Short Report for Clinical Oncology

Title: **Recent Developments in the Pathology of Thyroid Cancer**

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Abstract

The histopathological features of thyroid cancers can be used to risk stratify patients, allowing prognostication and treatment decisions. Detailed accurate histological assessment by experienced pathologists working within a multidisciplinary context is crucial. Experience is also essential for interpretation of pre-operative thyroid cytology specimens, which can be challenging. There is now more international harmonisation of numerical reporting systems for thyroid cytology. Understanding of the molecular basis of thyroid cancer has increased dramatically in recent years. Pre-operative molecular pathology testing, when available, can refine cytological diagnosis to rule in or out for surgery, as well as assisting prognostication and enabling targeted treatment for thyroid tumours.

Keywords: molecular diagnostics, thyroid cancer, pathology, recent advances, diagnosis, cytology.

Introduction

The most important aspect of thyroid cancer pathology is making a correct and timely diagnosis with provision of sufficient detailed histological information upon which to risk stratify patients for treatment decisions. Some aspects of thyroid pathology are, however, quite subjective and so there is often variation in pathological interpretation even between experienced endocrine pathologists. Recent important developments in thyroid cancer pathology include refinement of some prognostic features for assigning risk, a better understanding of the molecular basis of thyroid cancer and reclassification of one of the lowest risk tumours as non-cancerous tumour. Below is a brief summary of some key developments in the last few years.

Discussion

Increasing incidence of thyroid cancer. The incidence of thyroid cancer has increased in developed countries, but the mortality has remained unaltered. The rise is primarily due to increased use of thyroid ultrasound(1) and most of the increase is due to lower risk tumour types.(2)

Tumour categorisation and risk. Traditionally, primary thyroid cancer was classified into either follicular epithelial cell derived tumours: papillary carcinoma, follicular carcinoma (minimally or widely invasive), and anaplastic carcinoma, with progressively worse prognosis; or C-cell derived medullary thyroid carcinoma (familial and sporadic), lymphoma and other rarer tumour types. Poorly differentiated thyroid carcinoma (PDC) is now recognised as an intermediate risk cancer between differentiated thyroid carcinoma (follicular and papillary) and anaplastic thyroid carcinoma (ATC).

For thyroid carcinomas, precise tumour typing, as well as accurate TNM staging and assessment of other prognostic features, is crucial in determining the patient's risk group and therefore the appropriate treatment. Histopathology reports need to be accurate and complete with all the relevant diagnostic and prognostic features; this is aided by proforma reporting using datasets or templates of the type issued by the UK Royal College of Pathologists (RCPath)(3) or by The College of American Pathologists (CAP)(4).

Papillary thyroid carcinoma (PTC) subtypes. There are many subtypes of PTC but perhaps the most confusing are papillary microcarcinomas and the "follicular" variant of papillary thyroid carcinoma (FVPTC).

Papillary microcarcinomas are tumours up to 10mm in greatest dimension. These may be the diagnostically targeted lesion or, more commonly, found incidentally either on ultrasound or histology. Various additional histological factors enable assessment of their risk level, and personalised decision making may be required.(5)

PTC is diagnosed primarily by its nuclear features, and may show either a papillary (classical) or follicular architecture, or a mixture of both. FVPTCs show an almost exclusively follicular architecture, and are classified into subtypes including *infiltrative* (non-encapsulated) with molecular genetics and behaviour similar to classical PTC, and *encapsulated* (eFVPTC) where the molecular genetics and behaviour are more akin to follicular neoplasms.(6) The distinction of eFVPTC from follicular thyroid carcinoma (FTC) therefore hinges on whether the nuclei show PTC-type nuclear features or not, which is notoriously subjective even among experienced endocrine pathologists.(7). The diagnosis of FTC has also evolved with time. In a very recent study, a group of American pathologists reviewed a series of 66 thyroid tumours originally diagnosed in 1965-2007 as FTC. 47 (71%) cases were reclassified: 24 (36%) to PTC, 18 (27%) to follicular adenoma (FA), and 5 (8%) to PDC. Nine of 23 (39%) cases from 2000-2007 were reclassified as benign (FA).(8)

Poorer prognostic variants of PTC include tall cell, columnar cell and diffuse sclerosing types, and any foci of such change needs documentation.(3,9)

NIFTP An international consensus group of pathologists led by Prof Yuri Nikiforov, University of Pittsburgh, reviewed 109 cases of non-invasive eFVPTC followed up for 10 to 26 years (median 13 years). Using consensus pathological criteria, this study showed these tumours have a very good prognosis with less than 1% risk of death or recurrent disease on long-term follow-up, implying that that this tumour should be regarded as of very low malignant potential. In 2016, these lesions were re-designated “NIFTP”, *non-invasive follicular thyroid neoplasm with papillary-like nuclear features*.(10) Diagnosis of NIFTP requires application of strict pathological criteria including examination of the entire tumour capsule, confirmation of the nuclei according to the NIFTP nuclear scoring system, absence of psammoma bodies, and no capsular or vascular invasion. If these criteria are not met, is not possible to make a diagnosis of NIFTP and the default diagnosis remains eFVPTC. NIFTPs are follicular-derived tumours, they are *RAS*-driven lesions and they do not show evidence of *BRAF* V600E mutations.(10) The UK RCPATH has produced an addendum to the 2014 *Dataset For Thyroid Cancer Histopathology Reports* detailing the diagnosis of NIFTP.(11) This could have a major impact, because in some centres up to 20% of newly diagnosed thyroid cancers are non-invasive eFVPTC.(12)

Minimally Invasive FTC. These are single encapsulated nodules of tumour with a follicular architecture but the cells lack the nuclear features of PTC. Carcinoma is diagnosed when there is invasive growth, either through the tumour capsule (capsular invasion)(13) or within the blood vessels of the tumour capsule or adjacent thyroid tissue (vascular or angioinvasion),(13,14) both of which can be subjective histologically.(7) Vascular invasion carries higher risk than capsular invasion, so there should be thorough examination of the tumour capsule histologically and the type of invasion present should always be clearly stated. Precise criteria for vascular invasion in the literature are also conflicting (4,14). Minimally invasive FTC with capsular invasion only and no other adverse risk factors has a very low risk of recurrence or metastasis and may not require completion thyroidectomy.(15-17) Frequent vascular invasion is an adverse prognostic feature and should be commented upon, with the 2016 CAP Protocol requiring distinction between focal

(less than 4 vessels) vs extensive (4 or more vessels) whereas the 2014 UK RCPATH Dataset does not.(4)

PDC. These uncommon tumours have an intermediate prognosis between PTC/FTC and ATC. A previous term was “insular carcinoma”, but not all PDC have an insular architecture. Diagnosis is aided by the Turin consensus criteria.(18,19) Diagnosis requires a follicular cell derived tumour with a solid/insular/trabecular growth pattern, lacking the nuclear features of PTC, and with necrosis and/or a mitotic count of three or more per 10 high-power fields. The entire tumour should be designated as PDC if more than 50% of the tumour shows this appearance, but even a minority component of PDC in an otherwise well differentiated tumour should also be mentioned in the pathology report because this may worsen the patient’s prognosis.(20)

Other newly described pathological entities. These are extremely rare and are well reviewed by Eloy.(21) They include meningioma-like tumour of the thyroid, glomeruloid variant of FTC, angiomatoid PTC, hobnail/micropapillary variant of PTC, small cell primary non-neuroendocrine thyroid carcinoma, other primary thyroid tumours with Ewing-like elements, the very rare small cell neuroendocrine carcinoma of the thyroid, and other very rare primary thyroid neuroendocrine tumours which are calcitonin-negative and resemble well differentiated neuroendocrine tumours seen at other sites.

Staging. Worthy of discussion in an oncology review are some very simple points around staging. The use of extrathyroidal extension (ETE) in TNM assumes that the thyroid has a capsule but anatomically the thyroid has no capsule.(22) Assessment of ETE therefore has poor interobserver agreement.(23) ETE has been described as macroscopic vs microscopic, the former having a worse prognosis; “minimal” ETE (pT3) does not affect relapse-free survival.(24)

Metastases to lymph nodes are frequently seen with PTC but quite rarely with FTC. There is evidence that the size of lymph node deposits, the ratio of involved to non-involved lymph nodes, and extranodal extension have prognostic importance.(4,25-28)

Thyroid cytology. Thyroid FNA cytology is the principal means of pre-operative diagnosis of thyroid nodules. Numerical categorisation facilitates communication and audit, but does not replace the text of the report. In the UK, The RCPATH updated The Guidelines for Reporting Thyroid Cytology in January 2016.(29) Many countries have similar numerical systems including The Bethesda System (TBS) and these now align well with one another, enabling comparison of the international literature.(29) There is interobserver variation in interpretation of thyroid cytology, and central review of cytology increases accuracy, especially in the atypical categories.(30)

Cytology can yield a definite diagnosis of thyroid cancer, permitting therapeutic surgery, or a non-neoplastic/benign diagnosis, permitting reassurance. There is a continuing problem with the indeterminate or “grey zone” cytology categories, which require either follow-up or diagnostic hemithyroidectomy for a histological diagnosis, but these are to some extent unavoidable due to the overlap of cytomorphological

features and the need for histology to distinguish benign from malignant follicular neoplasms.(31) The new diagnostic entity NIFTP usually yields indeterminate cytology but can produce cytology that is reported as diagnostic of PTC (Thy5/TBS Class VI). Clinicians need to be aware of this and exercise caution, taking into consideration the ultrasound appearances as well as the cytology findings.(11) Molecular testing for *BRAF* V600E mutations may also be helpful as presence of *BRAF* V600E mutation excludes a diagnosis of NIFTP in an otherwise malignant Thy5/TBS Class VI cytology.

Molecular genetics of thyroid cancer. Understanding of the genetic basis of thyroid cancer is developing rapidly, and has important diagnostic, prognostic and therapeutic implications. Many different mutations and gene rearrangements can be seen in thyroid cancers but classical/high risk variant PTCs are predominantly driven by mutations of *BRAF*-V600E, and follicular tumours, including eFVPTC and NIFTP, predominantly by *RAS* mutations, as shown in the 2014 Thyroid Cancer Genome Atlas.(32) MTCs show *RET* gene mutations, either sporadic or familial. ATCs also show molecular phenotypes with a higher percentage of unfavourable gene mutations.

As a “rule-in” test, the best single test is *BRAF* V600E; this mutation is not seen in benign thyroid nodules and is therefore almost 100% specific for PTC (unless the thyroid tumour nodule is a metastasis) but has low sensitivity because only around 50% of PTCs have the mutation.(33) Other gene abnormalities such as *RET*/PTC mutations or *PAX8*/*PPAR* gamma translocations are also fairly specific for malignant thyroid nodules but not exclusively so. In 2015, The American Thyroid Association produced a statement on using molecular profiling for thyroid nodules, making the important point that the diagnostic value depends on the relative risk of malignancy in the cohort. Therefore, a molecular test applied to lower risk thyroid cytology (eg. UK Thy3a/TBS Class III), if positive for gene mutation(s) in the absence of a *BRAF* V600E mutation, would have a different significance compared to the same mutation(s) identified in a Thy4/TBS Class V FNA.(34)

A “rule-out” test, to reliably identify nodules as benign on FNA cytology, is more difficult. There are several competing commercial systems available but none are currently available in the UK. The system that is most widely known is the *Afirma Veracyte* system which is a proprietary micro-RNA based test.(36) A nodule classified as “benign” has a risk of malignancy of about 5%, but a “suspicious” result with *Afirma* has comparatively low specificity for cancer.(33)

The *Thyroseq 2* system, developed by Nikiforov and colleagues in Pittsburgh, USA,(36-38) uses a series of known thyroid cancer gene mutations on a commercially available Next Generation Sequencing (NGS) platform to target known gene mutations present in malignant thyroid nodules. This is highly effective as both a “rule-in” and a “rule-out” test, although as a “rule-in” test it is not totally specific because some of the mutations present in malignant thyroid nodules are also seen in benign nodules, specifically *RAS* gene mutations.(33)

BRAF V600E mutations are also targeted by tyrosine kinase inhibitors (TKIs), although there is no evidence that *BRAF* V600E testing is predictive of response to

some of the newer TKIs. Targetable mutations have also been identified in ATC and MTC, with anecdotal reports of response to crizotinib in tumours with *ALK* fusions.

Conclusion

Accurate pathology by experienced pathologists is crucial to the diagnosis and risk stratification of thyroid cancer. The future will no doubt include more widespread molecular testing for the diagnosis of thyroid nodules, and for targeting therapies to specific action gene mutations in radio-iodine refractory thyroid cancer and also the rarer tumour variants such as anaplastic thyroid cancer.

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