Management of thyroid nodules with indeterminate fine-needle aspiration cytology: histogram analysis of greyscale sonograms and molecular assay of residual tissue from fine-needle aspiration biopsies

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Determinate cancer screening enhances accurate cancer diagnosis and timely decision-making for treatment. Neck ultrasonography (US) has become the first routine evaluation of all patients with a known or suspected thyroid nodule to stratify its risk of malignancy. The morphological features of thyroid nodules observed during the US are reported via scoring classification systems for malignancy risk stratification based on the Thyroid Imaging Reporting And Data System (TIRADS) or 2015 American Thyroid Association (ATA) guideline (1,2). Different versions of TIRADS have been developed in different countries in line with their national conditions and medical status, such as Chinese TIRADS (3), European TIRADS (4) and Korean TIRADS (5). The sonographic features/patterns of thyroid ultrasound are widely used to identify nodules for further biopsy or follow-up ultrasound. Nodules with the more suspicious features of US are recommended to undergo thyroid biopsy. The Chinese TIRADS guideline recommends ultrasound-guided fine-needle aspiration (FNA) biopsy for TIRADS 4A nodules >15 mm and TIRADS 4B or 4C or 5 nodules >10 mm, but not for TIRADS 2 and 3 nodules (3). Needle biopsy was first introduced for diagnosis of thyroid nodules using an 18-gauge-needle puncture and aspiration by Martin and Ellis in 1930 and then fine needle (22–27-gauge) in the 1960s (6,7). This technique came into widespread use in North America in the 1980s and has become a gold standard to distinguish malignancy from benign thyroid nodules (7-9). Advances in diagnostics, including increasing use of computed tomography and magnetic resonance imaging, allow the detection and biopsy of thyroid nodules as small as 2 mm (10). Thyroid FNA cytological findings are classified across six diagnostic categories by the Bethesda System for Reporting Thyroid Cytopathology (BSRTC) with risk of malignancy in each category, including (I) nondiagnostic or unsatisfactory (ND), (II) benign, (III) atypia of undetermined significance (AUS)/follicular lesion of undetermined significance (FLUS), (IV) follicular neoplasm/suspicious for a follicular neoplasm (FN/SFN), (V) suspicious for malignancy (SM), and (VI) malignant (11). A nodule may be considered for resection if its cytology is malignant or SM (1,11) or for active surveillance for select papillary thyroid carcinomas and microcarcinomas (12). FNA cytology is the most accurate and cost-effective method for evaluating thyroid nodules. However, discrepancies exist between the US and FNA results in real world clinical practice. Up to 30% of the FNA biopsies are reported as indeterminate cytology: ND, AUS/FLUS, FN/SFN, or SM. According to the 2015 ATA guidelines, a repeat ultrasound-guided FNA is recommended to re-do the cytology examination or perform molecular testing when a nodule was predicted highly SM by US but

classified as indeterminate one on FNA examination (1). With a repeat FNA, many cases may receive a more definitive cytological diagnosis, whereas up to 30% of nodules are still diagnosed as AUS/FLUS. Molecular testing of FNA specimens has shown clinical potential in risk stratification for nodules with AUS/FLUS cytology. Currently there is no optimal molecular test yet that makes a definitive diagnosis of malignancy in all cases of indeterminate cytology. Considering options of repeat FNA or diagnostic surgery, attempts have been made to examine if the sonographic features of indeterminate thyroid nodules is able to guide decision-making. US assessment for thyroid nodules initially classified as AUS/FLUS has shown a malignancy rate of 7.7% in sonographically benign or very low risk nodules, 58% in sonographically low or intermediate suspicion nodules, and 100% in sonographically high suspicion nodules (13). A significantly high risk of cancer was shown in the AUS/FLUS nodules presenting with either the high suspicion pattern US or just one suspicious ultrasound feature (irregular margins, taller than wide shape, marked hypoechogenicity or microcalcifications) (13,14).

Sonographic patterns or features of nodules with AUS/FLUS cytology may aid in risk stratification. In this issue, Zhang *et al.* (15) performed histogram analysis to identify the independent predictive factors for malignancy via re-analyzing ultrasound images of thyroid nodules in BMP format on an open-source software MaZda 4.6 (http://www.eletel.p.lodz.pl/mazda/). Histogram analysis allows objective and quantitative determination of the spatial change of gray regions of the lesion which is very difficult to discern by the naked eyes. Using a cohort containing 123 nodules suspicious on US but not on cytology, the authors identified that the sonographic features and the histogram parameters associated with malignancy were skewness (OR =25.800; 95% CI: 1.034–76.422), taller-than-wide shape (OR =15.165; 95% CI: 3.157–72.854), irregular margins (OR =11.492; 95% CI: 1.747–75.573), and microcalcifications (OR =5.107; 95% CI: 1.455–17.927) (15). A second review and histogram analysis of ultrasound images of thyroid nodules suspicious on initial US may reclassify many of these patients into the benign or high suspicious categories, hence improving overall diagnostic accuracy. Better understanding of the characteristics of benign nodules may help decrease the need for a biopsy in some patients. Furthermore, the subjective bias and relatively high variability among observers in US diagnosis could be overcome by the objective histogram analysis. Such a technique shows promise as there is no extra visits or biopsies for the patient and there are potential

economic savings for the healthcare system. However, the results are inevitably influenced by the prevalence of cancer in the reservoir of tested nodules suspicious on ultrasound. The study would be considered preliminary due to the small sample size cohort. Further clinical validation in a larger cohort is needed to verify the utility of this method in managing the thyroid nodules with indeterminate cytology and even its extended utility in initial US diagnosis.

Compared to US, FNA is relatively more complicated, time-consuming, expensive and invasive. However, a diagnosis of cancer can rarely be made by US alone without a thyroid biopsy. A repeat FNA is still necessary for nodules with indeterminate FNA cytology that are identified with highly suspicious ultrasound features by histogram analysis.

Several molecular tests are commercially available for risk stratification of nodules initially classified as AUS/FLUS (16-18), but most of them also require another thyroid tissue sample from a full FNA pass via a repeat FNA procedure. Recently, we re-defined the clinical value of the residual tissues from FNA biopsies, which are considered discards after cytological examination, in assisting in diagnosis of AUS/FLUS biopsies by molecular assays (19). A sensitive digital polymerase chain reaction based molecular assay was established for accurate detection of *BRAF* V600E variation using residual specimens of FNA biopsies post cytology evaluation as an auxiliary approach for malignancy diagnosis of nodules falling into the AUS/FLUS or ND categories (19). Variant assay may be informative in AUS/FLUS and, in particular, ND samples in which there is quantitative or qualitative inadequacy for cytological examination due to insufficient number of cells or poor sample preparation. This assay showed an analytical sensitivity at 0.02 copies/µL and specificity at 100% in detecting *BRAF* V600E variant, and variant allele frequency may be reproducibly quantified in a wide range of tumor DNA input (1 ng/well to 200 ng/well), which allows for detection of tiny amounts of clinical specimens. Molecular assays of more actionable targets using the residual tissue from routine FNA biopsies may aid cytological interpretation to reach a conclusive diagnosis, hence likely sparing these patients from the need for a repeat FNA biopsy or diagnostic operation.

Thyroid nodules are common clinical findings and the major concern is to rule out malignancy. Different approaches for detection of malignant nodules keep evolving to provide better management for patients with thyroid nodular disease. An US with improved analysis is a

safe, non-invasive, and fast imaging technique for screening and selecting those with a higher risk of malignancy. FNA examination with enhancements in 2018 BSRTC may further stratify high or low incidence of cancer by distinguishing cytomorphologic features. In addition, molecular assays, which are mainly under development, can be informative to identify more aggressive cancers at a much earlier stage by detecting oncogenic variations or other genetic alterations among patients with suspicious or indeterminate thyroid nodules.

Footnote

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