% lidocaine was instilled into the trachea and larynx via cricothyroid puncture. The core needle was inserted into the skin in a parallel manner and advanced toward the margin, of or into the mass, under real-time US monitoring. After the biopsy route was confirmed, the stylet and cutting cannula of the needle were sequentially fired (Fig. 2, Supplementary Movie 1) (10-12). Generally, two passes were made with the core needle.

After US-FNAC/CNB, the needle puncture site was immediately compressed manually for 10–20 minutes,

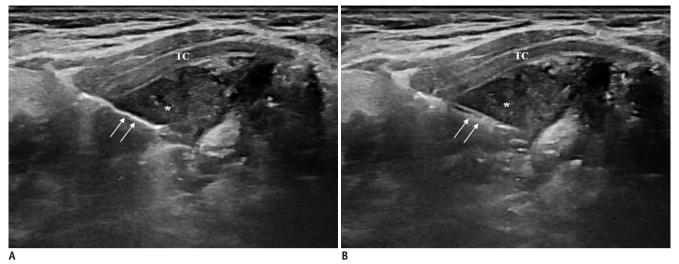
and subsequent US and laryngoscopic examinations were routinely performed to monitor for laryngeal swelling, bleeding/hematoma, and vocal cord immobility.

If FNAC/CNB failed to target the mass or to obtain conclusive pathological results, additional office-based laryngoscopic biopsy, under local anesthesia, or DLB, under general anesthesia, was performed to make a pathological diagnosis.

### **Assessment Parameters**

According to the study protocol, the patient's age and sex, and the location, side, size, and characteristics of the mass on US, such as the solidity, echogenicity, and vascularity, were evaluated, as well as the clinical tumor (T) and nodal (N) stages based on laryngoscopic and US examinations. To evaluate the technical feasibility and diagnostic performance of the procedure, the success of targeting and the pathological results of FNAC/CNB were evaluated. Possible complications and adverse reactions after FNAC or CNB, such as dyspnea, dysphonia, bleeding/ hematoma, subcutaneous emphysema, and infection, were evaluated immediately after CNB and at the follow-up visit. The elapsed time between the first visit and pathological

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**Fig. 2. CNB via a lateral approach for right pyriform sinus cancer (asterisks).** After confirming the biopsy route, the stylet (arrows) is fired first **(A)**, followed by the cutting cannula (arrows) **(B)**. TC = thyroid cartilage

diagnosis/start of treatment, as well as cost defined as submitted charges for US-FNAC/CNB, were also assessed.

### RESULTS

# Baseline Patients' Characteristics and Mass Detection by US

Table 1 shows the baseline characteristics of the 28 enrolled patients; of whom, a laryngo-hypopharyngeal mass was identified by US in 26 (92.9% detection rate; 95% confidence interval [CI], 76.5–99.1%). No mass was identified by US in 2 patients (7.1%), due to severe ossification of the thyroid cartilage, which prevented the penetration of US into the cartilage.

#### **Results of US-FNAC/CNB and Clinical Progress**

For the 26 patients with US identified masses, US-FNAC or CNB was performed for pathological diagnosis of the mass (Table 2). Among these patients, the procedure was successfully completed in 25 patients, while it was unsuccessful in 1 patient (3.8%) in whom US-FNAC was attempted; the 25-gauge needle for FNAC could not penetrate the thyroid cartilage due to focal ossification of the cartilage at the site of the needle puncture. Therefore, the successful targeting rate of US-FNAC/CNB was 96.2% (25/26; 95% CI, 80.4–99.9%).

In the 25 patients with successful US-FNAC/CNB, the pathological results were squamous cell carcinoma (SCC) in 23 patients (92.0%), atypical cells in 1 patient (4.0%), and benignity in 1 patient (4.0%). Hence, the rate of conclusive

#### Table 1. Baseline Patients' Characteristics

Variables	Patients $(n = 28)$	
Age (years)	70.3 ± 7.9	
Sex		
Male	22 (78.6)	
Female	6 (21.4)	
Primary site		
Larynx	17 (60.7)	
Glottis	6	
Supraglottis	10	
Subglottis	1	
Hypopharynx	11 (39.3)	
Pyriform sinus	11	
Side		
Right	15 (53.6)	
Left	9 (32.1)	
Midline or bilateral	4 (14.3)	
Clinical T category		
1	2 (7.1)	
2	10 (35.7)	
3	12 (42.9)	
4	4 (14.3)	

Continuous variables are presented as mean  $\pm$  standard deviation or n (%). T category was determined according to the 8th American Joint Committee on Cancer staging system based on laryngoscopic examination. T = tumor

results for guiding appropriate treatment was 96.0% (24/25; 95% CI, 79.6–99.9%). From the initial 40 patients with suspected laryngo-hypopharyngeal mass, US-FNAC/CNB replaced DLB in 60% of patients (24/40), ultimately.

For the 23 patients diagnosed with SCC by US-FNAC/CNB, subsequent staging work-ups were performed, and they were

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### Table 2. Results of US-FNAC/CNB

Variables	Patients $(n = 26)$
Modality	
FNAC	9 (34.6)
CNB	17 (65.4)
Approach	
Thyrohyoid	6 (23.1)
Cricothyroid	2 (7.7)
Lateral	11 (42.3)
Trans-cartilage	7 (26.9)
Success of targeting	
Success	25 (96.2)
Failure	1 (3.8)
Pathological results (n = 25)	
SCC	23 (92.0)
Atypical cells	1 (4.0)
Benign	1 (4.0)
N category in patients diagnosed as SCC ( $n = 24$ )	)
0	10 (41.7)
1	2 (8.3)
2	11 (45.8)
3	1 (4.2)
Time from first visit to final pathological diagnosis (days)	7.8 ± 4.5
Time from first visit to start of treatment in patients diagnosed as SCC (days) (n = 24)	25.2 ± 3.4

Continuous variables are presented as mean  $\pm$  standard deviation or n (%). N category was determined according to the 8th American Joint Committee on Cancer staging system based on the results of US-FNAC/CNB for lymph node. CNB = core-needle biopsy, FNAC = fine-needle aspiration cytology, N = nodal, SCC = squamous cell carcinoma, US-FNAC/CNB = ultrasound-guided FNAC/CNB

primarily treated with surgery- or radiation-based therapy. For the patient with atypical cells in US-FNAC, subsequent DLB was performed, which confirmed SCC. This patient received radiation therapy. For the patient with benign results, no treatment was given, and the mass was stable during the 15-month follow-up period, without any adverse change in the mass.

Of the 24 patients finally diagnosed with SCC, initial US examinations also found suspicious neck lymph nodes in 14 (58.3%), and US-FNAC/CNB for neck lymph nodes demonstrated metastatic SCC. For the 26 patients who underwent US-FNAC/CNB, the time elapsed from their first visit to the final pathological diagnosis was  $7.8 \pm 4.5$  days. For the 24 patients finally diagnosed with laryngeal or hypopharyngeal SCC, the time elapsed from first visit to start of treatment was  $25.2 \pm 3.4$  days.

### **Complications and Costs**

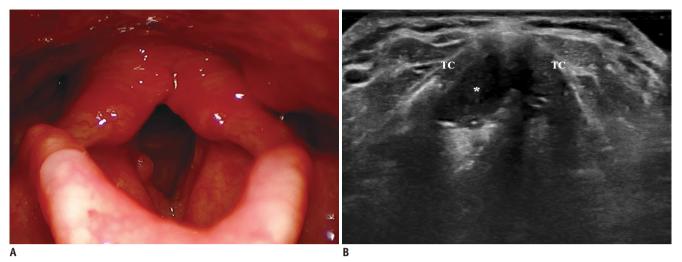
No major complications occurred during and after US-FNAC/CNB. However, subclinical minor hematoma, which was identified only on subsequent US after the procedure, was identified in 2 patients (7.7%) without signs or symptoms. The mean costs associated with US-FNAC/CNB was  $272 \pm 55$  under the Korean National Health Insurance Service System.

# DISCUSSION

In the present study, US detected 92.9% (26/28) of laryngo-hypopharyngeal masses involving or abutting preepiglottic, paraglottic, pyriform sinus, and/or subglottic regions. This rate is much higher than that of a previous study (57% detection rate for T1-T2 glottic cancers), supporting our criteria for eligibility for US examination, determined by the location and gross mass volume on laryngoscopic examinations (5). Although most previous studies on laryngeal US used clinical T staging as eligibility criterion for US examination, we believe that location and mass volume would be more important than T stage or diameter for determining eligibility for US examination (2, 5, 13). For example, in T2 glottic cancer involving both true and false vocal cords with a superficial depth of invasion, US cannot guarantee the detection of the lesion despite a sufficiently wide lesion to be categorized as T2 stage. However, in T1 glottic cancer limited to a single vocal cord, with sufficient volume of the mass to abut into the thyroid cartilage or paraglottic space, US can detect the lesion, even though the inner cortex of the thyroid cartilage or paraglottic space is not actually invaded (Fig. 3). In similar veins, T1 cancer of the pyriform sinus is more suitable for US examination than T3 cancer of the posterior pharyngeal wall, because the pyriform sinus is more accessible by US, via the thyroid cartilage.

For the 25 patients with successful US-FNAC/CNB, conclusive diagnosis to guide relevant treatment was determined in 96.0% (24/25) of patients, including 23 with SCC. These results were comparable to those of previous studies reporting 87.5–91.9% sensitivity in diagnosis of laryngeal and hypopharyngeal malignancy using US-FNAC/ CNB (2, 4, 8). Furthermore, compared with office-based flexible laryngoscopic biopsy (FLB), which has been used as the only alternative to DLB, US-FNAC/CNB sensitivity for laryngo-hypopharyngeal malignancy was superior to those of FLB (9-11). In effect, FLB sensitivity was only

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**Fig. 3. Image correlation between laryngoscopy and US.** Laryngoscopic findings of T1 glottic cancer at the right vocal cord **(A).** US can depict the exact boundary and depth of invasion of the mass (asterisk) **(B)**.

69.2–77.3% and FLB frequently yielded false-negative results, due to limitations of deep tissue harvesting (8, 9, 14). Conversely, in US-FNAC/CNB, real-time US monitoring allowed the operator to identify the exact boundary of the mass, even in submucosal tumors, along with the needle location during FNAC or CNB (10, 12, 15, 16). Furthermore, FNAC and CNB technique per se warrants harvesting specimens from internal contents of the target mass unlike in the punch biopsy technique (10, 12, 15, 16). Therefore, although FLB was used as a good alternative to DLB in laryngo-hypopharyngeal mass diagnosis, US-FNAC/ CNB would achieve better diagnostic performance than FLB, particularly when the tumor is deep-seated or has endophytic characteristics.

Another benefit of US-FNAC/CNB for laryngohypopharyngeal mass investigations, other than avoiding general anesthesia and DLB-related complications, would be to facilitate early pathological diagnosis by reducing the waiting time for a diagnostic procedure. Previous studies reported that the time to pathological diagnosis, when using DLB for upper airway lesions, was usually 40–50 days (8, 14, 17). Considering that a 4-week delay in treatment was reported to result in a significant increase in tumor volume and progression and that a 40-day delay increased the risk of local failure in head and neck SCC, delay in pathological diagnosis caused by awaiting DLB may lead to worse treatment response in patients with laryngohypopharyngeal SCC (18-20). In the present study, all US-FNAC/CNB procedures were introduced at the day of first visit for indicated patients based on the laryngoscopic

examination, and the final pathological diagnosis was completed in a mean of 8 days. Furthermore, regardless of the primary treatment modality used, treatments started within a mean of 4 weeks from the first visit in all patients, except for the patient who required subsequent DBL due to inconclusive US-FNAC results. From this point of view, US-FNAC/CNB could not only be an alternative to DLB, but could also be considered a primary diagnostic modality to enhance treatment outcomes by shortening the time of the diagnostic process. However, in real clinical practice, most US-FNAC/CNB takes place in the domain of the radiologist, and clinicians refer indicated patients to radiology departments. This process involves possible diagnostic delay, which was not taken into account in the present study. Nonetheless, given that the mean availability of the DLB under general anesthesia was one month in a previous study, US-FNAC/CNB for laryngo-hypopharyngeal mass diagnosis could still facilitate early diagnosis of the disease, if it is performed within one month or earlier than the usually available DLB schedule (14). Furthermore, in patients with laryngo-hypopharyngeal SCC, overall staging processes can be simplified by evaluating their N stage simultaneously with US examination and US-FNAC/CNB, for primary tumors.

In the seventh case in our series, we failed to target the mass with US-FNAC via a trans-cartilaginous approach. Up until this case, we used only US-FNAC due to concerns about complications, particularly bleeding and nerve injury. However, since this failure, we started using CNB and found that it is also a safe and feasible technique,



even in patients with partially ossified thyroid cartilage, and can facilitate a more accurate diagnosis by means of immunohistochemical staining. In effect, considering that CNB is used safely for masses in the thyroid, liver, and kidney, which have much higher vascularity than the larynx and pharynx, and is also used for diagnosis of neurogenic tumors, major complications (bleeding and nerve injury) would be rare (21-23). However, the larynx and hypopharynx are airway structures, and major bleeding/hematoma at these sites could lead to serious medical situations, even though it would be extremely rare. Therefore, when using US-FNAC/CNB for the larynx and hypopharynx, operators should exert considerable caution to prevent bleeding/ hematoma, along with thorough preparations for any airway emergency.

Another concern of percutaneous US-FNAC/CNB for laryngeal and hypopharyngeal lesions would be a possible risk of biopsy tract seeding. This complication is extremely rare, and systematic reviews on CNB for the assessment of various head and neck lesions have demonstrated no evidence of clinical tumor cell-seeding based on up to 7 years of clinical follow-up in 438 lesions (22). However, since there are no studies evaluating biopsy tract seeding of US-FNAC/CNB for laryngeal and hypopharyngeal tumors, these oncological safety issues should be evaluated by a larger scale study with long-term follow-up.

Although no study compared the cost-effectiveness of office-based US-FNAC/CNB to operating room-based DLB, it is plausible that an office-based procedure would be less expensive than an operating room-based procedure given the reduction in cost associated with general anesthesia. In effect, in a study comparing cost-effectiveness of inoffice biopsies versus operating room biopsies for laryngopharyngeal tumors, the cost of in-office biopsy was less than 1/4 that of operating room biopsies (\$2054 vs. \$9024) (19). In the present study, the mean cost as a submitted charge for pathological diagnosis using US-FNAC/CNB was only \$272 under the Korean National Health Insurance Service System. Even including the additional cost incurred by DLB after US-quided procedures in 2 patients due to targeting failure and inconclusive results (atypical cells), the mean cost did not exceed \$350. Considering that the mean cost of DLB is around \$600-650, under the Korean National Health Insurance Service System, in our institution, the overall cost for pathological diagnosis using US-FNAC/ CNB was markedly lower than that of DLB. Therefore, taken together with the aforementioned diagnostic performance,

US-FNAC/CNB is considered a cost-effective tool for laryngohypopharyngeal mass diagnosis in well-selected patients.

Although US-FNAC/CNB for larvngo-hypopharyngeal mass has several benefits regarding diagnostic performance, safety, and cost-effectiveness, the most critical drawback of this technique is that it cannot be performed in all patients. Indeed, from the initial 40 patients with suspected laryngohypopharyngeal masses in the present study, US-FNAC/CNB was completed in 25 (62.5%) and a conclusive result was obtained in 24 (60.0%). Therefore, US-FNAC/CNB replaced DLB in 60% of patients only, ultimately. However, these results also imply that by adopting appropriate eligible criteria for US-FNAC/CNB, DLB could be avoided in 60% of patients with laryngo-hypopharyngeal mass. Thus, even if DLB remains the gold standard for the diagnosis of larvngohypopharyngeal mass, US-FNAC/CNB could be introduced as a first-line modality in well-selected patients, with the concept of individualized diagnostic approach.

In summary, US-FNAC/CNB for the evaluation of laryngohypopharyngeal mass is technically feasible in well-selected patients, resulting in good diagnostic performance. This technique could be used as a first diagnostic modality for laryngo-hypopharyngeal mass when using appropriate indications and could thereby lead to avoidance of general anesthesia and DLB-related complications. Further larger and randomized studies are needed to confirm the results of the present study and to clarify the true benefits of US-FNAC/CNB for laryngo-hypopharyngeal mass in comparison with routine DLB and FLB.

## **Supplementary Materials**

The Data Supplement is available with this article at https://doi.org/10.3348/kjr.2020.0396.

# **Supplementary Movie Legends**

Movie 1. CNB for right pyriform sinus cancer.

### Conflicts of Interest

The authors have no potential conflicts of interest to disclose.

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