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One nodule–one punction–one slide: Optimizing thyroid fine-needle aspiration for a digital workflow

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Abstract

Objective: Interventional pathologists have expanded their expertise by acquiring proficiency in ultrasound-guided thyroid fine-needle aspiration biopsy (FNAB) and are now required to optimize punction procedures due to low resources and digital workflows. The aim of this study is to compare FNAB sample adequacy in two series with one versus two slides available for cytopathological analysis and its influence on diagnosis categorization, time taken to reach a final diagnosis, scanning time and size of the digital files produced.

Methods: Patients were retrospectively selected based on the sampling of thyroid nodules using either two glass slides (two-slide group) or one slide only (one-slide group) and cytological diagnosis was performed using the second edition of the Bethesda system. For each group, the initial 15 cases were sorted to be scanned.

Results: From a total of 713 procedures, 328 were sampled into two slides and 385 on one slide only. No significant differences were found regarding nodule size, location or EU-TIRADS classification between the two groups. The one-slide group did not exhibit a higher prevalence of non-diagnostic or atypia of undetermined significance (AUS) categories. As expected, the mean time taken to finalize diagnoses in cases where only one slide was prepared was 1.2 days faster. Scanning time and total file size were also significantly smaller in the one-slide group.

Conclusions: Adopting the 'one nodule–one puncture–one slide' strategy for thyroid FNAB optimization enhances procedural efficiency in digital workflows, leading to cost savings without compromising diagnostic accuracy.

KEYWORDS
digital pathology, fine-needle aspiration biopsy, interventional pathologist, thyroid

1 | INTRODUCTION

Thyroid nodules are frequently detected in clinical practice. In a review regarding unselected patients examined by high-resolution thyroid ultrasound (US), 19 to 68% had at least one thyroid nodule.[1] These thyroid nodules are frequently benign, occurring in the setting of follicular nodular disease, while only 7%–15% are malignant.[2,3]

US-guided thyroid fine-needle aspiration biopsy (FNAB) is a reliable, minimally invasive diagnostic method for distinguishing malignant from benign nodules, with high sensitivity and specificity.[4] The Bethesda System is the most used reporting system for thyroid FNAB cytology, allowing terminology standardization and providing a malignancy risk for specific diagnostic categories.[5,6]

Several factors may influence FNAB accuracy, including biopsy technique, operator skills, sampling method and nodule

characteristics.[7,8] Although most reports agree on FNAB technique principles, clinical debate continues regarding the best needle calibre (22G to 27G needle), number of needle punctions (2–5), best sampling method (conventional smears/liquid-based cytology, air-dried/wet fixation, cell block preparation) and/or cost–benefit of rapid on-site microscopic evaluation (ROSE) by the pathologist, leading to procedure variations to optimize costs, diagnostic rate and patients experience.[9–11]

Physicians, other than radiologists, have diversified their skillset by gaining competency in the use of US for thyroid aspiration and those, so called, interventional pathologists are increasingly employing the technique with promising results.[12–14] Studies have demonstrated that compared to pathologists aspirating nodules under US guidance, non-pathologists tend to produce a greater number of slides per nodule and generate a higher percentage of unsatisfactory or non-diagnostic samples.[12,13] This suggests a tendency to submit excessive material prone to create technical artefacts, an approach that remains unlikely to be cost-effective.

On the contrary, as digital pathology becomes mainstream and more widely used in daily practice, the issues that have blocked the digital scanning of cytology slides must be tackled. Well known limitations to the production of cytological whole slide images (WSIs) comprehend, among others, the thicker and uneven content to be scanned (in comparison with tissue cuts) requiring a multilayer acquisition that is time consuming and generates files with larger sizes. This consumption of resources can be multiplied according to the number of slides prepared per each FNAB, threatening the institutional storage capacity. Reducing the number of slides prepared per each FNAB, without compromising diagnostic accuracy, can benefit the process of scanning and subsequent digital navigation by the pathologist.

The aim of this study is to compare FNAB sample adequacy in two series with one versus two slides available for cytopathological analysis. Additionally, we also tested the influence of the number of slides collected on cytopathological diagnosis categorization, time taken to reach a final diagnosis, scanning time and size of the digital files produced.

2 | MATERIALS AND METHODS

2.1 | Patient selection

Patients performing thyroid nodules FNAB at IPATIMUP were retrospectively selected from two different periods, October 2021 and October 2022, according to distinct sampling methods. Patients admitted in October 2021 ($n=280$) underwent one needle punction FNAB technique with sampling distributed into two glass slides—two-slide group—while in October 2022 ($n=341$) nodule sampling was performed using one slide only—one-slide group. Records of gender and age were undertaken for each patient. Records of largest size, location, US-classification EU-TIRADS (European Thyroid Imaging Reporting and Data System) and cytological diagnosis were obtained for each nodule.

2.2 | US-guided FNAB technique

US-guided FNAB of the thyroid nodules was accomplished in both groups by a single experienced pathologist. The FNAB of the nodules was requested by the assistant physician in those that, due to their US classification or other reasons, had indication for FNAB. The procedure was performed after neck hyperextension using a 23G needle placed in 10 mL syringe attached to a holder. The needle passed through the skin and punction the nodule, under US guidance. Immediate mild suction followed and, as soon as aspirated material appeared in the syringe, suction was released, and the needle was withdrawn from the patient. Subsequently, a drop of aspirated material was expelled into two or one glass slides. Smears were prepared with the help of an additional glass slide, left air-dried and transported to the laboratory for May Grunwald-Giemsa (MGG) Quick Stain (Bio Optica Milano Spa, Italy).

In all patients, one single needle punction was made to avoid discomfort and to ensure patients' confidence in case FNAB procedure required repeating. Rapid on-site evaluation (ROSE) was not performed. Cell blocks were prepared with material collected from rinsing the needle in a fixative (CytoRich Red Collection Fluid, Epredia, USA), in cases suspicious for medullary carcinoma, parathyroid lesion or metastatic disease.

2.3 | Cytological diagnosis

All slides were observed under the optical microscope by the same experienced pathologist (10 years' experience with US-guided FNAB) in thyroid cytological diagnosis using the second edition of the Bethesda system as a frame. The following diagnostic categories were used: non-diagnostic, benign, atypia of undetermined significance (AUS), follicular neoplasm, suspicious for malignancy and malignant. Adequate samples were considered if they contained at least six clusters of well-preserved cells, with each group consisting of at least 10 cells. The time taken to reach the cytological final diagnosis was determined by calculating the date difference between the end of the FNAB procedure and the date of the final diagnosis, recorded in days.

2.4 | Scanning outcomes

From each group—the group with collection of the sample into two slides and the group with collection of the sample into one slide—the first 15 cases were sorted to be scanned on the Pannoramic 1000® Scanner (3DHISTECH LTD, Hungary) at 20x (0.25 µm/pixel), using the current scanning protocol validated for cytological WSIs currently used in our laboratory and managed by Case Center software (3DHISTECH LTD, Hungary). The scanning protocol uses the extended focus mode, four scanned layers, with a layer distance of 0.25 µm, and a focus distance of 1973.4 µm. The time for scanning and the size file for each slide of the 15 cases of both groups were recorded.

2.5 | Statistical analysis

Statistical analysis was performed using IBM SPSS Statistics 24.0. Continuous variables were presented as mean ± standard deviation (SD) or as median, percentile 25-percentile 75, depending on the distribution normality or asymmetry determined through the Kolmogorov–Smirnov test. The chi-square test was utilized to evaluate hypotheses regarding categorical variables, such as analysing differences in cytomorphological Bethesda categories between the groups of two slides and one slide, while the *t*-test was used to examine differences in continuous variables between the two groups. The Mann–Whitney U test was employed to analyse differences in time to final diagnosis, dimensions of digital files and scanning time. Statistical significance was determined by accepting *p*-values <0.05.

3 | RESULTS

The study included a total of 713 US-guided thyroid FNAB procedures cytologically characterized by after one punction per nodule. Of these, 328 (46%) were sampled into two slides and 385 (54.0%) on one slide only. Most patients were female ($n=517$, 83.3%), with a mean age of 56.9 ± 14.4 years and a mean nodule size of 20.9 ± 10.3 mm (range: 5–89 mm). Patients' demographics and clinicopathological characteristics of the respective nodules were not different among groups and are summarized in Table 1.

Specifically, thyroid nodules size (20.7 ± 10.7 mm vs. 21.1 ± 9.9 mm, $p=0.599$; nodules with size >10 mm, 91.4% vs. 91.9%, $p=0.819$) and location were comparable between two-slide and one-slide groups, respectively. In addition, there were no statistical differences in all EU-TIRADs nodule categories between the two groups.

TABLE 1 Clinicopathological characteristics of study population.

	Two-slide group	One-slide group	*p* value
Patients (*n*)	280	341	
Female (*n*, %)	239 (85.4)	278 (81.5)	0.199**
Age (years)[a]	57.1 ± 13.3 (Range: 19–87)	56.7 ± 15.1 (Range: 12–87)	0.713**
Nodules (*n*)	328	385	
Size (mm)[a]	20.7 ± 10.7 (Range: 6–84)	21.1 ± 9.9 (Range: 5–89)	0.599**
Size>10 mm (*n*, %)	297 (91.4)	350 (91.9)	0.819*
Location (*n*; %)			0.470*
Right lobe	165 (50.3)	179 (46.5)	
Left lobe	147 (44.8)	190 (49.4)	
Isthmus	16 (4.9)	16 (4.2)	
EU-TIRADS classification (*n*; %)			0.892*
Benign	6 (3.0)	11 (4.1)	
Low risk	76 (38.4)	98 (36.3)	
Intermediate risk	90 (45.5)	122 (45.2)	
High risk	26 (13.1)	39 (14.4)	
Bethesda category (*n*; %)			0.250*
I. Nondiagnostic	31 (9.5)	28 (7.3)	
Insufficient material	26 (7.9)	24 (6.2)	
Cyst	5 (1.5)	4 (1.0)	
II. Benign	265 (80.8)	317 (82.3)	
Benign follicular nodule	250 (76.2)	291 (75.6)	
Lymphocytic thyroiditis	15 (4.6)	26 (6.8)	
III. AUS	15 (4.6)	13 (3.4)	
IV. Follicular neoplasm	10 (3.0)	19 (4.9)	
V. Suspicious for malignancy	4 (1.2)	1 (0.3)	
VI. Malignant	3 (0.9)	7 (1.8)	
Cell block preparation	4 (0.6)	2 (0.3)	

Abbreviations: *n*, number; %, percentage.

[a]Expressed in mean ± standard deviation, AUS, atypia of uncertain significance. Pearson's Qui-square test *, *t*-test**.

In respect of Bethesda System categorization, comparison between two-slide and one-slide groups was performed to evaluate if there was a difference in the adequacy ratio according to the number of slides available for cytopathological analysis. The one-slide group did not demonstrate higher prevalence of non-diagnostic category, but in fact there was an absolute slight reduction with no statistical difference from two-slide group to one-slide group (9.5% vs.7.3%, $p=0.293$). Additionally, no difference on AUS frequency was detected from two-slide group to one-slide group (4.6% vs. 3.4%, $p=0.412$), respectively, a result that was transversal to all categories of Bethesda classification. The number of cases that required cell block preparation was also similar between groups (Table 1).

Concerning the time taken to final diagnose, the mean time to finalize diagnoses in cases where only one slide was prepared was 1.2 days (33.3%) faster than the time consumed to finalize diagnoses in cases where two slides were prepared (2.4 ± 1.1 days vs 3.6 ± 1.3 days, respectively, $p<0.001$).

As expected, the total file size consumed by the scanning with obtention of cytology WSIs is significantly smaller if we prepare one slide rather than two slides (Table 2). The median total file size in one-slide group is 3006 Mb while in the two-slide group is 4037 Mb ($p=0.025$). However, the single slide prepared in group with one slide is significantly larger than the first slide of the group with two slides (median file size 3006 Mb vs. 2126 Mb, respectively, $p=0.017$).

Similarly, the scanning time consumed to obtain cytology WSIs is significantly smaller if we prepare one slide rather than two slides (Table 2). The median total scanning time in one-slide group is 221 s while in the two-slide group is 358 s ($p<0.001$). It is to be noted that for 100 nodules, 6.1 hours are needed for scanning when one slide is used, while 9.9 hours are needed for scanning when two slides are used. The time for scanning is reduced 38.4% if one slide is used instead of two. The single slide prepared in group with one slide and the first slide of the group with two slides took similar time to be scanned.

The quality of the slides in both groups was appropriated for diagnosis as demonstrated in Figure 1.

Regarding costs, and understanding this exercise is hard to be performed, the authors estimate that the costs saving would be in the 30%–40% range per thyroid nodule. Here, we present our rational: work spend with patient registration, FNAB procedure and expedition of the report remain the same; materials (such as glass slides and film), stains, technical work are fairly reduced by 40%; pathologist work is reduced in 33%; scanning time is reduced in 38%, and file size is reduced by one third.

4 | DISCUSSION

Wide clinical experience has confirmed the reliability of US-guided FNAB as a preoperative diagnostic tool in thyroid nodules.[4,5] Nevertheless, some centres report non-diagnostic rates greater than 40%.[15] At IPATIMUP, US-guided thyroid FNAB and respective cytological evaluation are performed by the so-called interventional pathologists with 7.3% to 9.5% cases non-diagnostic. This finding demonstrates that in pathology laboratories is possible to optimize thyroid sample collection with good results provided that US is used to guide FNAB of the nodules. The one nodule-one punction technique was applied to achieve proper cytologic diagnosis along with diminished patient discomfort related to the procedure. The second punction is reserved to situations when it is not possible to demonstrate the entrance of the needed within the nodule. In a study of Kuzan et al,[10] 121 patients were submitted to thyroid FNAB by a single radiologist, through a total of four punctions per nodule, and adequacy ratio per number of punctions was evaluated. Cytological evaluation demonstrated that, from the first to the last punction, there were no changes at diagnose and no statistically different rates of adequacy. Nonetheless, cumulative analysis verified a statistically higher adequacy rate for two punctions (87.6%), comparative to one punction (76.0%), without further significant improvement with a third or fourth punction. Applying one nodule—one punction strategy, we verified in our study that we obtained a more favourable non-diagnostic rate than that of the above-mentioned study (12.4%).

On the contrary, in a context of growing demand, limited pathology workforce, introduction of digital scanning and related storage constrains in laboratories, this study aimed to evaluate the impact of the number of glass slides prepared after FNAB of thyroid nodules

TABLE 2 Results from the scanning process in the first 15 cases of both groups.

	One-slide group $n=15$	Two-slide group $n=15$			*p* value		
	Slide 1 (total)	Slide 1	Slide 2	Slide 1 + 2(total)	p^{b}*	p^{c}*	p^{d}**
File size (Mb)[a]	3006 P[25–75]:2351–3639	2126 P[25–75]:1460–3199	1911 P[25–75]:1782–2351	4037 P[25–75]:3048–5486	0.017	0.025	0.191
Scanning time (s)[a]	221 P[25–75]:211–274	186 P[25–75]:132–227	174 P[25–75]:157–208	358 P[25–75]:299–433	0.074	<0.0001	0.268

Abbreviations: *n*, number; Mb, megabytes; s, seconds.

[a]Expressed as median (MW); P[25–75], percentile 25 – percentile 75.

[b]Comparing both Slides 1 of the two groups (MW).

[c]Comparing totals from both groups (MW) *.

[d]Comparing Slide 1 and Slide 2 of Two-slide group - Wilcoxon test **.

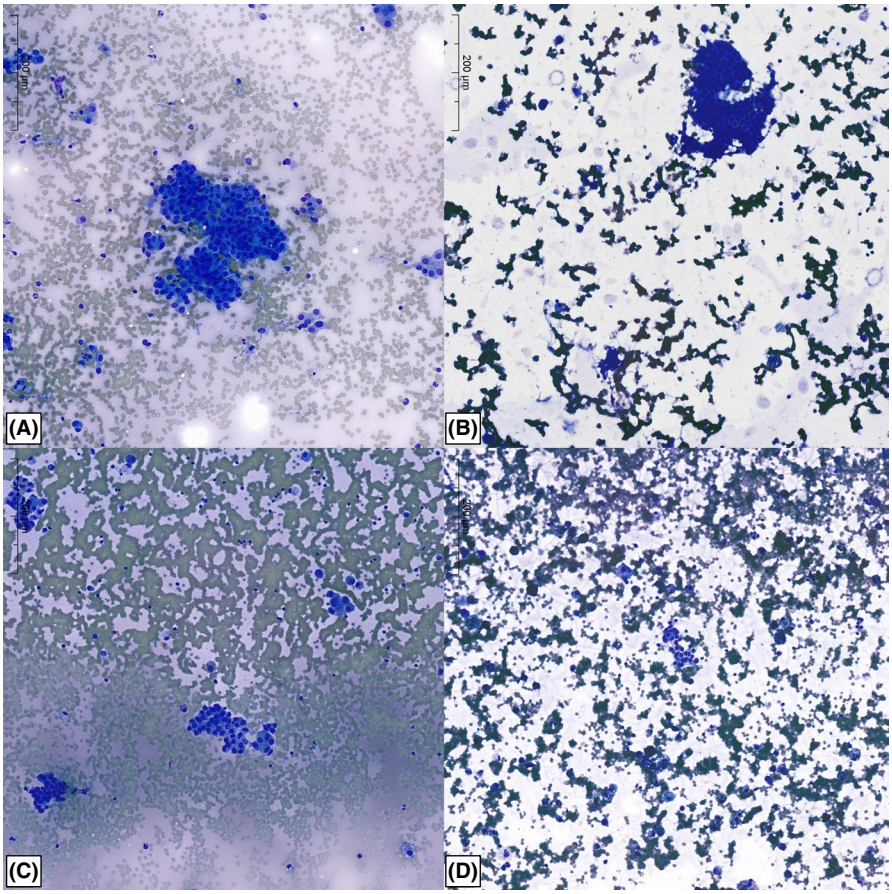

FIGURE 1 Examples from the scanned smears: Two-slide group—(A) Papillary carcinoma, (B) benign follicular nodule (MGG Quick Stain; 40x); one-slide group (C) papillary carcinoma and (D) benign follicular nodule (MGG Quick Stain; 40x).

on the adequacy ratio and diagnosis. We demonstrate that by preparing a single slide, the FNAB procedure time is optimized achieving concentration of the sample, scanning time is reduced in 38.4%, reporting time is reduced in 33.3%, and digital storage requirements are significantly decreased, without the need of additional cell block preparation and with no impact in the diagnosis. Our findings demonstrate that the one nodule–one punction–one slide sampling method, when performed by skilled and experienced pathologists, may not be inferior to the two-slide sampling method. Additionally, the one nodule–one punction–one slide sampling method allows sparing of precious resources enhancing the efficiency of pathology laboratory workflow and healthcare institutions in general.

A review article by Eccher and Girolami[16] describes the process of WSI acquisition and why it is more complex to implement for cytological slides, compared with histology slides. Cytology screening requires a more dynamic focusing since samples are thicker and more heterogeneous than those in tissue, requiring multiple focal planes (Z-stacking/extended focus). This results in much longer scan times and larger file sizes being generated for each slide. In our study, the total scanning time and file size of the cases were increased when two slides were used to collect the sample. Nevertheless, when assessing the median scanning time in the one-slide group in comparison with the first slide collected in the two-slide group we observed that the file size was larger in the one-slide group, without scanning time significative alterations. This fact may be due to an increased concentration of the sample in the first (and only) slide in the one- group in comparison with the two-slide group where the sample is distributed by two slides. The second slide in the two-slide group has a median file size smaller than the first one, suggesting that the majority of the sample is kept in the first slide making it the most relevant for observation.

In this study, the rapid on-site evaluation (ROSE) was not performed since it is not part of the regular FNAB procedure in our institution due to its marginal contribution to decrease the already low percentage of cases that need repetition (data not shown). The authors understand that in centres where ROSE is performed, namely by less experienced pathologists, there will be the need to collect more than one smear/slide for observation.

This retrospective study has some limitations to consider namely those related with patients' selection. By selecting a period, we cannot anticipate eventual bias of patients' selection, certainly attenuated by the fact that they were distributed among Bethesda System categories with similar frequencies in both periods. The basic procedure, operator and instruments remained the same in both periods of time as well. The data may not be generalizable to centres with less experience in thyroid FNAB and with other times of case load/features. Follow-up information of the patients included in the study is not available since our institution is not inserted in a hospital facility, preventing us to have immediate access to surgical data and to calculate accuracy of the procedure in these cohorts. Future studies may be needed to evaluate the impact on diagnostic accuracy and in contexts where ROSE is used as a standard procedure.

The procedures were performed in different patients at different times and the study aimed to show diagnostic non-inferiority of the 'one punction–one slide' method for thyroid nodule evaluation. The authors underly that even not having the possibility of scanning cytology slides or even choosing not to scan cytology slides due to the low efficiency of this process, the laboratories that choose the 'one nodule–one punction–one slide' strategy may still collect efficiency-related benefits from it.

We conclude that the use of the 'one nodule–one punction–one slide' strategy for thyroid FNAB comprehends an optimization of the procedure that leads to increase efficiency for a digital workflow, and cost savings without compromising diagnostic accuracy.

AUTHOR CONTRIBUTIONS

CE performed study concept and design; CE, JV and MC performed development of methodology AM and CR wrote the paper first draft; BC, MC, AM, JV and CE performed observation and data collection; AP and CE provided analysis and interpretation of data, and statistical analysis. All authors read and approved the final paper.

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CONFLICT OF INTEREST STATEMENT

C.E. consulted for Mindpeak, MSD and Leica. A.P. consults for Indica Labs. Other authors declare no conflict of interest.

DATA AVAILABILITY STATEMENT

Data generated or analysed during this study are partially included in this published article and may be provided after reasonable request.

ETHICS STATEMENT AND CONSENT TO PARTICIPATE

Formal ethical approval was not required for this study, as it did not involve any patient data collection or impact on patient care.

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