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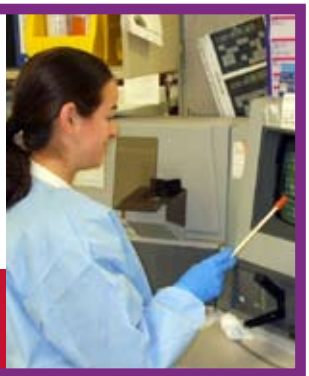

Variability in the Diagnosis of Thyroid Follicular Neoplasms

Despite advances in the use of ancillary studies such as immunohistochemical stains or molecular probes to improve the accuracy of morphologic interpretation, light microscopic evaluation of "routine" hematoxylin and eosin (H&E) stained tissue sections remains the "gold standard" for most surgical pathology specimens. As a result there is a degree of subjectivity involved in many interpretations that can produce clinically significant differences in patient management. Pathologists, as well as clinicians, recognize this fact. Difficult cases are often reviewed by additional intradepartmental pathologists in an effort to assure an accurate interpretation. In some instances, difficult cases are referred to extradepartmental "experts" with the same goal in mind. While the "expert's opinion" is generally accepted as the correct interpretation, several studies have indicated that there is often a surprising lack of concordance in the diagnoses rendered by different experts in a variety of surgical pathology settings. [1, 3]

A recently published study looked at the concordance of expert thyroid pathologists for cases of suspected follicular variant of papillary thyroid carcinoma (FVPTC).[2] Papillary thyroid carcinoma (PTC) was originally recognized on the basis of the papillary architecture supporting the lesional cells. [3] Subsequently, characteristic nuclear features of the lesional cells in PTC were recognized including nuclear chromatin clearing (optically clear nuclei or "Orphan Annie eye" nuclei), ground glass nuclei, nuclear grooves, nuclear holes, and nuclear cytoplasmic pseudoinclusions. (Images 1 – 5) With the adoption of these nuclear features as part of the diagnostic criteria for PTC, cases of malignant thyroid tumors in which the neoplastic cells (with PTC nuclear changes) were arranged in follicles rather than papillae were described as FVPTC.

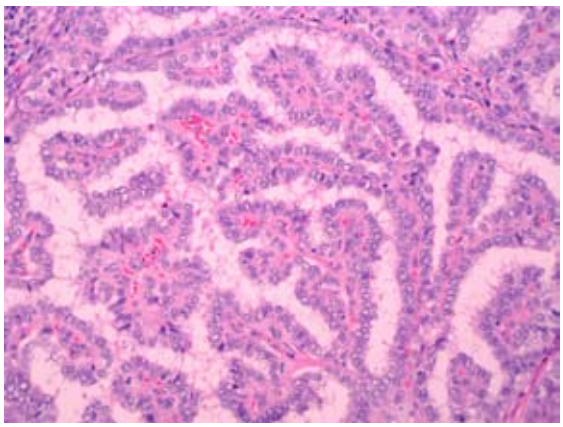

IMAGE ONE: Papillary thyroid carcinoma. Note papillary architecture and optically clear nuclei.

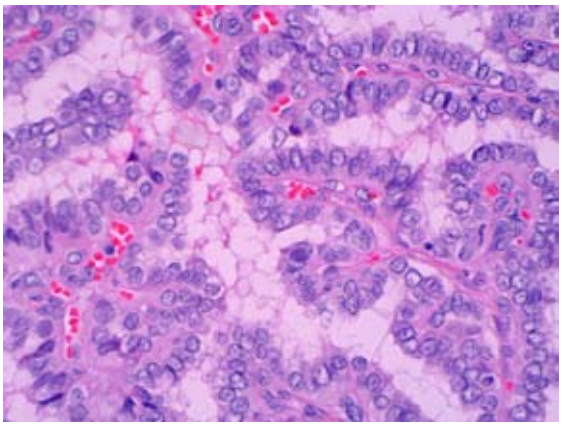

IMAGE TWO: Papillary thyroid carcinoma with optically clear ("Orphan Annie eye") nuclei.

Over time the emphasis on these nuclear changes has increased with a resultant expansion of the histologic spectrum of PTC to include a variety of configurations (encapsulated, minimally invasive or widely invasive), architectural patterns (papillary, follicular, solid, trabecular or cribriform) and cell types (small, tall, columnar, oncocytic, clear or spindled). [3] Unfortunately these nuclear changes are not entirely specific and may be observed in the setting of other thyroid lesions such as follicular adenomas, adenomatoid (colloid) nodules, and most notably, Hashimoto's thyroiditis. (Image 6) Furthermore these nuclear changes can be influenced by tissue fixation (and may not be apparent on frozen section

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slides). [4,5] Particularly problematic are cases involving a well encapsulated thyroid nodule in which a subset of the lesional cells displays PTC nuclear changes. In the past, most (if not all) of such cases would have been diagnosed as either follicular adenomas or adenomatoid nodules ("benign goiter"). The patient would be treated by lobectomy and appropriate follow-up without additional surgery or ^{131}I radiotherapy. Today a diagnosis of FVPTC may prompt total thyroidectomy with or without ^{131}I treatment, depending on the institution. Because of the "stakes" involved, such cases are often referred to "expert" thyroid pathologists for interpretation.

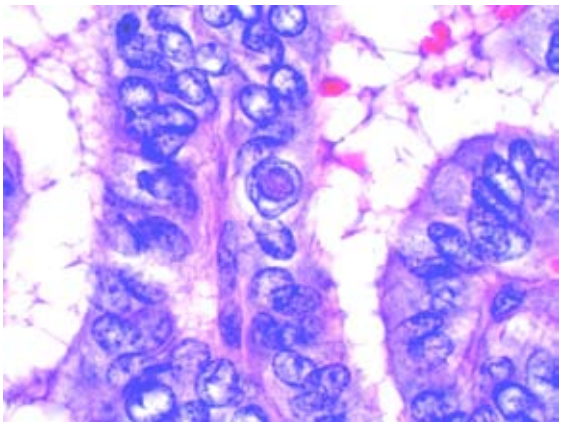

IMAGE THREE: Papillary thyroid carcinoma with optically clear nuclei and central prominent cytoplasmic pseudonuclear inclusion.

After noting some disagreement among expert pathologists on a few cases of possible follicular neoplasia sent out in consultation, a community pathologist (Tarik Elsheikh, MD) from Muncie, IN enlisted a panel of six expert thyroid pathologists to review a series of 15 thyroid surgical pathology cases where the differential diagnosis included FVPTC. [2] The panel included such luminaries as John KC Chan MD, Ronald DeLellis MD, Virginia Livolsi MD, and Bruce Wenig MD in addition to Clara Heffess MD (Chair of Endocrine Pathology at the Armed Forces Institute of Pathology). The cases were specifically chosen because they featured borderline (both qualitative and quantitative) nuclear features of FVPTC. The panelists were asked to assign one of the following diagnoses to each case: follicular adenoma, follicular carcinoma, FVPTC, or benign thyroid lesion after reviewing the histologic slides in a blinded fashion. Where interpretations of either "uncertain malignant behavior" or "suspicious for carcinoma" were given, the results were counted as follicular adenoma for statistical purposes. [2] To test intraobserver agreement, the same slides were returned to the same expert one year later with different labels and in a different order. The panelists were also asked to list in descending order the most important criteria used to arrive at a diagnosis in cases interpreted as FVPTC and to comment on the presence of capsular or vascular invasion when present. (Capsular and vascular invasion are considered crucial in the distinction of follicular carcinoma from follicular adenoma.) The patients were followed for a mean of 7.6 years (range = 7-13 years). Seven of the 15 had a completion thyroidectomy. One patient had a 1.3 cm "classic" PTC in the contralateral lobe on completion thyroidectomy. During the reported follow-up period there was no evidence of tumor recurrence or metastasis. [2]

Complete agreement among all six experts was achieved in only two of the 15 cases (13%) – a case of Hashimoto's thyroiditis and one case of FVPTC. [2] Interestingly the case unanimously interpreted as FVPTC was the only case in the series that had any possible clinical evidence of malignant behavior. (There was "classic" PTC in the contralateral lobe upon completion thyroidectomy, although it could be argued that this was not related to the index lesion.) Majority agreement (four or more panelists) on the interpretation of FVPTC occurred in six of 15 cases (40%). Separation of benign (adenoma, adenomatoid nodule, or thyroiditis) from malignant (follicular carcinoma or FVPTC) was accomplished unanimously in only four of 15 cases (27%). In eight cases (53%) there was majority agreement on a malignant diagnosis. There was considerable difference among the experts in the interpretation of FVPTC. One pathologist made this diagnosis twice (13%), while another invoked this interpretation in 14 of 15 cases (93%).

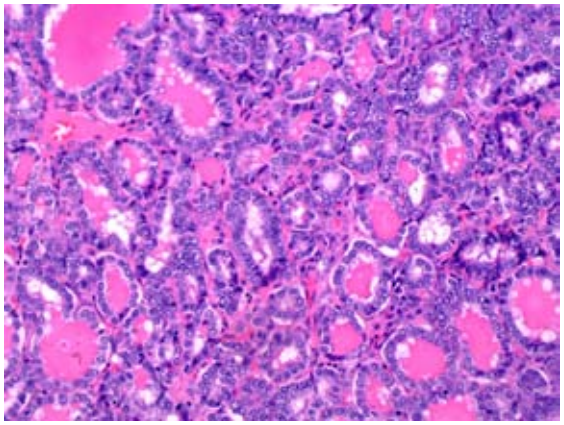

IMAGE FOUR: Follicular variant of papillary thyroid carcinoma. Note follicular architecture and optically clear nuclei.

Intraobserver agreement in the diagnosis of FVPTC ranged from 17 – 100%. Intraobserver agreement in separating benign from malignant processes ranged from 60 – 100%. The expert who had 100% intraobserver agreement with regard to FVPTC and benign vs. malignant diagnosed virtually every case as FVPTC (except for one case of Hashimoto's thyroiditis). [2] The panelists generally agreed on the relative importance of the criteria for FVPTC (nuclear clearing {"Orphan Annie Eyes"} > nuclear grooves > nuclear overlapping > nuclear irregularity), but this exercise documented the difficulties in applying some of these to actual cases.

With regard to invasive properties of the tumors, there was unanimous agreement on capsular invasion in only one of 10 cases (10%), but none on the extent (partial vs. complete) of capsular invasion or the presence of vascular space invasion. [2] Majority agreement on capsular invasion occurred in two of 10 cases (20%). Vascular space invasion was observed by a majority of experts in one of four cases.

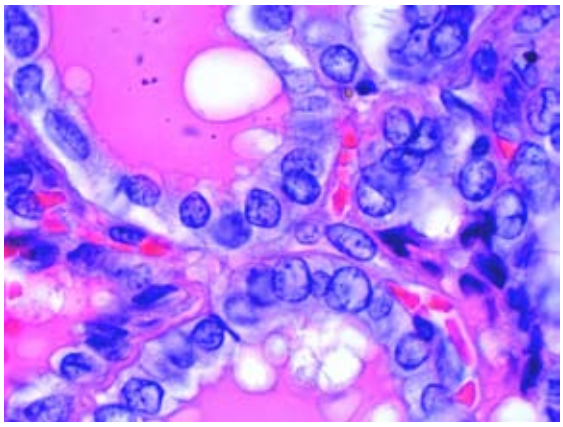

IMAGE FIVE: Follicular variant of papillary thyroid carcinoma with optically clear nuclei

The authors (which included all six panelists in addition to Dr. Elsheikh) concluded that the light microscopic evaluation of thyroid nodules has limitations due to inherent subjectivity, but remains the best method for achieving a correct diagnosis. A variety of immunohistochemical stains (various cytokeratins, CD15, CD44, CD57, fibronectin-1, HBME-1 for example) or molecular probes (BRAF, RET/PTC, ras, and microRNA overexpression) have been evaluated but lack sufficient specificity to achieve independent validation of PTC. [2]

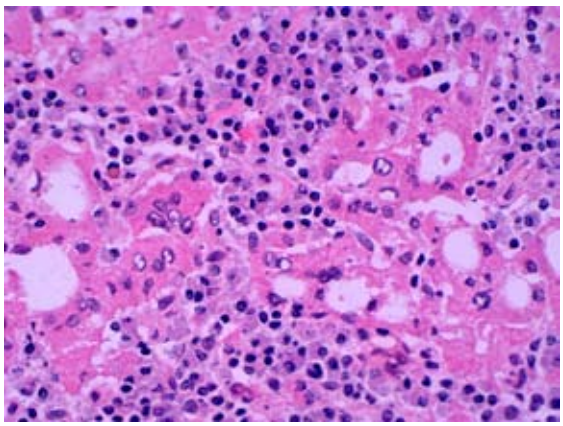

IMAGE SIX: Hashimoto's thyroiditis with scattered optically clear nuclei in follicular cells.

While it is acknowledged by many that the vast majority of these problematic lesions behave in a benign fashion, there are anecdotal reports of cases initially interpreted as follicular adenoma or adenomatoid nodule, which subsequently developed bone, lung or lymph node metastases. In these reported cases, retrospective review of the original lesion disclosed either subtle nuclear changes of FVPTC or capsular/vascular invasion suggestive of well differentiated follicular carcinoma. Given our current medicolegal climate, increased vigilance on the part of surgical pathologists has probably lowered the threshold for the diagnosis of both entities farther than the biologic behavior of such lesions requires. (For surgeons and endocrinologists, there is likewise greater incentive to err on the side of "overtreatment" than "undertreatment".) In an accompanying editorial, Dr. Juan Rosai (widely regarded as the world's greatest living surgical pathologist and a preeminent expert in thyroid pathology) reviews the history of FVPTC and points out that the nuclear changes described above, while widely applied are neither specific for nor pathognomonic of PTC. [3] He also notes that encapsulated thyroid nodules lacking diffuse nuclear changes of PTC, capsular invasion, or vascular invasion "practically never behave as cancer, in the sense that a conservative local excision (usually in the form of a lobectomy) will cure them permanently." [3] There is currently no solution to this problem which confronts practicing pathologists (expert or not) on a regular basis, although the idea of a consensus conference or revised classification scheme to try to reduce interobserver and intraobserver variation is proposed by the study authors and endorsed by Dr. Rosai. Stay tuned.

John D. Benson, M.D.

Photographic images courtesy of Dr. Keith Volmar and his new PaxCam.

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Skeletal Muscle and Nerve Biopsy

Skeletal muscle and nerve biopsies require special consideration for procurement, processing and diagnosis of neuromuscular conditions. The first step to assure appropriate processing and interpretation requires pertinent clinical information including the presence or absence of autoimmune disease, the distribution of the neuromuscular deficits, as well as laboratory and electromyography results. Copies of this information should be provided to the surgeon and accompany the biopsy to the pathology laboratory. Unfortunately, the majority of biopsies arrive in the laboratory without any clinical data other than the patient's gender and date of birth. It is also important that the physician (generally a neurologist or rheumatologist) requesting the study discuss the anatomic source of the biopsy with the surgeon to minimize the chance of "sampling error".

Skeletal muscle biopsies should include two pieces of skeletal muscle. The first should be a "dime size" (1.5 – 2.0 cm) unclamped piece of muscle taken fresh, placed onto a saline soaked Telfa pad. The second piece should be clamped in situ using either a plastic or metal muscle clamp prior to excision and subsequently placed onto a saline soaked Telfa pad. Both samples should be sent to the laboratory quickly after removal and given to either a histotechnologist or pathologist. The skeletal muscle is divided in such a way to allow full histochemical and ultrastructural evaluation. The tissue for these studies is transferred to Duke Medical Center Pathology Dept. The turnaround time for these special studies is one to two weeks, but may be longer if ultrastructural analysis is required, usually to exclude "inclusion body" myositis. A preliminary report may be generated from the Rex Pathology Department if there is sufficient tissue remaining for routine processing here at Rex.

Nerve biopsies require the use of a metal muscle/nerve clamp. The plastic clamp should never be used because of excessive artifact introduced into the tissue by the device and the difficulty of removing the nerve tissue from the device. The sural nerve is generally the specimen of choice for medical nerve biopsies. After isolating the nerve, it is clamped in situ and excised with an excess of 1.0-2.0 mm on either side of the clamp. (This "excess" tissue is used for immunofluorescence studies, while the tissue between the clamps is used for "nerve tease" preparations.) The clamped nerve biopsy should be placed onto a saline soaked Telfa pad and sent to the laboratory immediately. In the laboratory, the tissue on either side of the clamp is trimmed away from the clamped specimen and placed in Zeus media. The entire clamped nerve is placed into glutaraldehyde fixative. The entire specimen is transferred to Duke Medical Center Pathology Dept. The turn around time for this evaluation is two to three weeks. A preliminary report is not generated from the Rex Anatomic Pathology Department, since the entire nerve biopsy specimen is sent to Duke Medical Center.

To assure appropriate tissue preservation and specimen handling, muscle and nerve biopsies are best performed in the morning Monday – Thursday. Biopsies should not be scheduled on weekends or during the holiday period.

John P. Sorge, M.D.

New Laboratory Director – Stephen G. Finch

We are pleased to welcome Stephen G. Finch as the new Laboratory Director beginning January 5, 2009. Stephen comes to us after serving as the Director of Clinical Services for Emergency and Critical Care at Durham Regional Hospital since 2007. Originally from Canada, he moved to North Carolina in 1996. He worked as a staff nurse at Durham Regional Hospital from 1996 - 2003 and became Clinical Operations Director for the Emergency Dept. in 2004. He has a B.S. in Nursing from UNC-Greensboro and will receive a Masters in Health Administration from Pfeiffer University in May 2009. Under his leadership, the Emergency Dept. and Critical Care areas have expanded while increasing patient and employee satisfaction. He is a member of the Emergency Nurses Association. He and his wife Alyssa have two daughters, ages four and five. When time permits he enjoys hiking, playing hockey and golf. Stephen will assume responsibility for the day-to-day operations of the Laboratory and look to expand outreach services. Dr. Tim Carter will continue to serve as Medical Director of the Laboratory.