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ORIGINAL ARTICLE – ENDOCRINE TUMORS

Elastic Light-Scattering Spectroscopy for Discrimination of Benign from Malignant Disease in Thyroid Nodules

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ABSTRACT

Background. Thyroid cancer is the most common endocrine malignancy. The current standard of diagnosis, fine-needle aspiration biopsy, yields approximately 10–25% of indeterminate results leading to twice as many thyroidectomies for further diagnosis. Elastic scattering spectroscopy (ESS) is a new, minimally invasive optical-biopsy technique mediated by fiber-optic probes that is sensitive to cellular and subcellular morphological features. We assessed the diagnostic potential of ESS in the thyroid to differentiate benign from malignant thyroid nodules as determined by histology.

Methods. Under an IRB approved protocol, 36 surgical patients ($n = 21$ benign thyroid nodules, $n = 15$ malignant tumors) had collection of ESS data from their fresh *ex vivo* thyroidectomy specimens. Using surgical pathology as our gold standard, spectral analyses were performed using a training set; these data were used to assess the ESS diagnostic potential using the leave-one-out technique.

Results. Our test set was 75% sensitive and 95% specific in differentiating benign from malignant thyroid lesions, with a positive predictive value (PPV) of 0.92 and a negative predictive value (NPV) of 0.83.

Conclusions. The ESS can accurately distinguish benign vs malignant thyroid lesions with high PPV and NPV. With further validation ESS could potentially be used as an in situ real-time diagnostic tool or as an adjunct to conventional cytology.

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Thyroid cancer is the most common endocrine malignancy. In the United States, an estimated 37,200 new cases of thyroid cancer were diagnosed in 2009.[1] The yearly incidence has increased 2.4-fold from 1973 to 2002, mainly because of an increase in the incidence of papillary thyroid cancer (PTC).[2] This may be due to the increasing use of neck ultrasonography and other imaging modalities and the push for early diagnosis and treatment.[3,4]

The standard of care in the management of a patient with a thyroid nodule is fine-needle aspiration biopsy (FNAB) with cytological evaluation.[5] Cytological categories as outlined in the ATA Guidelines are: nondiagnostic (10–15%), benign (60–70%), malignant (5–10%), and indeterminate (10–25%).[5] While 5–10% of nodules are found to be malignant on FNA and approximately 70% are benign, up to 10–25% of FNAs are indeterminate. Patients with nondiagnostic FNA cytology should undergo repeat FNAB, with ultrasound guidance. Patients with benign cytology may undergo surgery for other indications including compressive symptomatology, but otherwise may be followed by physical examination or ultrasonography. Patients with a malignancy found on FNA require surgery, as do patients with indeterminate findings on FNA for the purpose of definitive diagnosis. Consequently, about twice as many patients who undergo thyroid surgery will have a benign nodule than a malignant lesion.

The American Thyroid Association, as well as most endocrinologists and endocrine surgeons, recommend near-total or total thyroidectomy for well-differentiated thyroid cancer.[5,6] Total thyroidectomy allows for better use of ^{131}I for postsurgical treatment, a statistically significant improved overall survival and decreased recurrence rate. Because patients with "indeterminate" thyroid nodules are not known to have the certain diagnosis of cancer preoperatively, clinical decision making incorporates surgical judgment; most surgeons prefer to treat these patients as

though they did in fact have a cancer and perform a near-total or total thyroidectomy as the initial operation, which subjects these patients to all of the risks of thyroidectomy including permanent bilateral recurrent laryngeal nerve injury requiring permanent tracheotomy and permanent hypoparathyroidism requiring calcium supplementation and its clinical consequences, as well as the need for life-time thyroid hormone replacement.

Many solitary molecular markers (e.g., galectin-3, cytokeratin, BRAF, PAX8-PPARgamma) have been evaluated to improve diagnostic accuracy for indeterminate nodules.[7–9] Large prospective studies have confirmed the ability of genetic markers (BRAF, Ras, RET-PTC) and protein markers (galectin-3) to improve preoperative diagnostic accuracy for patients with indeterminate thyroid nodules.[10–12] Likewise, mRNA-, qRT-PCR-, and microRNA-based arrays have been used to construct gene models for prediction of benign from malignant disease.[13–15] Most of these single-gene or multigene assays have not yet been widely applied in clinical practice; the results vary widely between studies and appear reliable in only a few selected laboratories. Additional diagnostic testing including 18FDG-PET scanning have been evaluated for use in differential diagnosis and appear to have relatively high sensitivity for malignancy but low specificity, although results vary among studies.[16] Clearly, a more accurate molecular- and ultrastructural-based algorithm would be useful to improve diagnostic accuracy, but it must also be cost effective.

Optical tissue diagnosis mediated by fiber-optic probes can be used to perform noninvasive or minimally invasive real time assessment of tissue pathology in situ. Elastic scattering spectroscopy (ESS) is a point spectroscopic measurement of subcellular micromorphology and cellular composition of a tissue, using a broad wavelength range (320–900 nm in our current system).[17] Markers include size and hyperchromaticity of cell nuclei, chromatin granularity, nuclear crowding, and changes in the size/density of mitochondria and other cellular organelles. ESS spectra derive from the wavelength-dependent optical scattering efficiency and the effects of changes in the scattering phase function (the angular probability distribution for scattering), caused by the optical index gradients (due to cellular and subcellular structures).

As a result of changes in nuclear size, density, granularity of the chromatin, mitochondrial swelling, and other subcellular structural changes, normal and abnormal tissues generate different scattering spectral signatures, which represent the optical-spectroscopy equivalent of histological appearances. The ESS method senses those morphology changes in a semiquantitative manner, without actually imaging the microscopic structure.[18] An important advantage of ESS is that it may provide a real-time objective and semiquantitative assessment of tissue pathology that may not require on-site special expertise and subjective image interpretation as in conventional histopathology. It is also convenient: the unit used is lightweight and mobile, and the ESS probe can be modified to fit through a conventional 21–23 gauge needle for a minimally invasive diagnosis. A measurement is rendered in less than 250 ms and can be triggered by a simple foot pedal.

In this study, our goal was to demonstrate that we can identify profiles of ESS pattern expression that can reliably differentiate benign from malignant thyroid nodules and that we can use these patterns to predict the difference between benign and malignant thyroid nodules.

METHODS

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

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.

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 were collected from sequential patients scheduled for surgical thyroidectomy, using immediate *ex vivo* thyroidectomy specimens in the frozen section pathology room within 5–10 min 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 s for each site measurement. We obtained readings from five separate sites within the nodule with five readings from each site.

Spectral Acquisition

The spectra were performed on a minimal number of planes, and the thyroid specimen was color-stained for future coregistration. Hemorrhage, degenerative cyst, and necrotic tissues were avoided. After the spectral data was obtained, thyroid specimens were fixed in formalin and sent for histopathological processing. Our gold standard was final histopathologic diagnosis, which was reviewed by an independent pathologist at the Boston University Department of Pathology. Individual hematoxylin and eosin slides of coregistered nodules were reviewed with a pathologist to confirm the histopathologic diagnosis and to confirm that ESS readings were taken from the nodule of interest.

Spectral Processing and Analysis

All the spectra used in the analysis underwent smoothing, in which each intensity point was replaced by the average of the 20 neighboring intensity points (namely, a moving average smoothing with a span of 20 points). The smoothed data were then reduced from the 1801 points, corresponding to the pixel density of the detector in the spectrometer, down to 180 intensity values to speed further manipulation. The wavelength window used was reduced from 320–920 nm to 340–900 nm 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. Each smoothed spectrum was then standardized by subtracting the mean intensity of the spectrum (i.e., the average intensity over the full spectral range) from each data point. Each point was then divided by the standard deviation of the smoothed spectrum. This method of standardization gave all the spectra a mean intensity of zero and a standard deviation equal to one. Using this standardization means that only the relative intensities across the whole wavelength range were important and not the actual light intensity, and as such the number of light pulses used to generate a spectrum did not have to be taken into account when analyzing the spectra. In the set of spectra used in this analysis, no obvious outliers were detected; however, a number of spectra (not yet standardized) were found to be negatively saturated (i.e., intensity of zero) at 420 or 550 nm, corresponding to the hemoglobin absorption peaks. In all cases the spectra were saturated only at the center of the peaks and not anywhere else. This saturation was considered to have a minimal effect on the rest of the spectrum, so these spectra were not removed from the analysis. Principal component analysis (PCA) was applied to the spectra to reduce the data to only those regions with large variability. This was carried out using the statistical package Systat. Linear discriminant analysis (LDA) was then carried out (also using Systat) on the principal components to improve on the discrimination between the benign and cancer nodules. Cross-validation was performed by using the "leave-one-out" technique.

RESULTS

A total of 180 thyroid nodule sites were sampled from 36 sequential patients who were undergoing surgical thyroidectomy; in total, 900 ESS spectra were analyzed. This included 500 spectra from 20 patients with benign nodules (Hurthle cell adenoma, $n = 5$; multinodular goiter $n = 13$, colloid nodule $n = 2$) versus 400 spectra from 16 patients with malignant nodules [papillary thyroid carcinoma (PTC) $n = 9$, follicular variant of papillary thyroid carcinoma (FVPTC) $n = 2$]. Of the 20 patients with benign nodules, 14 of them had preoperative fine-needle aspiration biopsies performed: 7 of the 14 had preoperative cytology

determined to be benign, and all 7 had benign histology; the other 7 of the 14 had preoperative indeterminate cytology and on final histology were determined to be benign. Of the 16 patients with cancer, all 16 had preoperative fine-needle aspiration biopsies performed: 9 of the 16 had preoperative cytology determined to be cancer, and all 9 had cancer on final histology; the other 7 had preoperative indeterminate cytology and on final histology were determined to be malignant.

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 accuracy of our model. The analysis was performed blinded to the benign versus malignant diagnosis. With *N* of 36, the sensitivity was 75%, specificity was 95%, positive predictive value (PPV) 92%, and the negative predictive value (NPV) of 83% for ESS diagnosis of benign versus malignant thyroid nodules (Table 1).

DISCUSSION

Our goal in this study was to verify whether ESS has the potential to reliably differentiate benign from malignant thyroid nodules. To our knowledge, our study is the first to date to use ESS optical spectroscopy to differentiate benign from malignant thyroid nodules. In our study, we have used an ESS system that has proved to be a promising method for differentiating benign versus malignant thyroid nodules with high sensitivity and specificity.

Most clinical applications using spectroscopy or microscopic imaging have been performed using laser-induced fluorescence, Raman spectroscopy, ESS, microendoscopy, and optical coherence tomography with varying success. For instance, Raman and fluorescence spectroscopic studies have yielded varying range of sensitivity and specificity between 80 and 95% in diagnosing colonic dysplasia.[19] Elastic scattering is a simple technology that may provide additional information about the cellular and subcellular changes in a suspicious lesion and is primarily sensitive to the subcellular architectural features that are used to make histological diagnosis. Furthermore, these morphology changes are sensed and registered in a semiquantitative manner and analyzed by computer without actually imaging the microscopic structure. 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 ESS in the endoscopic diagnosis of Barrett's esophagus and colonic polyps demonstrated an overall sensitivity and specificity between 70–75% and 85–90%.[19–23] Oral cavity lesions, lymph nodes, and bones have all been interrogated using ESS with promising results.[24–26] Breast cancer is another active field of ESS research for tissue, tumor/resection margin, and sentinel lymph node status assessment.[27–29] An intraoperative sentinel lymph node status assessment study with ESS revealed an average sensitivity of 84% and specificity of 91% in distinguishing cancer displaced vs normal nodes.[28,29] In these other clinical settings, optical diagnostics may be a more efficient and easier to use technology with the capacity for high-throughput compared with cytology and molecular diagnostics. In addition, as the only ESS probe currently available is a relatively large (2 mm) probe designed and used for surface measurements, there were no adverse events associated with its use *in vivo*. The FDA has therefore determined this system to be an electrical device with no significant risk. Our study showed similar success for ESS compared with studies in other organs.[19–21,25,27–30] In theory, such a model has the potential for greater utility and availability as an adjunct to fine-needle aspiration biopsy and cytologic evaluation.

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 19 of 20 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.

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 designed for use in surface measurements. A system for use *in vivo* should ideally fit through the center of a needle used for fine-needle aspiration biopsy. We are currently developing such a system, but none exists to date. In addition, blood, cystic

TABLE 1 "Leave-one-out" analysis for ESS data

ESS test	Histopathology diagnosis	
	Malignant	Benign
Malignant	12	1
Benign	4	19

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. Future prospective evaluation clearly is warranted. In summary, this strategy demonstrates the power of ESS as a technique for differentiating benign from malignant thyroid nodules. Once this model has been further validated *in vivo* we hope that it may prove useful in assisting in preoperative decision making for patients with thyroid nodules.

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