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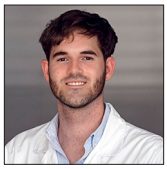
Editorial

Biochemical analysis of fine needle aspiration washout fluid: Redefining diagnostic precision in thyroid and parathyroid cytopathology

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Dear Editor,

The field of cytopathology stands at a critical juncture, transitioning from a discipline historically rooted in pure morphological interpretation to one that increasingly demands the integration of biochemical and molecular parameters to ensure diagnostic precision.

In this context, the immunometric analysis of fine-needle aspiration-washout fluid (FNA-WoF), often referred to as a "liquid biopsy" of the needle track, has emerged as a transformative adjunct that leverages the diagnostic accuracy of modern assays.[1-3] By detecting picogram or nanogram levels of tissue-specific biomarkers in the saline solution used to rinse the aspiration needle, it is possible to detect the functional signature of a lesion even when the cellular yield is sparse, compromised by blood, or morphologically equivocal.

Thyroid and parathyroid cytopathology arguably represent the most illustrative terrain where this significant breakthrough has been translated into clinical practice. Indeed, the measurement of thyroglobulin (Tg) in lymph node FNA-WoF has become the most extensively studied and widely adopted application of washout analysis, reshaping the staging and surveillance landscape for metastatic differentiated thyroid cancer (DTC), with particular emphasis on papillary thyroid carcinoma (PTC).[1,4] In a recent meta-analysis, Yu *et al.* (2025) acknowledged that ultrasound-guided FNA cytology (US-FNAC) may underperform in cystic or paucicellular PTC nodal disease, with sensitivity and specificity values ranging from 60–80% to 70–90%, respectively.[1] Nevertheless, the sensitivity and specificity approach 94–96% when combined with FNA-WoF Tg measurement.[1] A prospective study from Park *et al.* (2024) showed that combining Tg with cytokeratin fragment 21-1, a soluble fragment of the cytokeratin 19 protein that serves as a biomarker of lymph node metastasis and cell metastatic potential, in FNA-WoF of PTC lymph node metastases and thyroid nodules also yielded a >90% diagnostic accuracy, superior to either marker or simple FNAC.[5] However, cutoffs vary across studies and institutions, influenced by thyroid status (intact gland or

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post-thyroidectomy), serum Tg, assay calibration, and specimen dilution [Table 1].[1,6,7] For instance, in patients who have undergone total thyroidectomy (and often radioactive iodine ablation) for DTC, the production of Tg should be virtually absent; therefore, very low cutoffs are effective in these cases.[7] Conversely, interpretation is significantly more complex in patients who are either in the initial stages of assessment or have undergone a hemithyroidectomy.

Parathyroid hormone (PTH) measurement in FNA-WoF plays an important role in cases of primary hyperparathyroidism with negative imaging or disagreement between sestamibi and ultrasound. In this scenario, FNA-PTH can localize a nimble adenoma and allows for minimally invasive surgery rather than bilateral neck exploration.[8] It can also distinguish parathyroid tissue from thyroid lesions or central compartment lymph nodes in patients with multinodular goiter, prior neck surgery, or discordant imaging findings. Many groups favor a ratio-based interpretation (FNA-PTH relative to concomitant serum PTH) rather than relying solely on absolute concentration thresholds to define positivity. In a study conducted by Köroğlu *et al.* (2025), a PTH FNA-WoF/serum PTH ratio >1.0 was considered positive and yielded a sensitivity of 92.5% and specificity of 100% across 402 procedures.[3] When undiluted results were excluded to allow for quantitative analysis, the remaining 292 samples showed a sensitivity of 92.3% and specificity of 94.1% with a 0.99 PTH ratio cutoff. In addition, an absolute PTH-WO threshold of 99.5 ng/L was validated in the same subset, achieving a sensitivity and specificity of 93.1% and 94.3%, respectively. PTH washout also reopens an old question in modern clothing: When is parathyroid FNA justified? Some reports of hemorrhage, fibrosis, and potential seeding or parathyromatosis argue for selective use instead of reflex testing.[9] Therefore, a reasonable compromise would be to reserve PTH washout for cases where preoperative localization is genuinely limiting surgical planning (e.g., ectopic/atypical lesions, reoperative necks, or equivocal imaging) and to explicitly document the clinical rationale on the requisition so that the washout number is interpreted as a targeted localization tool rather than a general screening test in this case.

Medullary thyroid carcinoma (MTC) is frequently described as the "great mimicker" of thyroid pathology. Neoplastic cells typically produce calcitonin (Ct) and can display a bewildering array of morphologies on FNAC, including spindle-shaped, plasmacytoid, round, or polygonal. This phenotypic plasticity results in a dismal diagnostic performance when immunocytochemistry or a cell-block is unavailable, resulting in many cases still misclassified as indeterminate (Bethesda III) or suspicious for follicular neoplasm (Bethesda IV) nodules with diagnostic lobectomies that fail to clear the central compartment nodes. In patients with elevated Ct serum levels, Ct FNA-WoF measurement has repeatedly shown incremental value when directly compared with mere FNAC interpretation.[2,10] Cavallo *et al.* (2024) conducted a multicenter study where Ct washout levels were dramatically higher in MTC lesions (median ~3,100 pg/mL) compared to benign nodules (~0.5 pg/mL).[10] The FNA-Ct assay achieved an area under the receiver operating characteristic curve of 0.99, and using a threshold of ≥220 pg/mL yielded 100% sensitivity and 98% specificity for MTC diagnosis within nodular goiters. A meta-analysis by Trimboli *et al.* (2022) reported a pooled sensitivity of 98% (95% confidence interval [CI]: 96–100%), significantly outperforming mere FNAC, which showed a pooled sensitivity of 54% (95% CI: 35–73%) with marked heterogeneity.[2] It must be noted that studies commonly use small rinse volumes – often around 0.5 mL – and interpretation requires awareness of assay behavior – including dilution effects in very high concentrations – as well as the lack of a single universally accepted cutoff.

Table 1: Impact of key pre-analytical variables on washout accuracy.

Variable	Recommendation	Rationale	Potential pitfall
Saline volume	0.5–1.0 mL	Optimizes concentration for assay detection limits.	>2 mL: Dilutes the sample, causing false negatives. <0.5 mL: Insufficient volume for analyzer "dead volume".
Tube type	Plain (red/clear)	Prevents protein adsorption and assay interference.	SST (gel): may adsorb proteins. Heparin: May interfere with some immunoassays.
Processing	Immediate or ice	Prevents proteolytic degradation (critical for PTH).	Room temp>2h: Degradation of PTH, leading to false negatives.
Blood contamination	Measure serum levels	Distinguishes secretion from contamination.	Bloody tap: Introduces serum thyroglobulin/PTH/calcitonin. Must use washout/serum ratio interpretation.
High antigen load	Serial dilution	Detects "hook effect" in medullary thyroid carcinoma/PTH.	No dilution: Falsely low/normal results in samples with massive antigen excess ("hook effect").

PTH: Parathyroid hormone

In conclusion, biochemical analysis of FNA-WoF is a readily implementable ancillary technique that addresses the fundamental limitations of cytomorphology, low cellularity and morphological ambiguity, by querying the functional status of the tissue. Combined with US-FNAC, it can dramatically reduce indeterminate diagnoses, prevent unnecessary exploratory surgeries, and ensure that patients with thyroid and parathyroid malignancies receive precise, timely curative interventions. A pragmatic approach toward broader reliability would be to standardize the collection (consistent rinse volume and container), routinely report pre-analytical conditions, and locally validate interpretive thresholds using contemporary electrochemiluminescence immunoassays with paired serum values and outcome-based benchmarks.

AVAILABILITY OF DATA AND MATERIALS

Data sharing not applicable. No new data were created or analyzed in this study.

ABBREVIATIONS

AUROC: Area under the receiver operating characteristic curve
CI: Confidence interval
Ct: Calcitonin
CYFRA 21-1: Cytokeratin fragment 21-1
DTC: Differentiated thyroid cancer
FNA: Fine needle aspiration
FNAC: Fine needle aspiration cytology
MTC: Medullary thyroid carcinoma
PTH: Parathyroid hormone
PTC: Papillary thyroid carcinoma
Tg: Thyroglobulin
US-FNAC: Ultrasound-guided fine needle aspiration cytology
WoF: Washout fluid

AUTHOR CONTRIBUTIONS

AS: Conceptualization, writing of the original draft; IMM: Investigation, validation, writing - review and editing; FMD: Visualization, critical revision of the manuscript; FGM: Supervision, project administration. All author approve the final manuscript. All authors meet ICMJE authorship requirements.

ETHICS APPROVAL AND CONSENT TO PARTICIPATE

This is an editorial article and did not involve any new research with human participants or animals; therefore, ethics approval and consent to participate were not required.

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CONFLICTS OF INTEREST

The authors declare no conflicts of interest.

EDITORIAL/PEER REVIEW

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REFERENCES

1. Yu CY, Chu EH, Lin CH, Chen YC. Diagnostic efficiency of thyroglobulin in lymph node fine-needle aspiration washout: A systematic review and meta-analysis. J Clin Endocrinol Metab 2025;110:3569-87.
2. Trimboli P, Giannelli J, Marques B, Piccardo A, Crescenzi A, Deandrea M. Head-to-head comparison of FNA cytology vs. Calcitonin measurement in FNA washout fluids (FNA-CT) to diagnose medullary thyroid carcinoma. A systematic review and meta-analysis. Endocrine 2022;75:33-9.
3. Köroğlu EY, Evranos Öğmen B, Tural Balsak B, Yazicioğlu MÖ, Aydin C, Topaloğlu O, *et al*. Unlocking the diagnostic potential of parathormone washout: A path to accurate parathyroid tumor localization. Postgrad Med 2025;137:439-46.
4. Liu J, Wang R, Xu C, Zhao R, Wang S, Zhao Q, *et al*. FNA-Tg improves the diagnostic efficacy of FNAC for PTC lateral cervical LN metastasis. Sci Rep 2025;15:14057.
5. Park J, An S, Kim K, Kim JS, Jung CK, Bae JS. Diagnostic utilities of washout CYFRA 21-1 combined with washout thyroglobulin for metastatic lymph nodes in thyroid cancer: A prospective study. Sci Rep 2024;14:7599.
6. García-Molina F, Arense-Gonzalo JJ, Aguera-Sanchez A, Peña-Ros E, Ruiz-Marín M, Matínez-Perez M, *et a*l. Diagnosis of lymph node metastases from papillary thyroid carcinoma by measuring thyroglobulin in the puncture needle. Calculation of optimal cut-off point in our series. Rev Esp Patol 2024;57:258-64.
7. Cho A, Choe JH, Kim JH, Kim JS. Significance of washout thyroglobulin measurement in detecting thyroid cancer metastasis. Surgeries 2025;6:81.
8. Suzuki A, Hirokawa M, Kanematsu R, Tanaka A, Yamao N, Higuchi M, *et al*. Fine-needle aspiration of parathyroid adenomas: Indications as a diagnostic approach. Diagn Cytopathol 2021;49:70-6.
9. Balbaloglu H, Deniz O, Ozaydin RY, Tasdoven I, Karadeniz Cakmak G. Parathyroid fine needle aspiration with PTH

washout: Can it lead to parathyroid cell seeding in primary hyperparathyroidism? Medicine (Baltimore) 2024;103:e37754.
10. Cavallo AC, Pitoia F, Roberti J, Brenzoni P, Lencioni M, Jaroslavsky MJ, *et al*. Optimizing diagnostic accuracy of fine needle aspiration biopsy calcitonin measurements in detecting medullary thyroid carcinoma. Thyroid 2024;34:186-96.

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