Editorial

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

Guodong Fu[1], Ian J. Witterick[2,3]

[1]Lunenfeld-Tanenbaum Research Institute, Mount Sinai Hospital, Sinai Health, Toronto, ON, Canada; [2]Department of Otolaryngology-Head & Neck Surgery, Mount Sinai Hospital, Sinai Health, and University of Toronto, Toronto, ON, Canada; [3]Joseph and Mildred Sonshine Family Centre for Head and Neck Diseases, Mount Sinai Hospital, Sinai Health, Toronto, ON, Canada

Correspondence to: Dr. Guodong Fu, PhD. Lunenfeld-Tanenbaum Research Institute, Mount Sinai Hospital, Sinai Health, 600 University Avenue, Toronto, ON M5G 1X5, Canada. Email: David.Fu@sinaihealth.ca.

Comment on: Zhang R, Wang Q, Sun C, *et al.* Clinical utility of histogram analysis of grayscale sonograms in assessing sonographic suspicious malignant thyroid nodules. Transl Cancer Res 2022. doi: 10.21037/tcr-22-479.

Submitted Jul 20, 2022. Accepted for publication Aug 15, 2022.
doi: 10.21037/tcr-22-1919
View this article at: https://dx.doi.org/10.21037/tcr-22-1919

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

© Translational Cancer Research. All rights reserved.

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

© Translational Cancer Research. All rights reserved.

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.

Acknowledgments

Funding: None.

Footnote

Provenance and Peer Review: This article was commissioned by the editorial office, *Translational Cancer Research*. The article did not undergo external peer review.

Conflicts of Interest: Both authors have completed the ICMJE uniform disclosure form (available at https://tcr.amegroups.com/article/view/10.21037/tcr-22-1919/coif). IJW reports that he receives payment or honoraria for lectures, presentations, speakers bureaus, manuscript writing or educational events from Glaxo Smith Kline, Sanofi Regeneron and Novartis, served as President of the Canadian Society of Otolaryngology-Head & Neck Surgery 2019–2021 and Chair of the Skull Base Section, American Head and Neck Society-2021 to present as leadership or fiduciary role-unpaid, and holds Stock from Proteocyte Diagnostics Inc. The other author has no conflicts of interest to declare.

Ethical Statement: The authors are accountable for all aspects of the work in ensuring that questions related to the accuracy or integrity of any part of the work are appropriately investigated and resolved.

Open Access Statement: This is an Open Access article distributed in accordance with the Creative Commons Attribution-NonCommercial-NoDerivs 4.0 International License (CC BY-NC-ND 4.0), which permits the non-commercial replication and distribution of the article with the strict proviso that no changes or edits are made and the original work is properly cited (including links to both the formal publication through the relevant DOI and the license). See: https://creativecommons.org/licenses/by-nc-nd/4.0/.

References

1. Haugen BR, Alexander EK, Bible KC, et al. 2015 American Thyroid Association Management Guidelines for Adult Patients with Thyroid Nodules and Differentiated Thyroid Cancer: The American Thyroid Association Guidelines Task Force on Thyroid Nodules and Differentiated Thyroid Cancer. Thyroid 2016;26:1-133.
2. Tessler FN, Middleton WD, Grant EG, et al. ACR Thyroid Imaging, Reporting and Data System (TI-RADS): White Paper of the ACR TI-RADS Committee. J Am Coll Radiol 2017;14:587-95.
3. Zhou J, Yin L, Wei X, et al. 2020 Chinese guidelines for ultrasound malignancy risk stratification of thyroid nodules: the C-TIRADS. Endocrine 2020;70:256-79.
4. Russ G, Bonnema SJ, Erdogan MF, et al. European Thyroid Association Guidelines for Ultrasound Malignancy Risk Stratification of Thyroid Nodules in Adults: The EU-TIRADS. Eur Thyroid J 2017;6:225-37.
5. Shin JH, Baek JH, Chung J, et al. Ultrasonography Diagnosis and Imaging-Based Management of Thyroid Nodules: Revised Korean Society of Thyroid Radiology Consensus Statement and Recommendations. Korean J Radiol 2016;17:370-95.
6. Martin HE, Ellis EB. Biopsy by needle puncture and aspiration. Ann Surg 1930;92:169-81.
7. Bäckdahl M, Wallin G, Löwhagen T, et al. Fine-needle biopsy cytology and DNA analysis. Their place in the evaluation and treatment of patients with thyroid neoplasms. Surg Clin North Am 1987;67:197-211.
8. Walfish PG, Hazani E, Strawbridge HT, et al. Combined ultrasound and needle aspiration cytology in the assessment and management of hypofunctioning thyroid nodule. Ann Intern Med 1977;87:270-4.
9. Gharib H. Fine-needle aspiration biopsy of thyroid nodules: advantages, limitations, and effect. Mayo Clin Proc 1994;69:44-9.
10. Brito JP, Morris JC, Montori VM. Thyroid cancer: zealous imaging has increased detection and treatment of low risk tumours. BMJ 2013;347:f4706.
11. Ali SZ, Cibas ES. The Bethesda System for Reporting Thyroid Cytopathology: Definitions, Criteria, and Explanatory Notes. 2nd edition. New York, NY, USA: Springer, 2018.
12. Haser GC, Tuttle RM, Su HK, et al. Active surveillance for papillary thyroid microcarcinoma: new challenges and

© Translational Cancer Research. All rights reserved.

opportunities for the health care system. Endocr Pract 2016;22:602-11.
13. Gweon HM, Son EJ, Youk JH, et al. Thyroid nodules with Bethesda system III cytology: can ultrasonography guide the next step? Ann Surg Oncol 2013;20:3083-8.
14. Yoo WS, Choi HS, Cho SW, et al. The role of ultrasound findings in the management of thyroid nodules with atypia or follicular lesions of undetermined significance. Clin Endocrinol (Oxf) 2014;80:735-42.
15. Zhang R, Wang Q, Sun C, et al. Clinical utility of histogram analysis of grayscale sonograms in assessing sonographic suspicious malignant thyroid nodules. Transl Cancer Res 2022. doi: 10.21037/tcr-22-479.
16. Patel KN, Angell TE, Babiarz J, et al. Performance of a Genomic Sequencing Classifier for the Preoperative Diagnosis of Cytologically Indeterminate Thyroid Nodules. JAMA Surg 2018;153:817-24.
17. Steward DL, Carty SE, Sippel RS, et al. Performance of a Multigene Genomic Classifier in Thyroid Nodules With Indeterminate Cytology: A Prospective Blinded Multicenter Study. JAMA Oncol 2019;5:204-12.
18. Livhits MJ, Zhu CY, Kuo EJ, et al. Effectiveness of Molecular Testing Techniques for Diagnosis of Indeterminate Thyroid Nodules: A Randomized Clinical Trial. JAMA Oncol 2021;7:70-7.
19. Fu G, Chazen RS, MacMillan C, et al. Development of a Molecular Assay for Detection and Quantification of the BRAF Variation in Residual Tissue From Thyroid Nodule Fine-Needle Aspiration Biopsy Specimens. JAMA Netw Open 2021;4:e2127243.

Cite this article as: Fu G, Witterick IJ. 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. Transl Cancer Res 2022;11(8):2483-2486. doi: 10.21037/tcr-22-1919

© Translational Cancer Research. All rights reserved.