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### **Histopathologic Risk Factors in Retinoblastoma in India**

**Roshmi Gupta; Geeta K. Vemuganti; Vijay Anand P. Reddy; Santosh G. Honavar**

Retinoblastoma is the most common primary ocular malignancy in children. While it is possible to salvage the eye and optimize the residual vision in less advanced tumors, enucleation is still the primary treatment of choice for advanced unilateral retinoblastoma. Enucleation is also the standard approach in patients with neovascularization of iris, secondary glaucoma and anterior chamber seeds, and in those who have failed conservative therapy.

Systemic metastasis occurs in less than 10% of patients with retinoblastoma in developed countries. Postmortem studies have shown that central nervous system, orbital and cranial bones, long bones, and other viscera are the common sites for metastases. Known risk factors for systemic metastasis include involvement of the choroid, orbital tissues, and retrolaminar optic nerve and involvement of the optic nerve to the line of transection. The need to identify histopathologic risk factors (HRFs) for metastasis after enucleation and to provide appropriate adjuvant therapy has been emphasized.

The incidences of HRFs and metastasis are likely to be higher in developing countries where patients generally present with advanced disease. However, there are no data available on the exact incidence of HRFs in eyes enucleated for retinoblastoma in developing countries. We reviewed our series of patients to determine the frequency of HRFs and analyzed the related clinical features that may predict HRFs.

#### **COMMENT**

The histopathologic risk factors for metastatic spread of retinoblastoma include optic nerve invasion, particularly retrolaminar or to the transection line, and choroidal, scleral, and extrascleral (orbital) involvement. The published rates of occurrence of these factors show a wide range: 7% to 56% for invasion of retrolaminar optic nerve and optic nerve to the transaction line; 12% to 42% for choroidal involvement; and 3% to 30% for scleral and extrascleral spread. The incidence of HRFs has declined over time, possibly because of earlier detection of cases. Our combined rate of histopathologic risk factors was 54.2% (confidence interval, 47%–63%), which is much higher than that found in the recent Western literature, with the exception of 1 series. This difference may be due to comparatively late presentation of our patients, at a higher mean age, and with more advanced disease.

The degree of optic nerve invasion is associated with rate of survival; the incidence of metastasis rises sharply with retrolaminar involvement (14%–42%) and involvement to the transection line (41%–78%). In our series, 52.1% of eyes had optic nerve involvement when all degrees of invasion of retinoblastoma, including prelaminar, were considered. The higher frequency of optic nerve involvement was probably due to the fact that, in our setup, patients at first presentation had a more advanced stage of ocular disease. The mean extent of retrolaminar optic nerve involvement on histopathologic examination was 2.8 mm; this measurement cannot be directly

compared to the intraoperative optic nerve stump length, as formalin preservation would cause the tissue to shrink by about 30% to 40%. However, because 25% of eyes had retrolaminar invasion or invasion of the optic nerve to the transection line, it is advisable to obtain the longest possible optic nerve stump during enucleation.

Our case series had a 40% frequency of choroidal involvement, taking into account any degree of HRF involvement. In 18 eyes (12.7%), there was minimal involvement, with massive invasion of the choroid in 39 eyes (27.5%). We did not review serial sections of peripheral calottes for choroidal invasion, which may have yielded still higher rates of histopathologic risk factors. Our current practice is to study serial sections in eyes enucleated for retinoblastoma. Some authors have suggested that isolated choroidal invasion is not a risk factor for metastasis. However, more recent studies have found choroidal involvement to be a risk factor, especially if associated with optic nerve involvement.

On univariate analysis, we found that elevated intraocular pressure, neovascularization of the iris, and age greater than 24 months were predictors for histopathologic risk factors. The laterality of the tumor was not significantly associated with optic nerve or choroidal invasion. On multivariate analysis, only age greater than 24 months and iris neovascularization were predictive of choroidal invasion, and only iris neovascularization was associated with optic nerve invasion. Previously, Shields et al have found that iris neovascularization and glaucoma are predictors of both optic nerve and choroidal involvement. Tumor thickness greater than 15 mm and exophytic tumors have also been associated with HRFs. One would expect a larger tumor to involve more extensively the ocular structures; however, we did not find size to be significant in our study—except in anterior segment involvement—probably because most of our patients (77.5%) had massive tumors that almost filled the globe.

We also found that age at presentation greater than 24 months was a predictor for HRFs. Previously, age greater than 24 months has been associated with a greater mortality rate and more advanced Reese-Ellsworth staging but has not been correlated with HRFs.

At initial examination of CSF and bone marrow, we had only 1 positive result from 45 examinations of each of these specimens (2.2%). We request these investigations only for patients who show definite HRFs on enucleation such as choroidal, extrascleral, or retrolaminar optic nerve involvement. This low incidence is at variance with previous studies, which reported positive yields of 4.3% with CSF cytology and 9.9% with bone marrow biopsy.

For patients with HRFs on enucleation, our current practice is to administer 6 cycles of adjuvant chemotherapy with vincristine, carboplatin, and etoposide, with additional external beam radiotherapy for patients with involvement of optic nerve to the transection line or scleral and extrascleral invasion. The protocol of chemotherapy is the same as that used for chemoreduction of intraocular retinoblastoma; the protocol is changed to 12 cycles of high-dose chemotherapy for patients with involvement of the optic nerve to the transection line and scleral and extrascleral extension.

This is the first large clinical series reporting the histopathologic risk factors associated with retinoblastoma for patients in a developing country. We found a higher rate of histopathologic

risk factors and definite clinical predictors of such risk factors. Presence of any such clinical predictor in a patient warrants a high index of suspicion on the part of the pathologist as well as a meticulous evaluation of histopathology slides. The detection of the HRF and administration of appropriate adjuvant therapy may improve the chance of metastasis-free survival of the patients.

**Conclusion.**—Histopathologic risk factors are present in a significant proportion of patients enucleated for retinoblastoma and have identifiable clinical predictors.

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## **Pathology of Soft-Tissue Tumors: Daily Diagnosis, Molecular Cytogenetics and Experimental Approach**

**Hiroshi Iwasaki, Kazuki Nabeshima, Jun Nishio, et al**

This article reviews problems in diagnostic pathology and molecular cytogenetics of soft-tissue tumors. Also discussed are the origin of soft-tissue sarcomas and the molecular basis of effective target therapy for sarcomas. Molecular cytogenetic analysis of tumor-specific chromosomal translocations and associated fusion gene transcripts offers a useful adjunct to the diagnosis of soft-tissue tumors, but recent studies have indicated a growing number of fusion gene variations in each tumor type. In pleomorphic sarcoma/malignant fibrous histiocytoma, the alternative lengthening of telomeres (ALT) mechanism may result in formation of anaphase bridges and marked nuclear pleomorphism. The histogenesis of soft-tissue sarcomas has been a matter of controversy. In the present experimental model using s.c. injection of 3-methylcholanthrene in C57BL/6 mice pretreated with bone marrow-transplantation from green fluorescent protein (GFP)-positive green mice, the bone marrow-derived mesenchymal stem cells as well as the tissue-resident mesenchymal cells in the peripheral soft tissues are possible originators of sarcomagenesis. Little is known about a molecular basis of target therapy for sarcomas. Platelet-derived growth factor-BB (PDGF-BB) enhances the invasive activity of malignant peripheral nerve sheath tumor (MPNST) cells through platelet-derived growth factor receptor (PDGFR) phosphorylation, whereas imatinib mesylate inhibited such activity, suggesting that targeting PDGFR- $\beta$  may result in the establishment of novel treatment for MPNST. In addition, emmprin is a transmembrane glycoprotein on tumor cells that stimulates peritumoral fibroblasts to produce matrix metalloproteinases (MMP), playing a crucial role in tumor progression, invasion and metastasis. The MMP upregulation mechanism mediated by tumor-associated emmprin may be a potentially useful target in anti-tumor invasion therapy for sarcomas.

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## **Poorly Differentiated Carcinoma of the Thyroid Gland**

Poorly differentiated thyroid carcinomas are a heterogeneous group of tumors occupying an area intermediate between well-differentiated follicular/papillary and anaplastic carcinomas. Although they have been incorporated in the 2004 World Health Organization classification of thyroid tumors, a long debate is still going on regarding their nature, morphologic diagnostic features, clinical significance, and optimal treatment. Common pathologic features of poorly differentiated carcinomas are solid/trabecular/insular growth, large size, frequent extrathyroidal extension, extensive vascular invasion, presence of necrosis, and increased mitotic activity. They may be associated with well differentiated components of either follicular or papillary type, and less frequently with anaplastic carcinoma. Literature data, although limited by the heterogeneous case series analyzed, suggest a distinct molecular pathway in poorly differentiated carcinomas, almost exclusively involving RAS gene alterations. The present report aims to describe the various aspects of this tumor type, from morphology and immunohistochemistry to molecular insights, from a practical and daily practice oriented point of view.

## **DISCUSSION**

The existence of a group of thyroid tumors lying from a morphologic, biologic, and prognostic standpoint in between well differentiated and anaplastic carcinomas, and designated poorly differentiated thyroid carcinomas (PDTC), was first proposed independently by Sakamoto et al and by Carcangiu et al in the early 80s. However, the concept of a type of thyroid carcinoma intermediate within the spectrum of thyroid carcinomas was already incorporated into the 1974 O.E.R.T.C. classification of thyroid cancer (the “moderately differentiated” vesicular [follicular] carcinomas). The controversy that still surrounds, at least partially, the concept of poorly differentiated carcinoma occurred because its definition was based, since the original descriptions made by the respective authors, on substantially different diagnostic criteria. In fact, 2 major diagnostic approaches have been used to recognize and classify these tumors. On the one hand, they have been defined according to their growth pattern, solid/trabecular/insular areas usually being predominant among the variable histologic patterns recognized in such tumors. Conversely, some authors pointed out that poorly differentiated carcinomas are characterized by unequivocal high grade histology, with atypia, high mitotic count, and necrosis, rather than by a specific growth pattern, thus including-in some instances-high grade variants (ie, tall cell or columnar) of papillary carcinomas in the poorly differentiated tumor group. Such different views were made plainly evident from the various presentations on the subject at the meeting of the Endocrine Pathology Society that took place at the USCAP meeting in Vancouver on March 2004.

From a classification point of view, PDTCs were just mentioned in the World Health Organization (WHO) Histologic Classification of Thyroid Tumors published in 1988, whereas they were introduced for the first time as a separate entity in the most recent WHO classification of Tumors of Endocrine Organs in 2004, that aimed at proposing a uniform terminology and diagnostic criteria that consider both architecture and high-grade features.

Pathological Aspects of Poorly Differentiated Carcinoma

The present review aims at the description of the most common pathologic features of poorly differentiated carcinoma, with special emphasis on established facts that are relevant for their recognition and classification and a critical reappraisal of the most relevant controversial issues.

### Gross Pathology

Common macroscopic features of PDTCs are large size, greater than 4 cm in nearly half cases, incomplete tumor capsule with pushing borders, and frequent extension to the perithyroidal soft tissues. The cut surface is firm, gray to white, with occasional macroscopic evidence of necrosis.

### Histopathology

Following the 2004 WHO classification, poorly differentiated carcinoma definition relies on the presence in an otherwise malignant thyroid lesion (recognized by the presence of vascular and/or capsular invasion) of insular, trabecular, and solid growth patterns in the majority of the lesion, together with high grade features such as infiltrative growth pattern, necrosis, and increased mitotic activity. Generally, cytologic atypia is mild with small round to oval dark nuclei in the majority of cases, whereas marked pleomorphism is rare and rather suggests the presence of an undifferentiated carcinoma component. In a minority of cases, clear nuclei with irregular shape and “raisin-like” contours (so-called convoluted nuclei, see below) are present.

Concerning the architectural features, solid/trabecular/insular growth patterns are more commonly admixed within the same lesion and may coexist with more or less extensive differentiated components of the follicular or papillary type. Moreover, poorly differentiated carcinoma may be associated with an anaplastic (undifferentiated) component, and, although this latter event is extremely rare (representing less than 1% of cases at least in our series from a mountainous area of Northern Italy), it should be mentioned in the pathologic report since it unfavorably affects prognosis. According to the WHO criteria, solid/trabecular/insular growth pattern should be recognized in the “majority” of the tumor since the presence of this predominant architecture was found to be associated with a more aggressive behavior, even in recent reports. However, the issue is still open, since other reports claim that even a minor solid/trabecular/insular component in the presence of high grade features may affect prognosis.

As for other types of thyroid cancer, an oncocytic variant of poorly differentiated carcinoma is recognized based on the presence of predominant oncocytic changes in an otherwise poorly differentiated carcinoma, but it is not a relevant diagnostic or prognostic feature requiring a different tumor categorisation.

Concerning high grade features, several studies based on large case series identified high mitotic index and presence of necrosis as relevant parameters associated with adverse prognosis in thyroid cancer, and therefore considered them a distinctive hallmark of poorly differentiated carcinoma. Necrosis can be extensive with a typical peritheliomatous appearance or punctate, and adequate sampling is necessary to detect it. Mitoses are usually heterogeneously distributed within the lesion with “hot spot” areas intermingled with mitotically inactive fields, and a careful search in at least 50 high power fields is recommended.

Indeed, in diagnostic practice, WHO criteria for poorly differentiated carcinoma diagnosis are still difficult to apply and controversial, and therefore some overlap exists between other categories which enter in its differential diagnosis, such as the solid variant of papillary carcinoma and well-differentiated follicular carcinomas with predominant solid/trabecular growth patterns. Recently, a consensus study involving 12 pathologists from Japan, United States, and Europe was designed to evaluate the possible role that the geographic distribution of the tumor and the country-related interpretation differences might play in the diagnosis of poorly differentiated carcinoma, and, starting from WHO 2004 criteria, a schematic approach, summarized in the so called “Turin proposal,” was suggested using the following diagnostic criteria: (i) presence of a solid/trabecular/insular pattern of growth in an otherwise malignant thyroid lesion, (ii) absence of the conventional nuclear features of papillary carcinoma, to distinguish poorly differentiated carcinoma from the solid variant of papillary carcinoma, which is characterized by a solid/trabecular growth pattern, but bears a significantly better prognosis, (iii) presence of mitotic activity  $>3 \times 10$  high-power field or tumor necrosis or convoluted nuclei (at least 1 feature). Convoluted nuclei are round, hyperchromatic nuclei with convolutions of the nuclear membrane, and are generally smaller and darker than papillary carcinoma nuclei. A recent Japanese study aimed at investigating the prevalence and clinical significance of “three types of poorly differentiated carcinoma”-as defined by Sakamoto et al, WHO classification and the Turin proposal as well as of the tall cell variant of papillary carcinoma, in a large series of thyroid cancers with papillary features, confirmed that different tumor groups are identified by the different inclusion criteria, and showed that significant differences among the groups were also present in terms of survival, being cases identified according to the Turin proposal those associated to the worst survival rates..

### Diagnostic Immunohistochemistry

The immunohistochemical profile of poorly differentiated carcinoma is that of a follicular cell-derived tumor, including thyroglobulin production (generally, as intracellular paranuclear vacuoles) and thyroid transcription factor TTF-1 expression. Despite the name “poorly differentiated” carcinoma, the majority of tumors maintain their capacity to produce hormones, and, although the diagnosis of poorly differentiated carcinoma relies primarily on pure morphologic criteria, thyroglobulin is an important immunohistochemical marker for the differential diagnosis from other nonfollicular nonpapillary lesions of the thyroid gland including medullary thyroid carcinomas and metastatic tumors. Moreover, thyroglobulin serum determinations represent a useful postoperative marker in the patient's follow-up.

Markers of malignancy of follicular tumors (such as HBME-1, galectin-3) are usually expressed by poorly differentiated carcinomas (although generally to a lower extent than well differentiated neoplasms), but have no practical diagnostic application (except possibly for cytologic diagnosis) in this tumor category since morphologic signs of malignancy (ie, vascular invasion) are unequivocally present in most of the cases. Similarly, although an increase of proliferation index is associated with the decrease of differentiation in thyroid tumors, markers such as Ki-67 are of unproven utility in poorly differentiated carcinoma diagnosis, although they might possibly replace mitotic count as a more objective means of evaluating cell proliferation.

## Cytology

Most of cases of poorly differentiated carcinoma undergo FNAB prior to surgery, and although a definitive diagnosis can be rendered only at the histologic level, some cytologic features helping the recognition of this aggressive subset of neoplasms on FNAB have been described. According to WHO, cytologic features of PDTC include high cellularity with dyscohesive, small to intermediate-sized cells, some microfollicles and scant colloid; atypia is usually mild, while necrosis and mitoses are relatively common. These features were originally described by Pietribiasi et al, and subsequently confirmed in small series and in case reports. In a recent study, Bongiovanni et al reviewed 40 thyroid FNABs from poorly differentiated carcinomas compared with 40 FNABs from well differentiated thyroid tumors (15 papillary and 25 follicular neoplasms) to identify a specific set of cytomorphologic features that characterize these tumors. Within 32 different cytomorphologic parameters considered, the combination of cytoarchitecture (insular-solid-trabecular), severe cellular crowding, presence of single cells and high nuclear/cytoplasmic ratio was the most predictive feature of poorly differentiated carcinoma on FNABs. The diagnostic value of such parameters was confirmed in a series of 6 consecutive cases of FNABs of surgically removed poorly differentiated carcinomas from our institution, showing high cellularity and high nuclear/cytoplasmic ratio in all cases, solid groups of crowded cells in 5 cases, and single cells identified on smears in 3 cases (Fornari A, MD, 2009; unpublished observation). A positive galectin-3 immunocytochemical staining was found in 4 of 5 cases tested.

## Molecular Pathology and Histogenesis

PDTCs may arise *de novo* or as the result of dedifferentiation from preexisting well differentiated carcinomas, as originally postulated by Carcangiu et al. To define poorly differentiated carcinoma histogenesis, several molecular alterations have so far been investigated, but, as a result of the discrepancies in their diagnosis and classification, the molecular genetic data reported in the literature are limited by the heterogeneity of the case series analyzed. Generally, irrespective of the selection criteria applied, RAS point mutations appear to be a common molecular alteration in these tumors, although they were found with highly variable frequency and wide variation in specific types of RAS mutations,. N-RAS mutations were almost exclusively present in the majority of reports and associated with a worse outcome. Specific papillary cancer-associated molecular alterations such as BRAF mutations or RET-PTC translocations have been detected in poorly differentiated carcinomas having residual papillary carcinoma foci, but these data were not confirmed by others. Pax8/PPAR[gamma] translocations, a genetic event present in a subset of follicular carcinomas, are absent in poorly differentiated carcinoma. Taken together, all these data support the hypothesis that poorly differentiated carcinomas may de-differentiate from both well differentiated follicular and papillary carcinomas, as evident from morphologic features, but following distinct molecular pathways, involving RAS gene alterations, that are alternative to those classically implicated in papillary and follicular carcinoma tumorigenesis.

Poorly differentiated thyroid carcinomas are a heterogeneous group of tumors occupying an area intermediate between well-differentiated follicular/papillary and anaplastic carcinomas. Although they have been incorporated in the 2004 World Health Organization classification of



thyroid tumors, a long debate is still going on regarding their nature, morphologic diagnostic features, clinical significance, and optimal treatment. Common pathologic features of poorly differentiated carcinomas are solid/trabecular/insular growth, large size, frequent extrathyroidal extension, extensive vascular invasion, presence of necrosis, and increased mitotic activity. They may be associated with well differentiated components of either follicular or papillary type, and less frequently with anaplastic carcinoma. Literature data, although limited by the heterogeneous case series analyzed, suggest a distinct molecular pathway in poorly differentiated carcinomas, almost exclusively involving RAS gene alterations. The present report aims to describe the various aspects of this tumor type, from morphology and immunohistochemistry to molecular insights, from a practical and daily practice oriented point of view.

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## **The Partnership Between Surgery, Cytopathology and Surgical Pathology in Thyroid Disease**

**Udelsman, Robert; Prasad, Manju; Theoharis, Constantine**

Mutual respect forms the foundation for a successful partnership between surgery, cytology and pathology. The partnership between surgeons and their colleagues in cytopathology and surgical pathology forms the basis for the care of patients with thyroid nodular disease. The fundamental and underlying principle of the relationship is one of mutual respect based upon training and experience. The surgeon is required to make clinical decisions and perform procedures that in most cases are beneficial. Unfortunately, the surgeon's actions by acts of both omission and commission will at times result in harm to an individual patient. An inaccurate or inadequate cytopathologic evaluation can delay the initiation of definitive cancer treatment and has the potential to allow for tumor growth, upstaging and compromised outcomes. Conversely, an overzealous cytopathology report can result in overtreatment, unnecessary surgery, and loss of organ function necessitating life-long thyroid hormone replacement and its associated costs and inconvenience. In addition, all thyroid procedures have the potential for adverse outcomes including iatrogenic nerve injury, hypoparathyroidism, postoperative airway obstruction, and death. Finally, a surgical pathologist must attend to intraoperative frozen section consultation in the appropriate context as well as evaluate the resection specimen for tumor status/stage, confirming the cytopathologic diagnosis in most cases. Accordingly, the surgeon, the cytopathologist, and the surgical pathologist must formulate a working relationship where all parties understand and appreciate the potential for incremental information gain and its limitations. The surgeon must trust the information offered by the anatomic pathology team and act upon it in the patient's best interest. If this relationship is compromised, the potential for significant harm is likely to be realized.

Surgeons must have realistic and attainable expectations. They must understand that pathology, particularly cytopathology, is often more of an art form than a science. Surgeons with insufficient education, experience and insight all too frequently transpose their limitations upon their expectations of their colleagues.

## CYTOPATHOLOGY

Institutions should define who is competent and interested in performing thyroid fine needle aspiration (FNA), and how these procedures are performed. Thyroid aspirates can be performed under ultrasound guidance with or without on-site cytologic review. The individuals performing these procedures should routinely evaluate the quality and sufficiency of the sample.

The importance of appropriate labeling cannot be overly emphasized. The specimen and its accompanying requisition form must contain the patient's name, unique hospital or clinic number, specimen number, the location of and number of lesions biopsied, number of passes per nodule, and the technique of preparation. Once the specimen is received by the cytology department it is recorded, logged into a temporal schedule and sent to the cytopathologist for review. During this review the cytologist is required to discriminate between inadequate, marginal and adequate specimens. Many departments have developed formal definitions for these categories; however, at minimum every specimen report should indicate whether or not the specimen satisfies minimal qualitative and quantitative characteristics. Synoptic reporting is increasingly common and it is believed that this will become the standard of care in the United States. Positive and negative information should be recorded. A microscopic description is encouraged. The presence or absence of special studies including immunoperoxidase and flow cytometry studies should be noted. If additional data are pending, a preliminary report may be issued. The findings should be interpreted employing the diagnostic criteria of the recent National Institute of Health consensus conference.

Cytopathologists and Surgical Pathologists must also recognize their skills and limitations. Pathologists may feel compelled to offer clinical advice and thereby exceed the boundaries of their specialty. The pathologist is unlikely to have the full clinical context of a given patient including complete information about family history, external beam radiation exposure, aggressive growth of a nodule, information about symptoms and signs as well as functional thyroid abnormalities. Nonetheless, it is the responsibility of the referring physician to offer the cytopathologist as much clinical information as possible. At minimum this must include information about thyroid function, former radiation exposure, nodular growth and known genetic abnormalities. Unfortunately, practitioners occasionally compromise the cytopathologist by performing biopsies of lesions that are unlikely to be informative. For example, performing an FNA on a hyper-functioning (“hot”) dominant nodule is unlikely to be useful and likely to result in cytologic findings associated with an aggressive lesion.

Each institution must establish standards of care including intervals of reporting results. The patients and the clinicians desire results in a rapid and predictable fashion. The cytologist will require adequate time to log in, process, analyze, generate, and review the report. Once a standard time interval has been established, compliance should be reported on a routine basis for those individuals who rely upon this service. Individuals who do not adhere to this schedule undermine the credibility of the cytology department.

## OVERSIGHT REVIEW AND CONSENSUS

Cytology, as much as any area of medicine, is heavily influenced by individual experience, interest and commitment. Quality and accuracy of interpretation vary dramatically even within departments at academic medical centers. Accordingly internal mechanisms and metrics should

be established to assure that standards are achieved so that clinicians can develop confidence in the findings. Oversight mechanisms include junior faculty mentoring, consensus conference review, and the willingness to refer cases to outside experts. In addition, internal and external audits should be performed. Finally, each pathology department should develop a correlative process to compare permanent histopathologic findings with cytologic results. This will enhance intradepartmental education and collaboration and is likely to result in improved diagnostic skills. The results of internal and external reviews should be forwarded to institutional leaders as part of a routine quality enhancement initiative.

#### **OWNERSHIP OF RESPONSIBILITY**

All clinical disciplines, especially those that require interpretive skills, are subject to both false-positive and false-negative results. Some of these are attributed to sampling error or the presence of multiple nodules contained within an individual thyroid gland. Interdepartmental collaborative efforts are essential to respond to false-positive and negative-findings. It is the surgeon in concert with the endocrinologist who consults directly with patients and their families. Accordingly, these frontline direct caregivers have a responsibility to interpret and report accurate and critical information and therefore assist the patient in understanding the application and limitations of cytologic evaluation.

The recent National Cancer Institute Bethesda Classification System is the ground upon which the partnership between the surgeon and the cytopathologist must grow.

#### **Cytologic Diagnosis**

- Positive for Malignancy (Papillary, Medullary, Anaplastic, Lymphoma, Metastasis)
- Suspicious for Malignancy (Papillary, Medullary, Anaplastic, Lymphoma, Metastasis)
- Follicular or Hürthle Cell Neoplasm
- Indeterminate/Atypical Cells of Undetermined Significance
- Negative for Malignancy
- Unsatisfactory for Evaluation

It is of paramount importance that the cytopathologist and the surgeon understand the relative risk of malignancy implied by each cytologic classification category. Each classification in and of itself becomes not only a method of triaging patients for surgery but also a morphology-based suggestion as to what type of operation the surgeon may recommend for a given patient. With the exceptions of nonepithelial malignancies (eg, lymphoma) and metastatic carcinomas, a diagnosis of “Positive for Malignancy (Papillary, Medullary or Anaplastic Carcinomas)” is at least a suggestion for the clinical consideration of a total thyroidectomy. Similarly, given no other clinical parameters but relying solely upon cytomorphology, the category “Suspicious for Malignancy (Papillary, Medullary or Anaplastic Carcinomas)” becomes in effect a suggestion for a lobectomy with consideration of intraoperative touch imprint and frozen section consultation,

thus opening up the possibility of an initial total thyroidectomy rather than a subsequent completion thyroidectomy at a later date. “Follicular Neoplasm” and “Hürthle Cell Neoplasm” are related in that both categories most often result in lobectomy with the majority of the patients having ultimately benign histologic diagnoses. The recently proposed category of “indeterminate” or “cells of undetermined significance” may prove controversial in its clinical application. In effect, the atypical category is not a diagnosis but a declaration of uncertainty as to whether surgical intervention is justified in a given patient. Given the inherent subjectivity of a category that encompasses too much atypia for a benign diagnosis but too little atypia for even a suspicious diagnosis, it is important that each institution pursue internal mechanisms and metrics with vigilance to prevent this category from degenerating into a wastebasket term. At present, the atypical category carries a recommendation for consideration of repeat FNA. It is unclear at present, however, what clinical recommendation may be garnered from the re-FNA results. Does a re-FNA with a “Benign” diagnosis obviate the “Atypical” diagnosis of the original FNA? Do 2 “Atypical” diagnoses, 1 on the original FNA and 1 on the re-FNA, present enough clinical risk to justify lobectomy? At our institution, internal studies (unpublished data) have led to a philosophy regarding the atypical/indeterminate category. Currently, we believe that most nodules with an “indeterminate/cells of undetermined significance” FNA diagnosis should be rebiopsied and not be surgically resected. Given the often-borderline specimen adequacy of “indeterminate” samples, consideration is given for a targeted, well-sampled re-FNA with a diagnosis of benignity to be a reassuring finding indicative of conservative management. Naturally, clinical findings such as symptomatic growth of a nodule can in themselves be an indication for surgery. An interpretation of indeterminate on a re-FNA may justify surgical intervention in the appropriate clinical circumstances given that the repeat finding of indeterminate suggests that rather than being a sampling issue, the diagnosis may reflect a relatively underdeveloped atypia within the lesion itself, most especially in cases exhibiting nuclear atypia. Re-FNAs with Follicular Neoplasm/Hürthle Cell Neoplasm, Suspicious and Positive diagnoses should be managed according to standard protocols. Thus, the indeterminate/atypical category may lend itself to a multitude of clinical considerations emphasizing the ongoing communication between the surgeon and the cytopathologist.

To improve both the surgeon's expectations of the cytology report and the cytopathologist's understanding of the clinical parameters encompassing a given patient, at our institution we hold a weekly Thyroid Cytology-Surgery Preoperative Case Conference. Its purpose is to present the microscopic findings of all positive, suspicious and indeterminate cases, ie, all possible operative patients, as well as selected negative cases, to the Endocrine Surgery and Cytopathology Divisions. At the multiheaded microscope histories are summarized, laboratory/imaging results are discussed and the cytopathologic morphology is reviewed. It is in this unique collaborative setting that the decision to go to the operating room is made and the surgical intervention to be used is chosen. Consequently, both the surgeon and the cytopathologist share responsibility for the given patient's ultimate care.

Cytologic diagnoses do not exist in a vacuum but reflect histopathologic entities to the best ability of the cytologic diagnostician and the limits of the cytologic modality. These entities may be summarized:

Cytologic Diagnostic Categories Reflect Histopathologic Diagnoses

- Papillary Carcinoma
- Follicular Variant of Papillary Carcinoma
- Follicular or Hürthle Cell Neoplasm
- Medullary Thyroid Carcinoma
- Anaplastic Thyroid Carcinoma
- Primary Thyroid Lymphoma
- Metastatic Disease to the Thyroid Gland.
- Additional Diagnostic Material: Role of Core Needle Biopsy

The last 4 entities in the above “cytologic diagnostic categories” listing, ie, Medullary thyroid carcinoma, anaplastic thyroid carcinoma, metastatic carcinoma, and lymphoma, may lend themselves to ancillary testing such as immunocytochemistry, immunohistochemistry, and flow cytometry for precise diagnosis. In experienced hands, the cytomorphic criteria allow the diagnoses to be made in most cases, eg, medullary carcinoma with spindled and plasmacytoid cells. However, a small percentage of patients may require further sampling for additional testing. The sampling needs for these additional tests may be better served with a needle core biopsy fixed in 10% neutral buffered formalin rather than with additional FNA cytology fixed in alcohol. The 2 arguments in favor are: first, the sample is larger, ie, more likely to be adequate, and second, most immunohistochemical markers are validated on formalin fixed tissues and therefore, the results, especially negative results, are likely to be more reliable on formalin fixed core needle biopsies than on alcohol fixed cytology cell blocks. This procedure is controversial and has been challenged by the majority of cytopathologists. Core needle biopsies provide no additional advantage to aspiration cytology in follicular thyroid lesions, by far the majority of thyroid nodules.<sup>2</sup> Indeed, there are no guidelines comparable to the National Cancer Institute Bethesda guidelines for interpretation of thyroid aspiration cytology that are applicable to core needle biopsies of thyroid follicular lesions. In the absence of experience and guidelines for interpretation, the generated report may be inconclusive in the majority of the cases of follicular lesions. To summarize, core needle biopsy is an uncommon procedure in diagnosing thyroid pathology, and may be of value when more tissue is required for additional immunohistochemical or flow-cytometric work-up. It may be an investigative tool complementary to FNA cytology in carefully selected cases.

#### THYROID HISTOPATHOLOGY-INTRAOPERATIVE PATHOLOGIC CONSULTATION

The expectations of the thyroid surgeon are to a large extent predicated by the quality of the cytopathologic analysis. An unequivocal cytologic result will often obviate the need for intraoperative pathologic consultation. For example, the diagnosis of papillary carcinoma of the

thyroid determined by a well-prepared cytologic specimen is extraordinarily reliable and in itself dictates definitive operative therapy. Similarly, cytologic results in the setting of medullary carcinoma of the thyroid, especially in the setting of elevated serum calcitonin levels, are extremely accurate. Nonetheless, even with a clear cytologic diagnosis, the surgeon may request additional intraoperative information. This is particularly relevant when the surgeon is considering a dissection of either the central or lateral neck. It is also not unusual for the surgeon to encounter unanticipated findings in the operating room. If a surgeon detects direct transcapsular extension of a nodule into soft tissues including the strap muscle, a guided intraoperative frozen section can be helpful. If a surgeon encounters unanticipated enlarged or normal sized but suspicious lymph nodes, a frozen section can often demonstrate metastatic disease. However, the surgeon should also know that in the central neck rests of normal thyroid epithelial cells can occur within lymph nodes. During an operative procedure the pathologist may also be requested to freeze and bank pathologic material.

Frozen section analysis, especially combined with a touch preparation, can be extremely helpful in the setting of an equivocal cytologic diagnosis. In the setting of a cytopathology report suggestive but not diagnostic of papillary carcinoma of the thyroid, the pathologist should be able to render a definitive diagnosis. In the setting of a lesion suspicious but not diagnostic of the follicular variant of papillary carcinoma of the thyroid, a touch preparation can yield diagnostic information at the time of frozen section analysis. However, it is nonetheless difficult to establish this diagnosis without access to permanently fixed well-stained histologic specimens and, therefore, despite the best intraoperative efforts taken by the surgeon and the pathologist, the final diagnosis may still need to be “deferred” to permanent histologic analysis.

Pathologists must also be well versed in or have access to cytologic techniques including touch preparations. Each institution will have individual pathologists who have more or less expertise in endocrine pathology and cytology. The institution must establish a protocol so that an individual who does not have a relative comfort level reviewing intraoperative frozen sections of thyroid lesions has a mechanism to promote collegial assistance from more experienced pathologists in the frozen section suite. The elective operative schedule should be reviewed in advance to help make this process efficient. Similarly, surgeons should be aware that pathologists have their limitations. A surgeon who insists upon a definitive diagnosis in the setting of a follicular or Hürthle cell neoplasm will require educational counseling.

Any time an intraoperative consultation is requested from pathologists, a formal report should be issued and be made readily available to the surgeon at the time of diagnosis. The practice of conveying this information solely by telephone or intercom is hazardous and subject to miscommunication. At minimum a written or electronically generated report must be submitted and given to the surgeon at the time of the operative decision about the extent of surgery. Reports that appear solely in the medical record postoperatively are not acceptable. An often overlooked element in this interaction is the pathology form. This should be in the format of a “physician to physician request for consultation” and must include adequate clinical, radiologic, and laboratory findings that may be useful in arriving at the correct diagnosis, including line drawings when possible. This critical element in surgeon to pathologist communication is often relegated to a nonphysician member of the staff who then proceeds to fill out the request for pathology like an order for blood tests.

Despite the best educational backgrounds, intentions and diligence, there will arise occasions where there are inconsistencies between the cytologic and pathology reports for a given patient. Regular intradepartmental cytologic-histologic correlation conferences provide a mechanism that allows collegial discussion of more complicated lesions as well as an educational format for more junior faculty. Quality Assurance/Quality Control needs can be met, errors both individual and systemic should be identified and rectified. In short, regular feedback allows for collective and individual growth of the partnerships between surgeons, surgical pathologists and cytopathologists and will result in improved clinical outcomes.

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## **Collagenous Gastritis: Histopathologic Features and Association with Other Gastrointestinal Diseases**

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Collagenous gastritis (CG) is a rare disorder with a distinct presentation and association in pediatric and adult patients. Association with lymphocytic gastritis, celiac sprue, lymphocytic colitis, and autoimmune disorders are frequently seen in adult patients. An absence of associated intestinal and autoimmune diseases characterizes the pediatric population. Increased awareness of this diagnosis, in particular when patients have established sprue or lymphocytic/collagenous colitis, would be helpful in better understanding the natural course of this disease.

Collagenous gastritis (CG) characterized by the deposition of a subepithelial collagen band and accompanying inflammatory infiltrate is a rare disorder. The natural history and pathogenesis of CG remain unclear. We describe the histologic features (23 gastric, 18 duodenal, and 4 colonic biopsies) and clinical findings of an additional 12 cases. Histologic features including active or chronic inflammation, surface epithelial injury, intraepithelial lymphocytosis, intestinal metaplasia, and *Helicobacter pylori*, and measurement of thickness of subepithelial collagenous band were evaluated in gastric biopsies. The clinical features, endoscopic findings, and follow-up were obtained and correlated with histologic features. There was an even number of males (n=6) and females (n=6). Four patients were children/young adults, 3 of whom (75%) presented with anemia and gastric nodularity. Eight patients were adults, 6 of whom (75%) had an associated autoimmune disease (1 with Hashimoto thyroiditis and polymyositis) or other intestinal disease (3 with celiac sprue, 1 with collagenous colitis, 1 with collagenous sprue), in contrast to none in the 4 children/young adults,  $P=0.06$ . The range of subepithelial collagen thickness was 15 to 120  $\mu\text{m}$  in CG. The collagenous layer showed surface epithelial injury and entrapped inflammatory cells. On presentation, the thickened collagen distribution in the antrum and body was variably patchy and diffuse. Four (33%) patients showed lymphocytic gastritis (3 within the same biopsy); one of these patients also had celiac sprue and another had collagenous sprue. Three (25%) patients had celiac sprue (2 had duodenal biopsy proven and 1 had a clinical diagnosis of celiac sprue). An additional patient had duodenal biopsies showing collagenous sprue. Four patients had follow-up biopsies during a 3 to 119-month period after the diagnosis of CG. CG persisted on the follow-up gastric biopsies in 3 (75%) of the 4 patients, and the other

patient had lymphocytic gastritis, a finding not seen in previous biopsies. CG is a rare disorder with a distinct presentation and association in pediatric and adult patients. An absence of associated intestinal and autoimmune diseases characterizes the pediatric population. Association with lymphocytic gastritis, celiac or collagenous sprue, collagenous colitis, and autoimmune disorders are frequently seen in adult patients.

### Histologic Features

There were 23 sets of gastric biopsies (15 with antrum and body/fundus biopsies, 4 with body/fundus alone, 3 with antrum alone, 1 with cardia alone) from 12 patients (8 patients with a single biopsy and 4 patients with 2 to 7 follow-up biopsies). On the initial biopsies, the thickened collagen distribution in the antrum was patchy in 3 patients, diffuse in 6 patients, and not available in 3 patients; the collagen distribution in the fundus was patchy in 6 patients, diffuse in 4 patients, and not available in 2 patients. The 2 patients (cases 2, 7) that showed patchy involvement in the body without endoscopically noticeable pathology in the antrum were associated with lymphocytic gastritis on initial biopsy. Three patients (cases 4, 5, 6) show diffuse antral involvement with patchy disease in the body on initial biopsy. Three patients (cases 8, 9, 10) showed diffuse involvement in both antrum and fundus on their initial biopsies. One patient (case 12) showed only cardia-type mucosa sampled and involved by disease. In the cases with follow-up biopsies (cases 1, 4, 10, 11), case 1 did not show endoscopic antral disease and were not sampled on the first 2 sets of biopsies, however, subsequent biopsies showed diffuse involvement of the antrum whereas the fundus showed variable diffuseness and patchiness. Case 10 showed diffuse involvement of the antrum and fundus in the initial biopsy and follow-up biopsy 22 months later; however, a follow-up biopsy 119 months after the initial biopsy showed diffuse antral involvement without evidence of CG in the fundus. Case 11 showed patchy involvement of the antrum in 3 biopsies over 10 years without associated endoscopic involvement of the body. No CG was present 3 months after initial diagnosis in case 4.

The range of subepithelial collagen thickness was from 15 up to 120  $\mu\text{m}$ . All cases showed some degree of surface epithelial injury characterized by epithelial flattening, and epithelial detachment). The thickened collagenous layer often contained entrapped inflammatory cells and capillaries with a ragged edge at the interface with the lamina propria, in contrast to normal gastric mucosa. Each gastric biopsy showed a chronic inflammatory infiltrate composed of lymphocytes, plasma cells, and in 3 patients (cases 2, 3, 5) a prominent eosinophilic infiltrate in the lamina propria. Five patients (cases 1, 6, 8, 10, 12) showed mild acute inflammation in their initial biopsies. No *H. pylori* was seen on hematoxylin and eosin sections in any of the biopsies, 1 of which (case 1) had corresponding negative *H. pylori* serologies, 2 patients (cases 3, 6) with negative *H. pylori* immunohistochemistry, 2 patients (cases 2, 8) with negative immunohistochemistry and serology.

Three patients (cases 2, 7, 11) showed an increase in intraepithelial lymphocytes consistent with lymphocytic gastritis within the same biopsy. An additional patient (case 4) showed evidence of lymphocytic gastritis in a 3-month follow-up biopsy (without evidence of CG) but not at presentation. One of the 4 patients (case 7) also had a diagnosis of celiac sprue and another patient (case 11) had concomitant collagenous sprue; the other 2 patients (cases 2, 4) did not exhibit any evidence of additional GI disease or autoimmune disease. No evidence of atypical



lymphocytes or lymphoepithelial lesions, destruction of crypt epithelium, lymphoid follicles, or other features of lymphoma were found.

Three patients (cases 6, 7, 9) also had a diagnosis of celiac sprue., 2 of whom (cases 7, 9) exhibited some degree of villous blunting and increased intraepithelial lymphocytosis on duodenal biopsy; 1 case carried a clinical diagnosis of celiac sprue and had been on a gluten-free diet before to the biopsy available for review (case 6). An additional patient (case 11) had duodenal biopsies that showed collagenous sprue. One patient (case 8) also had collagenous colitis.

Four patients (cases 1, 4, 10, 11) had follow-up biopsies during a 3 to 119-month period after the initial diagnosis of CG persisted on the follow-up gastric biopsies in 3 of the patients. The exception was case 4 where histologic evidence of CG was not present in a stomach biopsy 3 months after the diagnosis. This biopsy did, however, contain lymphocytic gastritis, a finding that was not present in any of his previous biopsies. Case 10 has 3 sets of biopsies over 10 years, however, the patient had upper GI bleeds intermittently over 20 years. The collagen band thickness increased dramatically over a 10-year period, from 78 to 120  $\mu\text{m}$ , with diffuse involvement particularly in the antrum; the patient had been treated with transfusions and without gluten restriction or steroids. In addition, the most recent follow-up biopsy also showed focal intestinal metaplasia. Case 1 has 7 sets of biopsies over 4 years. The collagen band thickness decreased from 33 to 69  $\mu\text{m}$  in a diffuse distribution to 18 to 40  $\mu\text{m}$  in a patchy distribution over a 4-year period where the patient was on Budesonide. Case 11 has 3 sets of biopsies over a year. The patient, who also has collagenous sprue, had a collagen band that showed a slight increase from 20  $\mu\text{m}$  to 30 to 80  $\mu\text{m}$  over a period of a year where the patient was refractory to gluten-free diet and symptomatically dependent on steroids.

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