Flexible Endoscopic Biopsy: Identifying Factors to Increase Accuracy in Diagnosing Benign and Malignant Laryngopharyngeal Pathology

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Summary: Objectives. To assess the influence that several factors, such as the amount of obtained biopsies, difficult procedures, biopsy site and the experience of the attending physician, have on accuracy of flexible endoscopic biopsy (FEB).

Materials and methods. 203 FEB procedures for benign or malignant laryngopharyngeal lesions were prospectively included. During the procedure, three representative biopsies (macroscopically containing vital tumor tissue and not only necrosis or healthy tissue) were obtained. The accuracy of each biopsy was separately analyzed. Difficulties during the procedures leading to failure of acquiring three representative biopsies were recorded and classified into tumor, patient and procedural factors. Histological results of FEB were defined correct when consistent with clinical context, additional biopsies or Positron emission tomography-computed tomography (PET-CT) revealed equivalent pathology, or the lesion was stable or resolved in >6 months follow-up.

Results. The first representative biopsy yielded a correct diagnosis in 65% of the cases. After the second representative biopsy, 78% was correctly diagnosed. The contribution of the third and fourth representative biopsies to accuracy was 3%. The overall accuracy of FEB was 85%. Difficult procedures were more likely to result in misdiagnosis, whereas biopsy site or experience of the attending physician did not influence results.

Conclusions. FEB was accurate in diagnosing laryngopharyngeal lesions when at least two representative biopsies were obtained. Accuracy of FEB could be further improved by limiting possible constraints during the procedures, for example by selecting, informing, and anesthetizing patients carefully.

Key Words: Office surgery−Topical anesthesia−Laryngeal neoplasms−Pharyngeal neoplasms−Accuracy.

INTRODUCTION

In recent years, several studies have been published addressing the safety, effectiveness, and tolerance of flexible endoscopic biopsy (FEB) under topical anesthesia for laryngopharyngeal lesions.[1−13] Traditionally, laryngopharyngeal endoscopy with biopsy performed under general anesthesia (GA) has been the standard diagnostic procedure for (suspected) head and neck malignancy. FEB, however, is a less invasive alternative. Advantages of FEB include the elimination of GA,[5] negation of patients' anatomical limitations,[14] shortened time to diagnosis,[8,15] and reduction in health care costs.[2,7] Previous studies have demonstrated that FEB is safe,[1−6,9−12] feasible,[2,3,6,7] and tolerated by patients.[1,5]

Because FEB is a relatively novel technique, its diagnostic value remains under evaluation. As the procedure is performed in the awake patient and the biopsy forceps are smaller than conventional rigid biopsy forceps, reaching the targeted lesion and obtaining a representative biopsy (i.e., assumed to consist of vital tumor tissue) can be challenging. Accuracy rates of FEB in diagnosing benign and malignant laryngopharyngeal pathology have varied widely over existing studies; that is 64% to 98%.[2−4,9,10,12,13,15] Factors that influence the diagnostic value have yet to be investigated. For example, the number of obtained biopsies and their representativeness, difficulties encountered during the procedure, biopsy site, or the experience and skill level of attending physicians could impact the diagnostic value of FEB. Therefore, the aim of this prospective study was to identify factors that could influence the accuracy of FEB in order to maximize the success of FEB in the future.

MATERIALS AND METHODS

Patient selection

This prospective cohort study was conducted from January 2016 to January 2018 at the Radboud University Medical Center, a tertiary referral center for laryngology and head and neck oncology. FEB has been the first choice for diagnostic work-up for benign and (recurrent) malignant laryngopharyngeal lesions at our center since 2012, except for patients with submucosal tumors, tumors that cannot be completely visualized using flexible endoscopy, or when at risk for a compromised airway. All consecutive patients who underwent a FEB procedure under topical anesthesia for benign or malignant laryngopharyngeal lesions were included. FEB procedures were planned on short notice for patients with suspected benign pathology. For the group of

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patients referred to our clinic with suspicion of malignancy, the FEB procedures were performed on the same day as the first oncological consultation. Procedures were undertaken with the understanding and oral informed consent of each patient. This study was conducted in accordance with the principles stated by the Helsinki Declaration.

Biopsy technique

We used a flexible transnasal esophagoscope for hypopharyngeal biopsies (EE-1580K, Pentax Medical, Uithoorn, The Netherlands) and a flexible laryngoscope for all other laryngopharyngeal biopsies (VNL-1570STK, Pentax Medical, Uithoorn, The Netherlands). Both endoscopes incorporate a 2.0 mm diameter working channel. A flexible biopsy forceps was used (Single-Use Radial Jaw four with Needle, jaw diameter 1.8 mm, Boston Scientific, Costa Rica). The topical anesthesia and FEB technique were previously described by Wellenstein et al.[1] As an alternative to lidocaine injections through the cricothyroid membrane, anesthetics were applied via the working channel of the endoscope directly onto the laryngopharyngeal mucosa. This anesthesia technique was performed using an 18-gauge catheter (Perifix-Katheter, B. Braun Melsungen AG, Germany) connected to a syringe filled with 2.5 ml of 4.3% lidocaine solution. The catheter was passed through the working channel of the endoscope. The glottis was visualized and the tip of the catheter was placed above the vocal cords. While instructing the patient to phonate as long as possible, the solution was delivered onto the vocal cords. Next, the epiglottis and base of tongue were anesthetized in the same fashion. The location of administered anesthetics varied according to the biopsy site. After the procedure, patients were advised not to eat or drink for 1 hour.

In all patients, we aimed to obtain three representative biopsies per lesion. Each biopsy was consecutively numbered and separately sent for pathological evaluation. The representativeness of each biopsy was registered as judged by the attending physician(s). A biopsy was recorded as representative when it was assumed to consist of vital tumor tissue (i.e., taken from the targeted lesion while sufficient resistance was encountered when taking the biopsy, so not only healthy tissue or necrosis was biopsied). The pathologist was blinded to the clinically estimated representativeness of the biopsy specimens. If less than three biopsies were obtained, the reason was recorded. Difficulties leading to failure of acquiring a sufficient number of specimens were classified into tumor factors (necrosis or vertical localization of the tumor that hindered a firm grab with forceps on tumor tissue); patient factors (anxiety or persistent laryngeal sensitivity, such as gagging reflex and coughing, despite repeated administration of topical anesthesia); and procedural factors (insufficient anesthetics administered, or blood or mucus hindering endoscopic view). In cases of small and superficial lesions, less than three biopsies were taken when a substantial part of the tumor was removed after one or two biopsies. All FEB procedures were performed by two physicians, usually a head and neck surgeon, a fellow or a senior resident, together with a junior resident, a physician assistant or an experienced oncology nurse.

Diagnostic work-up

In order to assess factors of influence on the diagnostic value of FEB, the accuracy [(Σ true positive + Σ true negative)/Σ total population][16] of FEB was determined. The following definitions were used for these calculations. The results from FEB were defined as true positive or true negative when these results were consistent with the clinical suspicion based on the medical history of the patient and the clinical aspect of the lesion, when additional biopsies or Positron emission tomography-computed tomography (PET-CT) revealed equivalent pathology, or when the lesion was stable or resolved in at least 6 months follow-up (Figure 1). When results were inconsistent with the clinical context, that is if benign histology was found while malignancy was suspected, the FEB procedure was repeated or rigid endoscopy with biopsies under GA was performed. Alternatively, instead of repeated biopsies, some patients underwent PET-CT in case of suspicion of recurrent malignant disease or were closely followed up in the outpatient clinic by regularly performing recorded HD-video flexible (strobo-)laryngoscopy (VNL9-CP and VIVIDEO Video Processor CP-1000; Pentax Medical, Uithoorn, the Netherlands). The patients

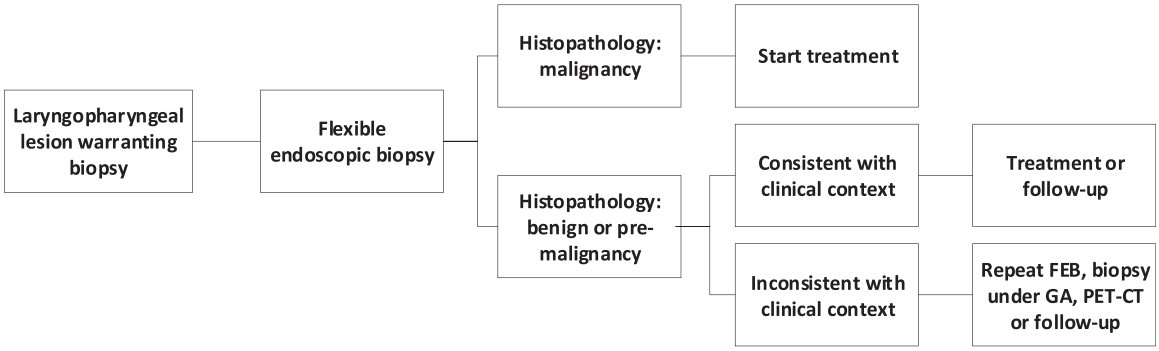

FIGURE 1. Flowchart diagnostic work-up flexible endoscopic biopsy.
Abbreviations: FEB, flexible endoscopic biopsy; GA, general anesthesia.

with inconclusive pathology who proceeded to follow-up were defined as benign cases only if follow-up was more than 6 months and the lesion did not exhibit progression. All patients with malignancies were reported in the multidisciplinary head and neck tumor board.

Data collection and analysis

We collected data regarding patient and biopsy characteristics, including sex, date of birth, biopsy site, number of obtained biopsies, biopsy representativeness, and whether difficulties occurred during the procedure. Furthermore, data on histological diagnosis for each separate biopsy and whether this was consistent with the final diagnosis, whether additional diagnostics (FEB, biopsies obtained under GA or PET-CT) were performed, and length of follow-up were recorded. Histology results were divided into two groups: the benign group consisted of reactive or infectious (eg, polyps, papilloma) histology, hyperkeratosis, leukoplakia, and mild to moderate dysplasia; the (pre-)malignant group consisted of severe dysplasia/carcinoma *in situ* (CIS) and invasive malignancy. These categories were chosen based on the clinical consequences of the histopathological results. The latter needs immediate intervention, whereas the first group can be monitored by follow-up and treated when the lesion is progressive. The name of the most experienced attending physician was recorded. For the statistical comparison, FEB procedures performed by first- to fourth-year residents were compared with FEB outcomes performed by fifth-year residents, and head and neck oncology fellows and surgeons. Statistical analyses were performed using SPSS version 22 (IBM Corporation, Armonk, NY) for Windows (Microsoft Corporation, Redmond, WA). Relative risk and chi-squared tests were used for comparative statistics.

RESULTS

During the study period, 222 consecutive procedures were performed. Of these procedures, 19 cases were excluded for the following reasons: the representativeness of biopsies was not recorded (n = 8); the lesion involved a lymphoma (n = 3); follow-up was less than 6 months in patients with benign histological diagnosis while malignancy was still suspected (n = 3); or a definitive histological diagnosis was lacking (eg, if FEB was inconclusive and the additional biopsy procedure was cancelled because distant metastases were found [n = 5]). In total, 203 FEB procedures performed in 184 patients were included (Table 1). Nineteen patients were included twice because of (suspicion of) a second primary or recurrent tumor that developed during the study period. The study population comprised 74% men and the mean age was 66 years (standard deviation [SD] = 10, range 31 − 91). The glottic larynx was the most common biopsy site. Figure 2 demonstrates the diagnostic trajectory that has been followed by the included patients.

TABLE 1.
Patient Characteristics

	N	%	Accurate Diagnosis After Initial FEB
Patients	184		
FEB procedures	203	100	
Sex (males)	151	74.4	
Age at time of FEB (mean ± SD (range))	65.5 ±10.5 (31.5–91.0)		
Tumor site			
– *Nasopharynx*	5	2.5	5/5 (100%)
– *Oropharynx*	37	18.2	32/37 (86%)
– *Hypopharynx*	31	15.3	26/31 (84%)
– *Supraglottic larynx*	62	30.5	52/62 (84%)
– *Glottic larynx*	64	30.0	54/64 (84%)
– *Subglottic larynx*	3	1.5	3/3 (100%)
– *Neopharynx*	1	0.5	1/1 (100%)
Histopathology			
– Normal	8	3.9	
– Benign[1]	6	3.0	
– Reactive[2]	26	12.8	
– Hyperkeratosis[3]	11	5.4	
– Mild dysplasia	1	0.5	
– Moderate dysplasia	2	1.0	
– Severe dysplasia / CIS[4]	35	17.2	
– Invasive carcinoma[5]	114	56.2	

Abbreviations: FEB, flexible endoscopic biopsy; SD, standard deviation.
1: Cysts, papillomas; 2: Ulcera, necrosis, hyperplasia; 3: Hyperkeratosis, leukoplakia; 4: Carcinoma in situ; 5: Squamous cell carcinoma, chondrosarcoma (n = 1).

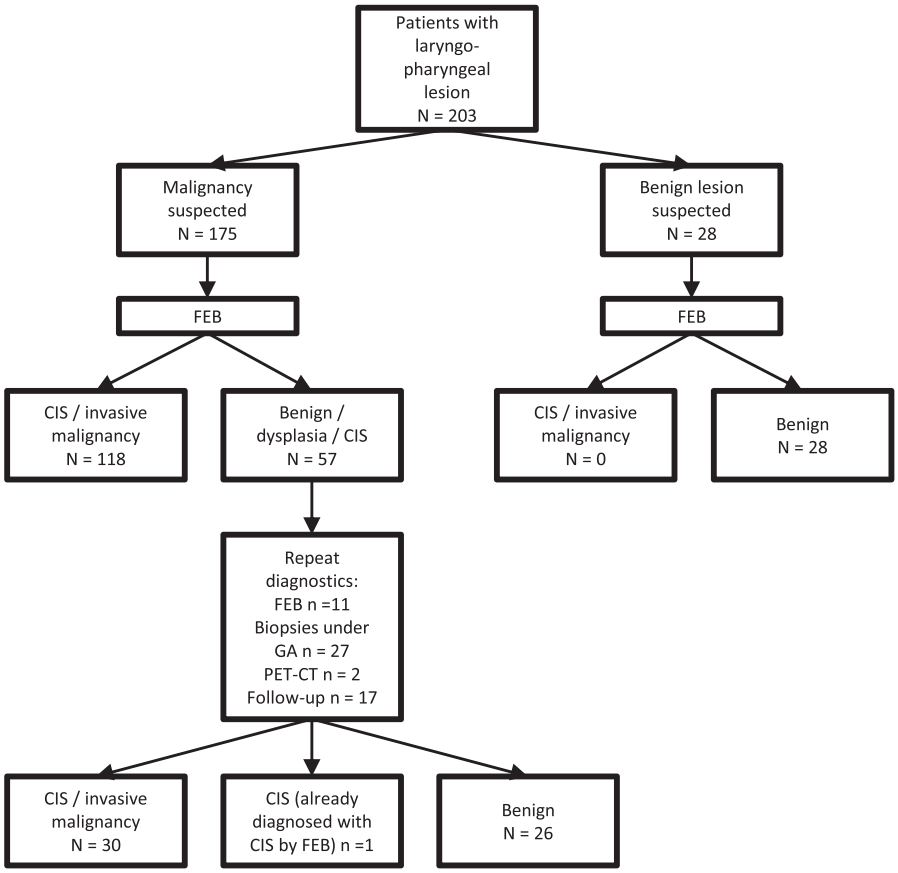

FIGURE 2. Flowchart of diagnostic trajectory followed by the included patients.
Abbreviations: FEB, flexible endoscopic biopsy; GA, general anesthesia.

The mean number of biopsies obtained per procedure was 3.1 (SD 1; range 1−6). In a total of 622 biopsies, 556 (89%) were defined as representative according to the attending physician(s). Non-representative biopsies were more likely to result in misdiagnosis compared with representative biopsies (RR 1.94 [95% CI 1.53−2.46]). When analyzing the accuracy of the first, second, third and fourth representative biopsy separately, accuracy rates of respectively 65%, 74%, 69% and 67% were found. When cumulating the results of the first and second biopsy, the second biopsy led to an increase of 13% in newly and correctly diagnosed patients. Thus, 78% of the included patients were correctly diagnosed after the second representative biopsy (Figure 3). The contribution of the third and fourth representative biopsy to the overall accuracy was 3%. In seven cases, the correct diagnosis followed from a non-representative biopsy. Overall, in 85% of the patients FEB was accurate in diagnosing benign and (pre-)malignant pathology. The sensitivity of FEB to detect CIS and malignancy was 80% and the specificity was 100% (Table 2). The positive predictive value was 100% and negative predictive value was 64%.

Difficult procedures (n = 28) resulted in more misdiagnoses (RR 2.68 [95% CI 1.37−5.24]) than unconstrained procedures. The chance of obtaining a correct diagnosis was not affected by biopsy site (χ^2 = 0.111, degrees of freedom 2) or the extent of experience of the attending physicians (RR = 0.98 [95% CI 0.84−1.14]). Additionally, surgeon's experience did not relate to the percentage of difficult procedures, as both groups of physicians encountered difficulties in 13% of the procedures (RR 0.98 [95%CI 0.37−2.6]).

DISCUSSION

In this study, FEB was accurate in diagnosing benign and malignant laryngopharyngeal lesions in 85% of the patients. To achieve satisfactory accuracy, at least two representative biopsies were required. We also demonstrated that difficult procedures led to a higher chance of inaccurate diagnosis, however, biopsy site and level of experience of the attending physician did not influence accuracy.

We found that a second representative biopsy was required to achieve an acceptable accuracy rate of 78%. In a previous study, in which only one biopsy was obtained in most cases, the reported accuracy was only 69%.[3] A previous report by our study group, in which a median of three biopsies per procedure was obtained, yielded an accuracy of 93% for CIS and squamous cell carcinoma.[15] In line with our results, this indicates that obtaining more than one

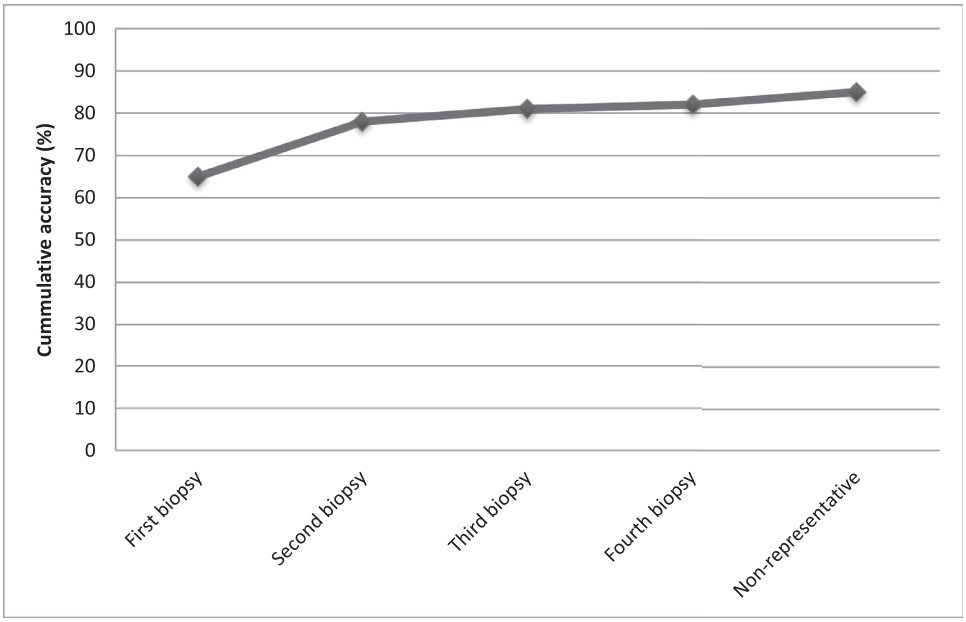

FIGURE 3. Cumulative accuracy per consecutive representative biopsy obtained during the initial FEB procedure (n = 203).

biopsy per FEB procedure leads to a higher diagnostic value. The possible explanation for this finding may relate to the size of the specimen, given that flexible forceps are smaller (1.8 mm) than conventional forceps used during rigid laryngoscopy under GA. In our clinic, whenever possible, the second biopsy is taken from the exact same location as the first biopsy in order to reach sufficient depth (except for superficial vocal fold lesions, which do not require much biopsy depth). However, based on the available data and literature, no causal statements can be made.

Furthermore, we found a higher risk for inaccurate diagnosis in difficult procedures. Examples of difficult procedures are procedures in which patients had a vertically localized tumor (e.g., posterior pharyngeal wall) or experienced discomfort due to anxiety or persistent laryngeal sensitivity. These findings probably explain why we found a lower accuracy (85%) than a previous study by our research group (93%).[15] Schutte et al. reported that the patients in this study were only selected for a FEB procedure if laryngoscopy was easily tolerated.[15] Currently, in our clinic, all patients with suspected benign and malignant laryngopharyngeal lesions initially undergo FEB after comprehensive instructions, unless a contraindication exists (ie, submucosal tumor, nasal cavity too narrow for the laryngoscope with working channel to pass through, incompletely visible tumor boundaries, or high risk of a compromised airway). Indications for FEB were stretched due to advantages of FEB compared to evaluation in GA. In other words, patient selection is less stringent in our study. This could lead to an increase in difficult procedures and thereby in lower accuracy. Our findings might better reflect true accuracy, as our population does not suffer from selection bias. In conclusion, our findings highlight that having a well-selected, well-informed and well-anesthetized patient increases the chance of success.

Differences in materials and equipment used for the performance of FEB is a potential factor of influence on accuracy that could not be assessed in this study. In this study, high-quality digital endoscopes were used; while in some other studies fiber-optic laryngoscopes were used,[3] resulting in lower image quality which might impedes the course of the procedure. Furthermore, the type and quality of biopsy forceps may influence the sampling success.[6] We used a flexible biopsy forceps that contains a needle in the middle when it is held in opened position. This leads to a better grip on the targeted tissue, in our experience. However, when comparing accuracy of studies using a similar biopsy

TABLE 2.
Accuracy of Initial FEB Procedure

	Final Result Positive for (Pre-) malignancy*	Final Result Negative for (Pre-) malignancy*	Total
FEB positive for (pre-)malignancy*	119	0	119
FEB negative for (pre-)malignancy*	30	54	84
Total	149	54	203

* (pre-)malignancy was defined as carcinoma in situ and invasive carcinoma.*Abbreviations:* FEB, flexible endoscopic biopsy.

forceps (64% [2]−93% [15]) with studies using a forceps without needle(69% [3]−83% [10]), no trend could be found.

Furthermore, inter-rater variability among pathologists analyzing the specimens could skew accuracy. Literature confirms that difficulties in diagnosing precursor laryngeal lesions exist due to significant interobserver variability.[17,18] As we used a two-grade system for this study, the inter-rater variability is likely to be reduced.[19] The scope of our study was not sufficient to analyze this possible factor of influence.

We found a PPV of 100% and a NPV of 64%. Compared to literature, the PPV is generally high (98% [3]−100% [7,10,15]), but the NPV can be disappointing: 33% [15], 20% [7], 57% [3]. These low values are likely to be the result of a selected study population of predominantly patients with high clinical suspicion of a malignancy, as the NPV is strongly dependent on the number of included patients with benign diagnoses. The only study in which the majority of the study population was diagnosed with benign lesions (68%) describes a high NPV of 87%.[10] Also for biopsies obtained by rigid endoscopy holds that NPV is low in a predominantly oncological population, that is 44% (unpublished data).[15] For both biopsy techniques holds that additional diagnostics should be performed when a biopsy reveals benign pathology while malignancy is suspected.

A potential limitation of the study design is that not all FEB results were verified by additional biopsies obtained under GA. The effect of this potential verification bias for malignancies, however, is considered to be negligible because false-positive results are unlikely. Concerning patients with benign pathology that was not verified by biopsies under GA; we closely followed them at least for 6 months, with a mean follow-up of 12 months. Still, indolent malignant tumors might have been missed in this follow-up period. As our study group demonstrated in a previous study that FEB was as accurate in diagnosing CIS/squamous cell carcinoma as biopsies taken under GA, we deemed it unethical to expose each FEB patient to GA as well. Another possible limitation of our study design was the subjective nature of the assessment of representativeness of biopsies. Nonetheless, we demonstrated that representative biopsies resulted in more accurate diagnoses than non-representative biopsies; therefore, we believe there is a general understanding of this judgment by practitioners.

In conclusion, our findings demonstrate that accuracy of FEB is satisfactory when obtaining at least two clinically representative biopsies per lesion. To further increase accuracy, we identified factors of influence on accuracy that can be anticipated on. Having a well-selected, well-informed and well-anesthetized patient could increase the success of FEB procedures.

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