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The intraoperative frozen section analysis of thyroid nodules categorized under Bethesda III-IV-V, accompanied by concurrent imprint cytology as a diagnostic technique

Ezgi Hacihasanoglu[a,*], Osman Melih Topcuoglu[a]

[a] *Yeditepe University, Faculty of Medicine, Department of Pathology, Istanbul, Türkiye*

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Abstract

Aim: Addressing therapeutic challenges posed by thyroid nodules classified under Bethesda categories III-IV-V is a primary concern. The aim of this study is to evaluate the diagnostic outcomes of intraoperative frozen section (IFS) when applied to these specific thyroid nodules, alongside evaluating the diagnostic performance of IFS when used in conjunction with concurrent imprint cytology (IC).

Materials and Methods: We conducted a comprehensive search in the electronic hospital database to retrieve IFS results and final diagnosis of cases with category III-IV-V thyroid nodules. These patients had undergone nodule resection or partial thyroidectomy with IFS. We gathered information regarding patient demographics, nodule dimensions, and the execution of concurrent IC.

Results: Our study comprised 81 eligible patients. Female/male ratio was 3.2. Mean patient age was 43.5 years. Average nodule size was 21.7 mm. IC was conducted in 20 cases as part of the IFS procedure. Sensitivity and specificity of IFS were 71% and 95%, respectively. Positive predictive value (PPV) was 83% and negative predictive value (NPV) was 90%. Diagnostic accuracy of IFS was 88%. We stratified cases into those with and without IC. IFS with IC exhibited a sensitivity, specificity, PPV, NPV of 80%, 93%, 80%, 93%, respectively. In contrast, IFS without IC had sensitivity, specificity, PPV, NPV values of 68%, 95%, 83%, 90%, respectively.

Conclusion: Inconsistent pathological assessments and varying malignancy rates present challenges when managing Bethesda category III-IV-V thyroid nodules. In addressing these challenges, IFS emerges as a potential effective method. Nonetheless, IFS exhibits restricted sensitivity in detecting malignancies within Bethesda category III-IV-V nodules. Inclusion of concurrent IC has the potential to improve the precision of IFS in identifying malignancies. A more extensive study in this regard is warranted.

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Introduction

Thyroid nodules represent one of the most frequently encountered clinical conditions. They are typically assessed through ultrasonography and fine needle aspiration biopsy (FNAB). These nodules are cytologically classified based on their expected malignancy risk, utilizing the Bethesda System for Reporting Thyroid Cytopathology, established in 2009 and updated in 2017 [1-3]. Category III nodules, also known as atypia of undetermined significance (AUS) or follicular lesion of undetermined significance (FLUS), represent a group of nodules that have some cellular abnormalities but do not meet the criteria for a definitive diagnosis of malignancy. Category IV nodules, classified as follicular neoplasm or suspicious for a follicular neoplasm, have a higher risk of malignancy compared to category III nodules. Category V nodules are labeled as suspicious for malignancy. The risk of malignancy for Bethesda category III, IV and V is 6-18%, 10-40% and 45-60%, respectively [1]. Bethesda category III, IV and V nodules are classified as 'indeterminate' due to their variable risk of malignancy. These categories play a significant role in guiding clinicians in the management of patients with thyroid nodules, helping to determine the appropriate next steps in their evaluation and treatment. A problem regarding these 'indeterminate' nodules is the low concordance among pathologists [2], which directly affects the treatment planning. Therefore, the clinical decision-making process becomes difficult in these lesions. The American Thyroid Association (ATA) guidelines recommend surgical management of Bethesda

*Corresponding author:
Email address: ezgihaci@yahoo.com (Ezgi Hacihasanoglu)

IV and V nodules. For Bethesda III nodules, repeating FNAB or molecular testing can be performed. Diagnostic surgical excision is another option in the management of Bethesda III nodules [3].

The surgical treatment of indeterminate nodules, which carry a variable risk of malignancy, can potentially lead to a significant number of unnecessary thyroidectomies, resulting in subsequent postoperative complications. Use of intraoperative frozen section (IFS) to determine the nature of the nodule and to decide the extent of surgery has been thought as a solution for a long time [4]. However, several recent studies suggest against this approach due to low to moderate diagnostic performance and inefficiency of IFS [5, 6]. Nevertheless, IFS of indeterminate nodules is still in routine use in some institutions. In centers where routine use of IFS continues, there is a need to enhance the sensitivity of this procedure while maximizing its economic efficiency for optimal patient benefit. The simultaneous use of IFS and imprint cytology (IC) may be a way to increase the sensitivity without causing significant additional time and cost, by providing a combined evaluation of the morphological and cytological features of the nodule.

The objective of this study conducted at a single center is to assess the effectiveness of IFS analysis on resection specimens from thyroid nodules classified under Bethesda categories III-IV-V. Additionally, the study aims to explore how the inclusion of IC influences the accuracy of IFS.

Materials and Methods

We retrospectively reviewed the IFS outcomes and final diagnostic reports of patients with thyroid nodules falling within Bethesda categories III-IV-V, who underwent nodule resection or partial thyroidectomy with IFS between January 2018 and December 2021. We extracted demographic data, nodule dimensions, and the availability of concurrent IC.

A total of 81 patients, each with Bethesda category III-IV-V nodules, underwent either nodule resection or partial thyroidectomy with IFS were included in the study. For IFS, samples were taken from the nodule depending on the nodule size and macroscopic evaluation. None of the nodules were totally sampled for IFS. In the most recent 20 cases, concurrent IC was conducted and assessed simultaneously with IFS (IFS+IC). Regarding cytological assessment, we prepared two slides: one was fixed in ethanol and stained with Papanicolaou, while the other was air-dried and stained with May Grunwald-Giemsa.

The results were communicated with the surgical team as three headlines: 1) malignant, 2) follicular neoplasm-favor malignant and 3) follicular neoplasm-favor benign. For follicular patterned lesions, if suspicion of capsular/vascular invasion was present in IFS, it was called follicular neoplasm (FN)-favor malignant; if not, it was called FN-favor benign. For all cases with a IFS diagnosis of FN, it was shared with the surgeon that it is not possible to sample the entire nodule in IFS and the final diagnosis will be given after the examination of permanent sections.

In the calculation of the sample size for this study, with a Type-1 error of 0.05 and an effect size of 0.8, the power value was determined as 87% for a total of 81 cases; 20 cases with IFS+IC and 61 cases with only IFS. The normal distribution of continuous measurements in the study was assessed using the Shapiro-Wilk (for $n<50$) and Skewness-Kurtosis tests. Since the measurements were found to follow a normal distribution, parametric tests were applied. Descriptive statistics, including mean, standard deviation, number (n), and percentage (%), were used to characterize continuous variables in the study. Independent T-test was employed to compare continuous measurements between groups. The relationship between categorical variables was determined using the Chi-square test. Sensitivity, specificity, positive predictive value (PPV), negative predictive value (NPV), and accuracy were calculated for diagnostic tests. A significance level of $p<0.05$ was considered in the calculations, and the statistical package program SPSS (IBM SPSS for Windows, ver. 26) was utilized for the analyses.

This study received approval from the ethical committee (Yeditepe University Non-invasive Clinical Research Ethics Committee, 202209Y0286-14/10/2022) and was conducted in accordance with the Declaration of Helsinki and the ethical guidelines of the institutional research committee.

Results

A total of 81 patients, each with Bethesda category III-IV-V nodules, underwent either nodule resection or partial thyroidectomy with IFS. Among these nodules, 26 (32%) were categorized as Bethesda III, 31 nodules (38%) as Bethesda IV, and 24 nodules (30%) as Bethesda V. The patients' ages ranged from 18 to 76 years, with a mean age of 43.5 years. Out of the total patients, 62 were female and 19 were male, resulting in a female-to-male ratio of 3.2. The nodule size ranged between 3 millimeters to 80 millimeters (mean: 21.7 millimeters). One to three samples (mean: 1.3) were taken from the nodule depending on the size and macroscopic suspicion. In 61 cases the nodules were assessed by IFS, whereas in the most recent 20 cases concurrent IC was also conducted and assessed simultane-

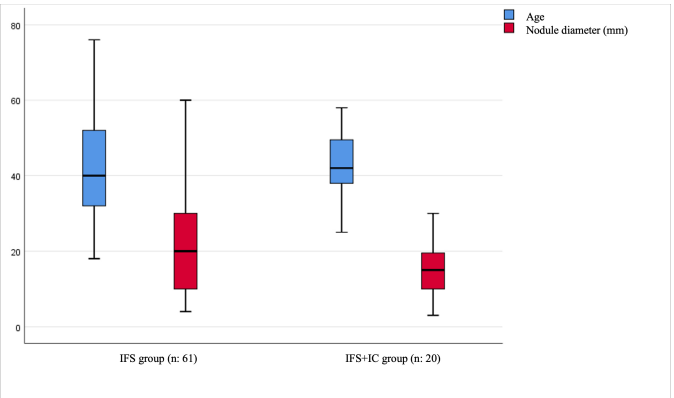

Figure 1. Distribution of patient age and nodule size in cases evaluated with only intraoperative frozen section and in cases evaluated with intraoperative frozen section and concurrent imprint cytology (IFS: Intraoperative frozen section, IC: Imprint cytology).

Table 1. Age, sex and nodule diameter data in cases evaluated with only intraoperative frozen section and in cases evaluated with intraoperative frozen section and concurrent imprint cytology. Use dot mark in stead of comma.

	Cases evaluated with only IFS (n:61)		Cases evaluated with IFS+IC (n:20)		*p
	Mean	Std. Dev.	Mean	Std. Dev.	
Age	43.82	13.65	42.90	11.06	0.786
Nodule diameter (mm)	23.53	17.44	16.26	10.52	0.091
	n	%	n	%	**p
Sex					
Male	12	63.2%	7	36.8%	0.160
Female	49	79.0%	13	21.0%	

IFS: Intraoperative frozen section, IC: Imprint cytology *Significance levels according to Independent T-test results. **Significance levels according to Chi-square test results.

Table 2. Intraoperative frozen section diagnosis and final diagnosis of all cases.

IFS diagnosis	Final diagnosis
Malignant (n=6)	3 PTC 2 PMC 1 FVPC
Follicular neoplasm favor malignant (n=12)	2 FC 2 PTC 4 FVPC 1 NIFTP 3 AN
Follicular neoplasm favor benign (n=63)	1 FC 1 PTC 4 PMC 57 AN

IFS: Intraoperative frozen section, PTC: papillary thyroid carcinoma, PMC: papillary microcarcinoma, FVPC: follicular variant papillary carcinoma, FC: follicular carcinoma, NIFTP: noninvasive follicular thyroid neoplasm with papillary-like nuclear features, AN: adenomatous nodule.

Table 3. Diagnostic performance of intraoperative frozen section and intraoperative frozen section combined with imprint cytology.

Method	Sensitivity (%)	Specificity (%)	PPV (%)	NPV (%)
IFS only	68.7	95	83.3	90.4
IFS+IC	80	93.3	80	93.3

PPV: positive predictive value, NPV: negative predictive value, IFS: frozen section, IC: imprint cytology.

ously with IFS (IFS+IC). The age, sex distribution, and nodule size were observed to be similar between the IFS group and the IFS+IC group ($p>0.05$) (Table 1, Figure 1). IFS diagnosis was rendered as FN-favor benign in 63 cases, FN-favor malignant in 12 cases, papillary microcarcinoma in 3 cases and papillary thyroid carcinoma in 3 cases. After FS, nodules were totally sampled. Final pathology reports revealed adenomatous nodule in 60 cases, noninvasive follicular thyroid neoplasm with papillary-like nuclear features (NIFTP) in 1 case, follicular variant papillary thyroid carcinoma in 5 cases (all five cases are currently classified as NIFTP), papillary microcarcinoma in 6 cases, papillary thyroid carcinoma in 6 cases, follicular carcinoma in 3 cases. IFS diagnosis and final diagnosis of all cases are presented in Table 2. Histopathological and cytological microscopic images reflecting two examples from the cases are presented in Figure 2.

For statistical analysis, the IFS diagnosis of FN-favor benign was accepted as benign and the IFS diagnosis of FN-favor malignant was accepted as malignant. The comprehensive performance metrics for IFS included a sensitivity of 71.4%, specificity of 95%, a positive predictive value of 83.3%, and a negative predictive value of 90.4%, resulting in an overall diagnostic accuracy of 88.8%. The specific sensitivity, specificity, positive predictive value, and negative predictive value for both IFS+IC and IFS alone are detailed in Table 3.

Discussion

IFS of thyroid nodule aim to provide information about the nature and malignancy potential of the lesion and to guide in deciding the extent of surgery. However; use of IFS has been highly discouraged over the years, due to low sensitivity, high discrepancy and inefficiency [5-7]. IFS of thyroid nodules may also cause tissue loss and significant artifacts which may affect the final evaluation [8]. An important development in thyroid pathology is the increasing accuracy of thyroid FNAB since Bethesda System for Reporting Thyroid Cytopathology is used as a standardized system. FNAB is currently the most useful method to assess the risk of malignancy in thyroid nodules. Nevertheless, within the framework of these six categories, Bethesda category III-IV-V nodules exhibit varying malignancy rates and a lack of consensus among pathologists. These factors contribute to challenges when making treatment and follow-up decisions. Although it varies from center to center, IFS is still used to decide the extent of the surgery in patients with Bethesda III-IV-V nodules and thought as an option to eliminate the diagnostic difficulties in these indeterminate nodules. In institutions like ours, where IFS is routinely used in Bethesda III-IV-V nodules, it becomes

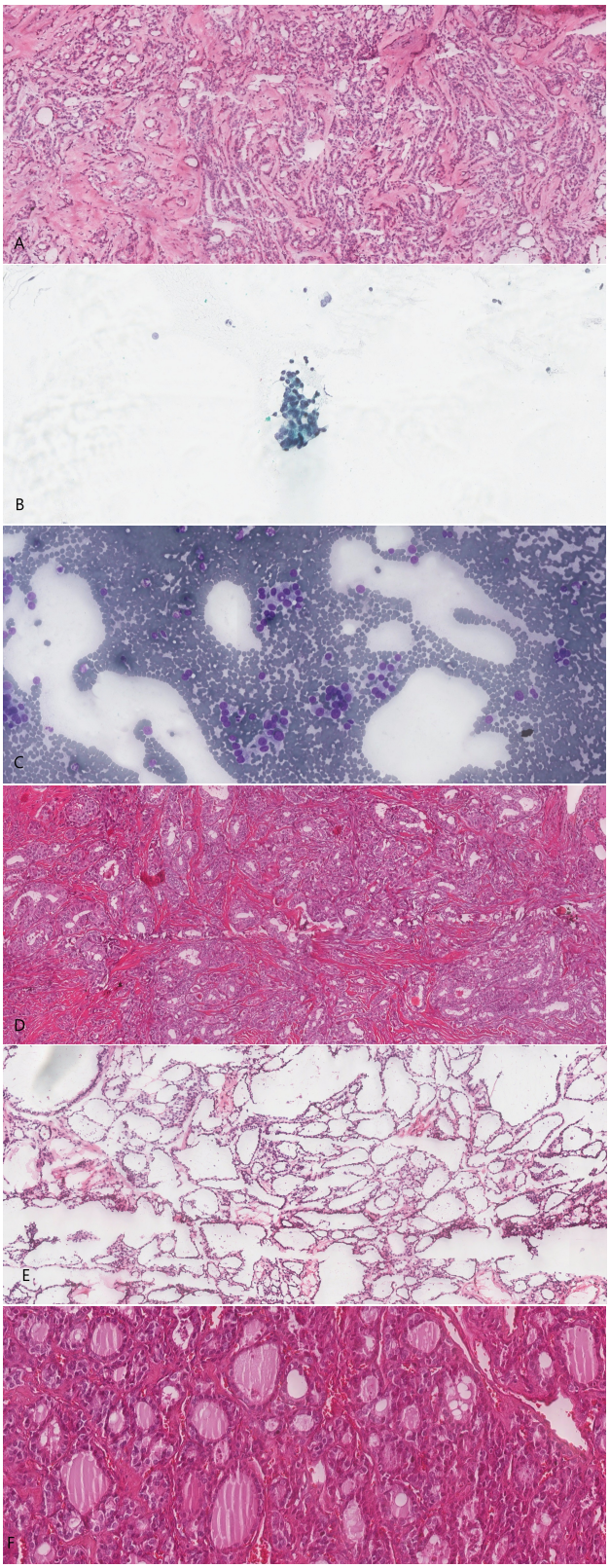

Figure 2. Microscopic images of IFS sections, IC specimens and paraffin block sections of two cases. First case (Figure 2A-B-C-D) shows an example of malignant category in IFS+IC and the final diagnosis was PTC. Figure 2A shows the IFS slide (hematoxylin-eosinx10). Figure 2B shows IC slide stained with Papanicolaou stain (x20) and Figure 2C shows IC slide stained with May Grunwald-Giemsa stain (x20). Second case (Figure 2E-F) constitutes an example for FN-B category in IFS. Figure 2E shows the IFS slide (hematoxylin-eosinx20). This case did not have IC. The final diagnosis is PMC, shown in Figure 2F (hematoxylin-eosinx20).

important to have high sensitivity and accuracy for optimal patient management.

In the literature, the sensitivity of IFS varies over a wide range (6%-100%) [9]. In a meta-analysis of studies from 1991 to 2018 that assessed IFS in Bethesda category IV nodules, the overall sensitivity was found to be 43% [9]. This means that in patients diagnosed as FN or suspicious for a FN in FNAB, IFS shows moderate diagnostic performance and has limited value for intraoperative decision-making process. In the most recent study on this subject by Goemann et al., the sensitivity, specificity and accuracy of IFS in 316 thyroid nodules were 80.9%, 100% and 94.9%, respectively [10]. However, in Bethesda III and IV nodules the sensitivity, specificity and accuracy have been reported as 25%, 100% and 88.7%, respectively. These results indicate a low sensitivity of IFS in indeterminate nodules and authors do not suggest the use of routine IFS for these types of nodules. In our study, consisting only of Bethesda III-IV-V nodules, the overall sensitivity of IFS was 71.4% and diagnostic accuracy was 88.8%. Our findings show that the sensitivity of IFS in indeterminate thyroid nodules in our institution is higher than most of the studies. This may be due to the low number of cases in our study but it may also reflect the long-term collaboration of the same surgical team and pathology team in our institution.

Other than low sensitivity, another important issue in thyroid IFS is the high deferral rate [9, 11]. For papillary thyroid carcinoma, the concordance between FNAB and IFS results is good due to the unique nuclear features of the tumor [12]. For follicular lesions however, evaluation of the nodular capsule and the vascular structures have diagnostic importance. Usually, the entire nodule needs to be sampled in order to determine capsular and/or vascular invasion. When operative time and cost is added to the equation, IFS is usually insufficient for the evaluation of follicular lesions. This situation causes high deferral rates, meaning that the definitive diagnosis cannot be given in IFS and it will be given after permanent sections. In Roychoudhury et al.'s research, they demonstrated that 55% of cases were deferred during the IFS analysis, particularly with rates of 68% for Bethesda III and 86% for Bethesda IV categories [11]. The authors underscored the substantial deferral rate and emphasized the challenge of providing a definitive diagnosis through IFS for nodules falling under Bethesda III and IV categories. In the meta-analysis by Grisales et al., it was reported that 58% of the studies had deferred results at varying rates [9]. In our study, in case of a FN in IFS, the surgical team was informed that the nodule consisted of a follicular patterned neoplasm and total examination of the nodule would be needed to make the final diagnosis. If suspicion of capsular/vascular invasion was present in IFS, IFS result was reported as FN-favor malignant; if not, it was reported as FN-favor benign. With this viewpoint, there were no deferred cases in our cohort; but from another perspective, all cases of follicular neoplasms, unless they are totally sampled in IFS, are in fact deferred.

IFS is still used to guide the treatment of indeterminate thyroid nodules despite the highly variable sensitivity and accuracy and high deferral rates. Using intraoperative cy-

tology in conjunct with IFS has been thought as a method of increasing the sensitivity of FS. Tworek et al. compared intraoperative cytology consisting of imprint or scrape preparations with IFS in 68 cases, and as a result they suggested employing intraoperative cytology as a supplementary tool alongside IFS [13]. In two separate studies by Basolo et al., significant increase in the accuracy of IFS with association of intraoperative cytology in the form of scrape preparation was reported [14, 15]. The authors' conclusion was that combining IFS with intraoperative cytological examination represents the most precise technique for managing thyroid nodules surgically. Chehrei et al. compared diagnostic performance of IFS, touch imprint, crash preparations and combinations of these diagnostic tools in 55 nodules [16]. The sensitivity of IFS alone was 92.9%, whereas IFS combined with intraoperative cytology (including touch imprint and crash preparations) was 100%. All of the above-mentioned studies included scrape/crash preparations for cytological evaluation, but not imprint preparations. In another study by Taneri et al., diagnostic performances of imprint cytology and IFS were compared and both methods showed similar sensitivity and accuracy rates [17]. Our study showed that concurrent IC increases the sensitivity of IFS in indeterminate thyroid nodules. Sensitivity of IFS+IC was 80%, whereas sensitivity of IFS without IC was 68.7%; meaning that combination of IC and IFS increased the sensitivity of IFS evaluation of indeterminate thyroid nodules.

Conclusion

The capacity of IFS to identify malignancy within Bethesda category III-IV-V nodules is restricted, prompting a recommendation against its routine use in cases involving indeterminate nodules. Nevertheless, the inclusion of concurrent IC holds promise for improving the accuracy of IFS in identifying malignancies correctly. It is crucial to emphasize that this study is limited by its modest sample size, highlighting the necessity for larger-scale studies to validate or challenge the results reported in this research.

Ethical approval

Ethical approval was received for this study from Yeditepe University Non-Interventional Clinical Research Ethics Committee (202209Y0286-14/10/2022).

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