Preoperative Discrimination of Benign from Malignant Disease in Thyroid Nodules with Indeterminate Cytology Using Elastic Light-Scattering Spectroscopy

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***Abstract*—Thyroid nodules are common and often require fine needle aspiration biopsy (FNAB) to determine the presence of malignancy to direct therapy. Unfortunately, approximately 15-30% of thyroid nodules evaluated by FNAB are not clearly benign or malignant by cytology alone. These patients require surgery for the purpose of diagnosis alone; most of these nodules ultimately prove to be benign. Elastic light scattering spectroscopy (ESS) that measures the spectral differences between benign and malignant thyroid nodules has shown promise in improving preoperative determination of benign status of thyroid nodules. We describe the results of a large, prospective, blinded study validating the ESS algorithm in patients with thyroid nodules. An ESS system was used to acquire spectra from human thyroid tissue. Spectroscopic results were compared to the histopathology of the biopsy samples. Sensitivity and specificity of the ESS system in the differentiation of benign from malignant thyroid nodules are 74% and 90% respectively, with a negative predictive value of 97%. These data suggest that ESS has the potential for use in real time diagnosis of thyroid nodules as an adjunct to FNAB cytology.**

***Index Terms*—Elastic light scattering spectroscopy, thyroid, indeterminate nodules, diagnosis, real-time**

I. Introduction

Thyroid nodules are common. While thyroid cancer is the most common endocrine malignancy, most nodules are in fact usually benign[1]. Current diagnostic methods have a modest sensitivity but poor specificity for cancer. The current gold standard for evaluation of a thyroid nodule is fine needle aspiration biopsy (FNAB) with cytological evaluation [2, 3]. Ultrasonographically guided FNAB yields cytologic material that is either nondiagnostic (10-15%) or diagnostic (85-90%)[4, 5]. Diagnostic aspirations are defined as benign (60-70%), malignant (5-10%) or in the category previously called "indeterminate" (15-30%) which includes three subtypes: "atypia (or follicular lesion) of undetermined significance," "follicular neoplasm or suspicious for follicular neoplasm," and "suspicious for malignancy [6, 7]." Patients with the diagnosis of malignancy require surgery. Patients with nondiagnostic FNA cytology often undergo repeat FNAB with ultrasound guidance if not performed previously and may necessitate surgery for diagnostic purposes if the repeat studies do not yield improved information. Patients with benign cytology may undergo surgery for indications including compressive symptomatology. The most concerning group are those patients with indeterminate findings, who require surgery for the purpose of diagnosis alone[8]. Only a minority of these patients ultimately are found to have thyroid cancer; therefore the majority will have undergone surgery for what was actually benign disease [8, 9]. These patients are exposed to the 2-10% risk of surgical complications and many require lifelong levothyroxine replacement therapy [10-14]. The surgical approach for patients known to have cancer preoperatively often involves a total thyroidectomy as per the American Thyroid Association guidelines and may also include a prophylactic or therapeutic central lymph node dissection[8]. The surgical approach for a patient with an indeterminate nodule is not usually as extensive, but may more commonly involve a lobectomy alone and require a return to the operating room for a completion thyroidectomy if the patient is found to have cancer on final histopathology. The difference is surgical management between patients known to have cancer preoperatively and those only found to have cancer postoperatively is therefore significant.

The issue regarding patients with "indeterminate" cytology is not simply an issue of sampling error; usually 4-6 passes of separate needles into separate locations in the nodule occur, and the diagnosis of "indeterminate" rests on the cytologists' interpretation of the morphology of the cells as being neither

Manuscript received October 18, 2012. This work was supported in part by a grant from the Coulter Foundation.

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clearly malignant nor benign[15]. Also, the prior thinking that it is margin assessment (as in the margin of the nodule within the thyroid gland) that is needed for determination of malignancy is more for the question of determining malignancy in patients with Hürthle cell carcinoma or follicular carcinoma[7, 16]. In fact, the most common cancer for patients with "indeterminate" cytology is papillary thyroid carcinoma, a type of thyroid cancer that does not rely on margin assessment for determination of malignancy [3, 17].

There are several techniques under investigation to improve the ability to diagnose cancerous thyroid nodules preoperatively. These include molecular analysis[18, 19], gene-expression classifiers using thyroid tissue[20-24], radiologic approaches including computed tomography and positron emission tomography[25], enhancement of ultrasonography including elastography[26-28], among many others. Most of the gene assays have not been widely applied in clinical practice and require repeat FNAB to obtain additional cellular material for a send-out test that takes weeks to return. The imaging technologies have varying exposure of patients to ionizing radiation, are expensive, and have low specificity.

Elastic scattering is a simple technology that may provide additional information about the cellular and subcellular changes in a suspicious lesion, and can be sensitive to the sub-cellular architectural features that are used to make histological diagnosis[29]. Morphologic changes are sensed and registered in a semi-quantitative manner and analyzed by computer without actually imaging the microscopic structure [30, 31]. This process potentially allows for minimally invasive and objective real time diagnosis in an operator-independent manner. This has been demonstrated in a number of additional clinical settings; preliminary data from using elastic light scattering spectroscopy (ESS) in the endoscopic diagnosis of Barrett's esophagus[32-36] and colonic polyps[37, 38] demonstrated an overall sensitivity and specificity between 70-75% and 85-90%. Oral cavity lesions[39], lymph nodes and bony neoplasms[40] have all been interrogated using ESS with promising results. Breast cancer is another active field of ESS research for tissue, tumor/resection margin, and sentinel lymph node status assessment [41-45].

Clearly there is a need for a point-of-care, more accurate and cost-effective real-time screening system to improve the preoperative diagnosis of malignancy in patients with thyroid nodules. Such a system would need to have high sensitivity and a high negative predictive value [20]. Recently, we demonstrated that a system using ESS can differentiate benign from malignant thyroid nodules with a sensitivity of 75%, a specificity of 95% and a high negative predictive value of 83% in a pilot study[46]. Here we describe the results of a large, prospective, blinded study validating this ESS classifier in patients with indeterminate thyroid nodules.

II. Materials and Methods

A. Elastic Scattering Spectroscopy System

The ESS instrumentation consists of a pulsed xenon arc lamp, an optical probe, a spectrometer, and a computer to control the various components and record the spectra. ESS involves directing short pulses (~1 ms) of white light (~320 to 920 nm) from the pulsed xenon arc lamp (Perkin Elmer, Inc., Fremont, California) through a flexible emission optical fiber (200 micron diameter, for this study) touching the tissue to be interrogated. Ultraviolet B (280 to 315 nm) and ultraviolet C (100 to 280 nm) light are filtered out to avoid any potential risk to patients. A collection fiber (also 200 micron diameter, for this study), with a fixed separation distance of 250 µm from the first fiber (center to center), collects light scattered from the nearest layers of the tissue and propagates it to the spectrometer (S2000 Ocean Optics, Dunedin, Florida), which outputs the spectrum to the laptop computer for recording and further analysis. The whole fiber assembly measures 1.5 mm in diameter, and the distal end is housed in a rigid stainless steel casing for easy handling and sterilization. The collection and recording of a single spectrum takes less than a quarter of a second, with the integration time of the detector (~20 to 40 ms per pulse), being the limiting factor.

B. System Calibration

Before any spectra of the nodules are taken, a white reference spectrum is recorded. This establishes the overall system response by recording the diffuse reflectance from a flat surface of Spectralon™ (Labsphere, Inc., North Sutton, NH), which has spectrally flat diffuse reflectance between 250 and 1000 nm. The reference spectrum allows spectral variations in the light source, spectrometer, fiber transmission, and fiber coupling to be accounted for. Each consequent nodule or normal tissue spectrum was divided by this reference spectrum to give the system-independent spectrum of the site being investigated.

C. Patient Selection and ex vivo Measurement Procedures

The study was approved by the Institutional Review Board (IRB) of the Boston Medical Center and informed consent was obtained prior to their participation from patients with thyroid lesions already scheduled to undergo thyroidectomy. ESS data was collected from sequential patients scheduled for surgical thyroidectomy, using immediate ex vivo thyroidectomy specimens in the frozen section pathology room within 5-10 minutes of surgical removal and prior to the formalin fixation. Specimens were bisected by a pathologist at site, and ESS readings were performed on either the most prominent nodule or preoperative FNA examined nodules. All nodules were bivalved, and spectra were obtained from five sites with five repetitive readings per site per nodule on average, depending on the size. At the time of measurement, the fiber probe is calibrated, and then the tip of the fiber probe is momentarily placed in contact with the suspect tissue, and the measurement is activated at the keyboard of the attached computer or with a

foot pedal. The system automatically takes a background measurement without firing the lamp, followed within 100 ms by an ESS measurement with the pulsed lamp being triggered, and then subtracts the background spectrum from the ESS spectrum. The entire measurement process is controlled by the built in computer allowing for accurate and reproducible measurements and providing a graphical display of the spectrum for inspection. Typical data acquisition and display time is less than 1 second for each site measurement.

D. Spectral Acquisition

The spectra were performed on a minimal number of planes and the thyroid specimen was color-stained for future co-registration. Hemorrhage, degenerative cyst and necrotic tissues were avoided. After the spectral data was obtained, thyroid specimens were fixed in formalin and sent for routine histopathologic processing. Our gold standard was final histopathologic diagnosis. Individual hematoxylin and eosin slides of co-registered nodules were reviewed with a pathologist blinded to the ESS results to confirm the histopathologic diagnosis to confirm that ESS readings were taken from the nodule of interest.

E. Spectral processing and Analysis

The spectra from the ESS measurements consist of ~800 pixels in the wavelength range 300nm to 800nm. All spectra were pre-processed before being analyzed. The five measurements taken at each site were averaged, smoothed, and then cropped. Smoothing is done by first using a moving average with a sliding window of a size of ten points (detector pixels), and then by averaging blocks of five points (corresponding to a spectral band of approx. 3nm). The spectra are then cropped from 330nm to 760nm, resulting in a spectrum of 126 points, to remove the regions of the spectra with low signal-to-noise ratios arising from the detector sensitivity and lower light intensity emitted by the Xenon arc lamp at the extremes of its output spectrum. Finally, each spectrum was normalized to the intensity at 650 nm, as we are interested in the spectral shape and not the relative intensity. In the set of spectra used in this analysis, no obvious outliers were detected; however, a number of spectra were found to be negatively saturated (i.e., intensity of zero) due to strong hemoglobin absorption at 420 and/or 550 nm. These spectra were removed from the subsequent analysis. Principal component analysis (PCA) was applied to the spectra to reduce the data to only those regions with large variability. Support vector machines (SVM) with linear kernels were used to classify the features extracted with PCA [38, 47, 48]. Cross validation was performed by using the "leave-one-out" technique to obtain performance estimates in the form of sensitivity and specificity. We then used the performance we obtained (sensitivity and specificity) to estimate the NPV based on an assumed incidence of 10% of thyroid cancer in the population sampled.

III. RESULTS

A total of 64 patients' thyroid nodules were sampled from sequential patients who were undergoing surgical thyroidectomy; in total, 193 ESS spectra were analyzed. This included 120 spectra from patients with benign nodules (Hurthle cell adenoma, n=11; multinodular goiter n=105, other benign pathologies n=4) versus 73 spectra from patients with malignant nodules (papillary thyroid carcinoma (PTC) n=71, micropapillary thyroid carcinoma (MPTC) n=2). The spectral differences between the unprocessed ESS spectra obtained from cancer nodules versus benign nodules demonstrated minimal variability between replicate measurements (data not shown).

Principal component analysis using linear discrimination and the "leave one out" technique was used to confirm the retrospective accuracy of our model. A sensitivity of 74%, specificity was 90%, and the negative predictive value (NPV) calculated as 97 % based on an incidence of 10%[7] was obtained for ESS diagnosis of benign versus malignant thyroid nodules. In a sub analysis of 31 malignant and 28 benign spectra from 25 patients found to have indeterminate nodules by preoperative cytology, our sensitivity was 65%, specificity was 79% and the NPV was 95% for ESS diagnosis.

TABLE I
"LEAVE-ONE-OUT" ANALYSIS FOR ESS DATA FROM ALL PATIENTS

ESS Test	HISTOPATHOLOGY DIAGNOSIS	
	Malignant	Benign
Malignant	54	19
Benign	12	108

TABLE II
"LEAVE-ONE-OUT" ANALYSIS FOR ESS DATA FOR PATIENTS WITH "INDETERMINATE" DIAGNOSES

	HISTOPATHOLOGY DIAGNOSIS	
	Malignant	Benign
Malignant	20	11
Benign	6	22

IV. DISCUSSION

This study describes the validation of an ESS system optimized to identify benign nodules in a population of patients whose cytology from FNAB are indeterminate. With the use of the ESS algorithm, the negative predictive value was 97%. The high negative predictive value for cytologically indeterminate nodules of 95% provides strong independent evidence of the performance of the ESS classifier. The high specificity of 90% for the cytologically benign lesions should be viewed with caution as this test should not be used in the analysis of samples with known benign cytologic features. These data could suggest that the ESS classifier can be useful in helping make important management decisions, including the cytology information, such as recommending watchful waiting in lieu of diagnostic surgery, as in the case of nodules with cytologically indeterminate features but benign findings on testing with the ESS classifier. In this clinical setting,

optical diagnostics may be a more efficient and easier to use technology with the capacity for high-throughput compared with cytology and molecular diagnostics. These properties would make ESS an ideal diagnostic tool in complementing FNA, which has a high sensitivity, ultimately minimizing thyroidectomies performed for the purpose of diagnosis alone. The most important goal of diagnostic tool is to have high negative predictable value so that more invasive diagnostic studies can be avoided. In our present study, ESS correctly identified 108 benign nodules yielding high specificity. When considering that fact that only 10% of the clinically significant thyroid nodules harbor malignancy in a given population, the ESS system would show even higher NPV when applied to a general population in clinical settings.

TABLE III
POSSIBLE SOURCES OF STUDY VARIABILITY

Variability	Impact on Study:	Minimization Effort:
Intra-probe	Spectral intensities	A reference was used to account for spectral variations from light source, fiber transmission, fiber coupling and spectrometer.
Intra-patient	Physiological changes of thyroid tissue	Normalization of spectra readings with reference.
Intra-nodule	Content of nodule	ESS Measurements were taken in multiple locations throughout the nodule.
Intra-site	Spectral noise	Measurements taken in a controlled environment to minimize background light.
Intra-operator	Probe stability and pressure	For each location within the nodule, 5 measurements were taken. On average, 25 readings were done per nodule.

There are limitations to our study, including the use of *ex vivo* thyroidectomy specimens and our relatively small N. We chose to use *ex vivo* thyroidectomy specimens to validate the use of ESS spectral analysis in differentiating benign from malignant nodules for several reasons. The first was that the ESS system used here was the only system available, and is a relatively large probe with a 2 mm diameter designed for use in surface measurements. A system for use *in vivo* should ideally be compatible with fine needle aspiration biopsy using a 25 g needle. We are currently testing such a system but none exists to date. We chose to use the leave-one-out cross-validation technique to obtain retrospective performance estimates in spite of its' property of being a high variance estimator because we wanted to make use of as much of the data as possible in the training set to mitigate the inherent variability and noise in our dataset. Of course, using this approach can result in optimistic results and thus a prospective validation will have to be made on a larger dataset in the future. In addition, blood, cystic degeneration and necrosis can affect the spectra; to address this we ensured careful choice of patients for inclusion, and coregistration of the ESS spectral measurements from the same nodule assessed by a conventional biopsy. A combined optical and conventional fine needle aspiration biopsy probe used with ultrasound guidance and used in combination with cytology would address this problem.

V. CONCLUSION

In summary, this study demonstrates that an ESS system can be used to identify patients with a low likelihood of cancer in a population of patients who might other require diagnostic surgery. These data suggest that combination of fine needle aspiration biopsy cytologic evaluation and a benign result on ESS testing may allow a more conservative clinical approach for patients with indeterminate cytology.

ACKNOWLEDGMENTS

This work was supported in part by a grant from the Coulter Foundation (JER, IB, and SL).

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