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
Figure 1 shows the study Standards for Reporting of Diagnostic Accuracy Studies diagram. From January 2018 to June 2019, 40 patients with untreated laryngo-hypopharyngeal 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 laryngo-hypopharyngeal 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 laryngo-hypopharyngeal 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 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 US-guided 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 laryngo-hypopharyngeal 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.
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
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...
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 ± 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 ± 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 ± 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 pre-epiglottic, 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

| 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 ± 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.
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 in-office 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-guided 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 laryngohypopharyngeal 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 laryngohypopharyngeal 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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REFERENCES

1. Marinone Lares SG, Allen JE. Safety of in-office laryngology procedures. Curr Opin Otolaryngol Head Neck Surg 2019;27:433-438
2. Preda L, De Fiori E, Rampinelli C, Ansarin M, Petralia G, Maffini F, et al. US-guided transcutaneous tru-cut biopsy of laryngo-hypopharyngeal lesions. Eur Radiol 2010;20:1450-1455
3. Ahn D, Lee GJ, Sohn JH. Ultrasonographic swallowing examination for early detection of neoplastic glottal fistula after salvage total laryngectomy: a preliminary study. Head Neck 2019;41:1804-1808
4. De Fiori E, Conte G, Ansarin M, De Benedetto L, Bonello L, Alterio D, et al. The role of ultrasound-guided transcutaneous tru-cut biopsy in diagnosing untreated and recurrent laryngo-hypopharyngeal masses. Eur J Radiol 2016;85:158-163
5. Kuribayashi S, Miyashita T, Nakamizo M, Yagi T, Kumita S. Utility of sonography for evaluation of clinical T1 and T2 glottic carcinoma. J Ultrasound Med 2009;28:1429-1440
6. Lopchinsky RA, Amog-Jones GF, Pathi R. Ultrasound-guided fine needle aspiration diagnosis of supraglottic laryngeal cancer. Head Neck 2013;35:E31-E35
7. Dedivitis RA, de Carvalho MB, Rapoport A. Transcutaneous fine needle aspiration biopsy of the preepiglottic space. Acta Cytol 2000;44:158-162
8. Wellenstein DJ, Schutte HW, Takes RP, Honings J, Marres HAM, Burns JA, et al. Office-based procedures for the diagnosis and treatment of laryngeal pathology. J Voice 2018;32:502-513
9. Cohen JT, Safadi A, Fliss DM, Gil Z, Horowitz G. Reliability of a transnasal flexible fiberoptic in-office laryngeal biopsy. JAMA Otolaryngol Head Neck Surg 2013;139:341-345
10. Ahn S, Jung S, Kim JY, Shin JH, Hahn SY, Oh YL. Evaluation of modified core-needle biopsy in the diagnosis of thyroid nodules. Korean J Radiol 2018;19:656-664
11. Chung SR, Baek JH, Choi YJ, Sung TY, Song DE, Kim TY, et al. The role of core needle biopsy for the evaluation of thyroid nodules with suspicious ultrasound features. Korean J Radiol 2019;20:158-165
12. Na DG, Baek JH, Jung SL, Kim JH, Sung JY, Kim KS, et al. Core needle biopsy of the thyroid: 2016 consensus statement and recommendations from Korean Society of Thyroid Radiology. Korean J Radiol 2017;18:217-237
13. Parasuraman L, Singh CA, Sharma SC, Thakar A. Ultrasonography guided fine needle aspiration cytology in patients with laryngo-hypopharyngeal lesions. Braz J Otorhinolaryngol 2020;86:237-241
14. Cohen JT, Bishara T, Trushin V, Benyamini L. Adverse events and time to diagnosis of in-office laryngeal biopsy procedures. Otolaryngol Head Neck Surg 2018;159:97-101
15. Kim SY, Chung HW, Oh TS, Lee JS. Practical guidelines for ultrasound-guided core needle biopsy of soft-tissue lesions: transformation from beginner to specialist. Korean J Radiol 2017;18:361-369
16. Lee YH, Baek JH, Jung SL, Kwak JY, Kim JH, Shin JH, et al. Ultrasound-guided fine needle aspiration of thyroid nodules: a consensus statement by the Korean Society of Thyroid Radiology. Korean J Radiol 2015;16:391-401
17. Lippert D, Hoffman MR, Danp P, McCulloch TM, Hartig GK, Dailey SH. In-office biopsy of upper airway lesions: safety, tolerance, and effect on time to treatment. Laryngoscope 2015;125:919-923
18. Chen Z, King W, Pearcy R, Kerba M, Mackillop WJ. The relationship between waiting time for radiotherapy and clinical outcomes: a systematic review of the literature. Radiother Oncol 2008;87:3-16
19. Teppo H, Hyynkangas K, Koivunen P, Jokinen K, Alho OP. Impact of patient and professional diagnostic delays on the risk of recurrence in laryngeal carcinoma. Clin Otolaryngol 2005;30:157-163
20. Hansen O, Larsen S, Bastholt L, Godballe C, Jorgensen KE. Duration of symptoms: impact on outcome of radiotherapy in glottic cancer patients. Int J Radiat Oncol Biol Phys 2005;61:789-794
21. Ahn D, Lee GJ, Sohn JH, Jeong JY. Fine-needle aspiration cytology versus core-needle biopsy for the diagnosis of extracranial head and neck schwannoma. Head Neck 2018;40:2695-2700
22. Novoa E, Gürterm N, Arnoux A, Kraft M. Role of ultrasound-guided core-needle biopsy in the assessment of head and neck lesions: a meta-analysis and systematic review of the literature. Head Neck 2012;34:1497-1503
23. Ahn D, Sohn JH, Yeo CK, Jeon JH. Feasibility of surgeon-performed ultrasound-guided core needle biopsy in the thyroid and lymph nodes. Head Neck 2016;38 Suppl 1:E1413-E1418
24. Naidu H, Noordzij JP, Samim A, Jalisi S, Grillone GA. Comparison of efficacy, safety, and cost-effectiveness of in-office cup forcep biopsies versus operating room biopsies for laryngopharyngeal tumors. J Voice 2012;26:604-606