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ORIGINAL ARTICLE

Could cytology supplant frozen section for intraoperative evaluation of thoracic lesions? A single institutional experience in a developing country

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Abstract

Background: The diagnostic performance of cytology was compared with the frozen results and its usability was evaluated as a rapid diagnosis method in intraoperative thoracic surgery in a single institution (Istanbul, Turkey).

Methods: All 197 subsequent patient specimens (cases) from 158 patients who were sent to our department from the thoracic surgery clinic for an intraoperative diagnosis request between the years 2016 and 2021 were evaluated. Obtained results from frozen and cytology were compared with final paraffin section diagnoses. Lesions were grouped into three different groups as nonneoplastic, benign, and malignant neoplasms.

Results: Diagnostic accuracy values of cytology and frozen sections in intraoperative consultation were 98.8% and 99.4%, respectively. Sensitivity values of cytology and frozen sections in intraoperative consultation were 96.3% and 98.7%, respectively. Specificity values of cytology and frozen sections in intraoperative consultation were 100% and 100%, respectively. Negative predictive values of cytology and frozen sections in intraoperative consultation were 96.7% and 98.9%, respectively. Positive predictive values of cytology and frozen sections in intraoperative consultation were 100% and 100%, respectively. Kappa statistics between cytology and frozen revealed a very high interrater reliability (Cohen's Kappa value: 0.911; $p = .001$; $p < .01$). The difficulty in distinguishing primary and metastatic carcinoma, which is mostly undecided in frozen sections and the definitive diagnosis is left to paraffin sections, seems also be a problem in the cytological examination.

Conclusions: Cytological diagnosis can be used in the evaluation of small biopsy specimens that require tissue preservation in intraoperative consultation, especially for immunohistochemical and advanced genetic studies.

KEYWORDS
intraoperative diagnosis, pulmonary pathology, scratch-imprint cytology

1 | INTRODUCTION

Lung cancer is the most commonly encountered malignancy globally, both in terms of deaths (1.6 million) and new cases (1.8 million) per year.[1,2] Successfully accomplished on site assessment for diagnosis and staging of lung cancer necessitates not only the capability to recognize the diagnostic specimen but also to determine when sufficient material has been obtained to provide the full range of ancillary tests required.[2] Especially during cancer surgery, the surgeon requires to determine the extension of the resection, and on-site evaluation about the true lesion extension and involvement of the regional lymph nodes is essential for such decisions.[3] The cryosections for intraoperative pathology consultation are generally utilized to determine the adequacy of the specimen for diagnosis, to confirm a previous diagnosis, to define the benign or cancerous nature of the operated lesion, to reveal the disease spread and if the resection margins are free of cancer cells, and to find out the lymph nodes involved (sentinel node procedure and N staging).[3,4] Intraoperative frozen section is a plausible option superior to preoperative biopsy because, since it provides larger amount of tissues for pathologist evaluation but it has some disadvantages.[5] Cryosection technique is lasting about 10 to 11 min, and the section quality is lower than the conventional histopathology with paraffin embedding.[6] Cryosections harbor limitations, artifacts of frozen investigation might be misinterpreted (nuclear chromatin alterations due to frozen technique), special stains cannot be employed to distinguish tumor subtypes resembling other lesions, and the technician skills may alter obtaining proper slides for an accurate diagnosis.[3] In frozen section, it is intriguing to interpret the lung tissue due to total collapse of the alveolar spaces with highly distorted architecture, and ice crystal formation during cryosection.[7] This uncertain frozen section diagnosis delays the exact diagnosis and may subject the patients to a second surgery after ultimate pathologic diagnosis.[7] Frozen procedure is more labor intensive and time-consuming when multiple specimens are sent for analysis simultaneously or within a short period, as especially occurs in staging procedures.[6] Imprint cytology is faster than the frozen (about 2 min) and provides sampling of the specimen more extensively.[6]

Targeted treatments for lung cancer are on the rise; hence, more molecular studies and immunostaining analyses may be necessary to decide for the proper adjuvant therapies. Therefore, cytology conservation of tissues for future analyses may save patients from the potential morbidity of repeat biopsies.[8] Intraoperative cytology may be used to augment or, in certain circumstances, supplant the conventional frozen section analysis and additively to the routine stains utilized for cytomorphology, the prepared smears can be used for special immunohistochemical and histochemical studies.[9] Due to all the above cited reasons, it is necessary to define whether cytology can supplant frozen investigations as a general strategy in diagnosing lung lesions. Hence, in this study, we aimed to define the sensitivity, specificity, negative and positive predictive values, diagnostic accuracy, and specific diagnostic features of cytology versus frozen investigations for lung lesions in comparison to definitive paraffin diagnosis as golden standard. We also defined interrater reliability between cytology and frozen inspection with kappa statistics.

2 | MATERIALS AND METHODS

Patients undergoing chest surgery at Siyami Ersek Chest, Heart and Vessel Surgery Research and Education Hospital from 2016 until 2021 were evaluated during surgery by intraoperative cytology and frozen section analysis. All subsequent cases submitted to the pathology team for intraoperative diagnosis were included and no cases were excluded in the mentioned period. Ethical approval was obtained from the responsible ethical committee with the decision number HNEAH-KAEK 2022/KK/132. The study complies with the principles of the Declaration of Helsinki. All patients signed informed consent forms for participation into the study. There were 48 women and 110 men patients aging between 26 and 81 included in the study and 197 specimens taken from these patients were analyzed in the statistics. Based on definitive paraffin diagnoses, a malignancy diagnosis for a nonneoplastic or benign neoplasia, was evaluated as false-positive. Malignant cases where a malignant neoplasia was diagnosed as benign or nonneoplastic were considered false-negative. Cases with indecision in the differentiation of primary/metastasis or carcinoma/malignant mesothelioma and cases that could not be evaluated due to inadequate methods were also discussed. The indecisive group of cases included those where benignity or malignancy cannot be discriminated, where primary and metastatic tumors cannot be distinguished and where the diagnosis was deferred to definitive paraffin diagnosis.

2.1 | Operative technique

Case specimens were obtained either via mediastinoscopy or open surgical procedure.

2.2 | Cytology technique

Smear preparations were prepared by scraping and/or imprinting from fresh tissue samples sent in plastic containers. The scraping was performed with the edge of the slide, not the knife. The specimens were then immediately spread on a glass slide and fixed in 95% ethanol. The critical step in the process is fast smearing and fixation to hinder air-drying artifact. Slides are then stained with a rapid hematoxylin and eosin stain: hematoxylin for 60 s followed by water rinse and eosin counterstain for 30 s followed by dehydration in 95% and 100% ethanol and clearing in xylene. Standard cytologic categories were utilized to interpret the findings. A pathology specialist experienced in lung pathology examined first the cytology and then the frozen slides. Before the evaluation of the pathological findings, the surgeon informed the pathologist about the clinical history of patients including any previous malignancy, any suspicion of nonneoplastic lesions such as tuberculosis, clinical prediagnosis, and radiological findings. The

sample was regarded as inadequate/nonrepresentative if cellular material from the anatomic site was not identified. The existence of four to six groups of cells on each slide was required for proper assessment of the nonlymphoid specimens. The benign category included reactive and inflammatory changes or completely normal cells. The gray zone included cells with suspicious or atypical features, and the malignant category had definitive cancer criteria. Clinically benign lesions with inadequate aspirates were either followed or reaspirated.

2.3 | Frozen-section technique

Tissue specimens no larger than 1.0 cm and 2 to 3 mm thick are frozen in optimal cutting temperature medium on a brass chuck in a −23°C cryostat. Six-micron sections are cut, placed on glass slides, and fixed for 1 min in 95% ethanol. They are then stained by the same rapid hematoxylin and eosin technique used for imprint slides.

2.4 | Permanent-sections and final pathologic analysis

All remaining tissue after imprint preparation is placed in 10% neutral-buffered formalin for processing as permanent sections. Frozen tissue is thawed and placed in 10% neutral-buffered formalin for permanent sections. Diagnoses rendered on imprints or frozen sections were compared with those for permanent sections. Imprint or scratch cytological specimens were compared with frozen specimens for sensitivity, specificity, diagnostic accuracy, negative and positive predictive values. According to the intraoperative diagnoses, specimens were classified as nonneoplastic, benign, malignant, indecisive/deferred to paraffin diagnosis and as insufficient. False positive results are those which specimens were classified as malignant despite they were non-malignant/benign in paraffin definite diagnosis.

2.5 | Statistical analyses

Kappa agreement test was used to evaluate the concordance between cytology and frozen pathology results for diagnoses, which was calculated with NCSS (Number Cruncher Statistical System) program. Statistical significance was accepted as $p < .05$.

3 | RESULTS

A total of 197 cases (specimens) from 158 patients including 110 male and 48 female (aging between 26 and 81) were evaluated. The preoperative clinical diagnoses of the patients are summarized in Table 1. Ninety-five cases (48.22%) were nonneoplastic. Among those, 10 were reactive/inflammatory parenchymal changes, 72 were reactive/hyperplastic lymph nodes, 8 were granulomatous inflammation and 5 were others. An example of granulomatous lesion that was correctly identified in cytology and frozen was shown in Figure 1, which depicts necrotizing granulomatous vasculitis in a pleura specimen from a patient with bilateral lung nodules and with multiple mediastinal lymphadenopathies. One case (0.51%) was a benign neoplasia (chondroid hamartoma); 101 cases (51.26%) were malignant neoplasias; 76 of those were primary lung malignancy, 18 were metastatic carcinomas or sarcomas and 7 were others. Among 76 primary lung malignancies, 60 were non-small-cell lung carcinomas (subtypes listed in Table 2) and cytology and frozen correctly determined 39 and 40 of these malignancies, respectively. This means that 35% and 33.3% of non-small-cell lung cancers cannot be subtyped either by cytology and frozen, respectively. Figure 2 depicts such a nonkeratinizing squamous cell carcinoma case which was evaluated as adenocarcinoma in the intraoperative cytology and which was evaluated as non-small-cell carcinoma—unable to be subtyped—in frozen. Figure 3 depicts another such case which was an acinar-pattern lung adenocarcinoma which cytological features mimicked squamous cell carcinoma. One malignant case was a large cell neuroendocrine carcinoma correctly determined both by cytology and frozen. One malignant case was a combined large cell neuroendocrine carcinoma and adenocarcinoma. Four cases were small cell carcinomas correctly diagnosed both by cytology and frozen. One of the lung malignancies was a sarcomatoid carcinoma, which could not be diagnosed either by cytology or frozen.

TABLE 1 Prediagnosis of cases

Prediagnosis of cases	
Lung nodule or mass	137
Pleural thickening and/or effusion	12
Pleural nodules	1
Mediastinal/Hilar lymph nodes	5
Operated lung adenocarcinoma	2
Operated lung squamous cell carcinoma	7
Operated breast carcinoma	5
Operated breast and colorectal carcinoma	1
Nasopharyngeal carcinoma	1
Oral cavity tumor (squamous cell carcinoma)	1
Hepatocellular carcinoma	1
Pancreatic ductal adenocarcinoma	1
Colorectal carcinoma	6
Colon adenocarcinoma and bladder carcinoma	1
Renal cell carcinoma	2
Renal mass	1
Prostate cancer	2
Ovarian cancer	3
Bladder cancer	2
Multiple metastasis of unknown primary	1
Carcinomatous peritonitis	3
Synovial sarcoma	1
Melanoma	1
Total	197

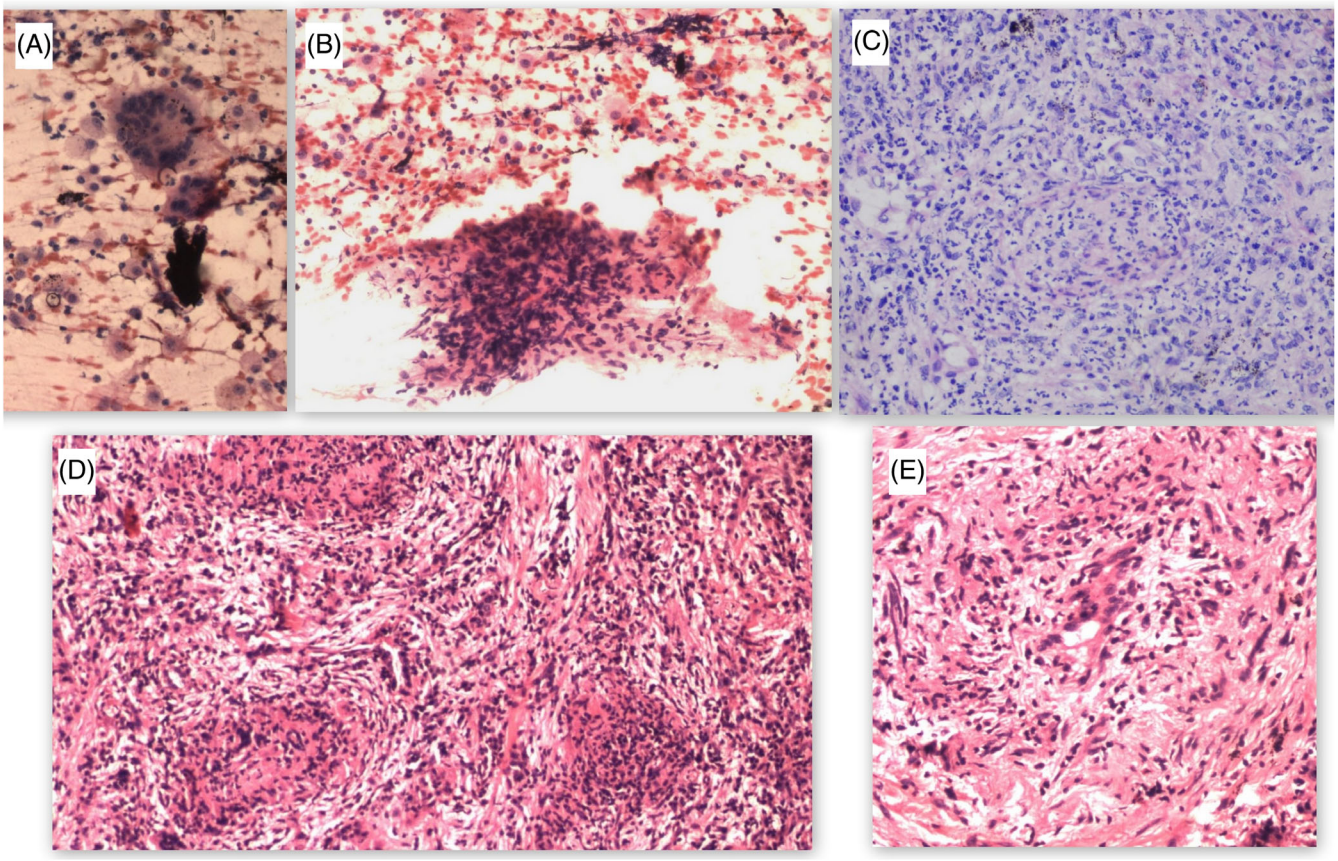

FIGURE 1 Necrotizing granulomatous vasculitis in a pleura specimen from a patient with bilateral pulmonary nodules and with multiple mediastinal lymphadenopathies. (A, B) Multinuclear giant cells (A) and granuloma formation (B) in cytology (A, B: both stained with H&E and ×200 magnification). (C) Granuloma in frozen sections (stained with H&E, ×400 magnification). (D, E) Necrotizing granulomatous vasculitis in small and medium-sized vessel walls (d and e stained with H&E, D: ×100, E: ×200 magnification) [Color figure can be viewed at wileyonlinelibrary.com]

Distinguishing malignant mesothelioma was also difficult in some cases. Figure 4 depicts an epithelioid malignant mesothelioma case which diagnosis was deferred to paraffin sections due to the necessity of differential diagnosis from well-differentiated adenocarcinoma. In certain circumstances, definite diagnosis of carcinoid tumors was also difficult. Atypical/typical carcinoid tumors constituted five of the lung malignancies and both cytology and frozen determined three of these cases. Figure 5 depicts a carcinoid tumor case, which was diagnosed as round-spindle cell tumor in cytology and frozen and which definitive diagnosis was deferred to paraffin investigation. Lastly, distinguishing malignancy and reactive changes was also intriguing in certain cases. Figure 6 depicts such an invasive mucinous adenocarcinoma case which cannot be distinguished as malignancy versus reactive changes during intraoperative pathological examination.

Table 2 summarizes paraffin diagnoses of cases obtained from resection pieces, primary masses and lymph nodes and their corresponding histopathological subtypes. Table 3 demonstrates true positive rates in intraoperative cytology and frozen diagnosis of malignant cases according to paraffin diagnosis. There were 60 cases belonging to non-small-cell lung carcinomas according to paraffin diagnosis. Among these, cytology revealed 55 cases (90.16%) as true positive and frozen analyses revealed 56 cases (91.8%) as true positive. There were 18 cases belonging to metastatic specimens according to paraffin diagnosis. Among these, cytology revealed 10 cases (55.56%) as true positive and frozen analyses revealed 11 cases (61.1%) as true positive. In total, among a total of 101 malignant cases, cytology revealed 80 cases (79.2%) as true positive, while frozen revealed 82 cases (81.19%) as true positive.

Table 4 demonstrates the sufficiency and true negative ratios in intraoperative cytological and frozen diagnoses in nonneoplastic/benign specimens according to paraffin diagnosis. There were 96 nonneoplastic specimens, 91 of these were sampled sufficiently for cytology, 88 specimens (96.7%) were determined as true negative with cytology, and 96 specimens (100%) were determined as true negative with frozen examination. Five cases were left out of cytological assessment due to insufficient cytological sampling. Three of these were reactive lymphoid hyperplasia with widespread fibrosis which provided peripheral blood elements, one was fibrinous pleuritis which provided acellular smears and one was necrotizing granulomatous inflammation which provided necrotic smears. Table-5 demonstrates 21 specimens which were deferred to paraffin diagnosis after cytological evaluation. Table-6 demonstrates 17 specimens which were deferred to paraffin diagnosis after frozen evaluation. The distribution of 3 false negative specimens were

TABLE 2 Paraffin diagnoses of cases obtained from resection pieces, primary masses, lymph nodes, and their corresponding histopathological subtypes

Paraffin diagnoses of resection pieces obtained via wedge resection, lobectomy, pneumonectomy, and decortication		
Non-mucinous adenocarcinoma	27	
	Acinar dominant	13
	Solid dominant	9
	Papillary dominant	3
	Solid dominant	1
	Clear cell and signet ring cell findings[a]	1
Squamous cell carcinoma	12	
	Keratinized	8
	Non-Keratinized[b]	3
	Basaloid	1
Minimal invasive non-mucinous adenocarcinoma	1	
Invasive mucinous adenocarcinoma	1	
Colloid adenocarcinoma	1	
Pleomorphic carcinoma with adenocarcinoma	1	
Paraffin diagnoses of biopsy samples obtained from mass lesions of non-small-cell lung cancers		
NSCLC favoring adenocarcinoma	6	
NSCLC favoring squamous cell carcinoma	2	
NSCLC not otherwise specified	1	
Paraffin diagnoses of lymph node metastases from non-small-cell lung cancers		
Non-mucinous adenocarcinoma metastasis	6	
Squamous cell carcinoma metastasis	1	
Metastasis of NSCLC not otherwise specified	1	

[a]Intraoperative diagnosis was requested for the pleural thickening and effusion in this case. Mesothelioma was ruled out for diagnosis. No immunohistopathological and radiological signs of metastasis and other primary foci were found and the lesion was considered as lung adenocarcinoma.

[b]In one case, there was adenocarcinoma component for less than 10% of the tumor, which was mentioned in the pathological report.

as follows: One was obtained from a solitary lung nodule of a patient operated due to squamous cell carcinoma on the controlateral lung lobe. In this sample, cytology did not distinguish reactive atypia and malignancy due to hypocellular smears. One specimen was obtained from mediastinal lymph node sampling which paraffin diagnosis revealed adenocarcinoma micrometastasis which both the cytology and frozen did not reveal a diagnosis due to insufficient sampling. One specimen was obtained from a mediastinal mass with a definite paraffin diagnosis of thymoma type B2 which the cytology only demonstrated reactive lymphoid elements.

Diagnostic accuracies were 98.8% and 99.4% for cytology and frozen section, respectively. Sensitivities were 96.3% and 98.7% for cytology and frozen section, respectively. Specificities were 100% for both cytology and frozen. Positive predictive values were 100% for both cytology and frozen. Negative predictive values were 96.7% and 98.9% for cytology and frozen section, respectively. Kappa statistics between cytology and frozen revealed a very high interrater reliability (Cohen's Kappa value: 0.911; $p = .001$; $p < .01$). Specific diagnostic rates in primary lung carcinomas were as follows: Out of 76 total primary lung carcinomas, 61 were non-small-cell lung carcinoma, 4 were small cell carcinomas and 11 were other carcinomas. For primary lung carcinomas, cytology diagnosed 39 of 61 non-small-cell carcinoma specimens precisely (meaning capable of specific subtyping), while frozen diagnosed 40 non-small-cell carcinoma specimens precisely. For small cell carcinomas, both cytology and frozen correctly diagnosed all specimens ($n = 4$, 100%). In sum, cytology precisely diagnosed 47 (61.84%) carcinoma specimens while frozen section precisely diagnosed 48 (63.16%) carcinoma specimens. Five specimens were insufficient in cytology and 1 sampling error occurred in frozen. Cytology provided specific diagnosis in 141 out of 197 specimens (71.57%) while frozen provided specific diagnosis in 156 of 197 specimens (79.19%).

4 | DISCUSSION

Lung nodules are generally first diagnosed with frozen section, promptly followed by lobectomy or other processess.[10] The principal aims are to diagnose and stage the patient's lung cancer simultaneously, preferably by employing the least invasive, safest, and least costly analyses.[11] A positive cancer finding is especially important if the patient is not a candidate for surgery because physiological limitations or anatomic lesion location. In a prospective investigation, Clarke et al. determined the diagnostic accuracy of imprint cytology performed on 121 mediastinal lymph node specimens from 38 patients.[6] Their specificity, sensitivity, positive and negative predictive values are very close to findings in our current study. Orki et al. investigated 1050 mediastinal lymph node specimens obtained from 255 NSCLC patients.[12] Specificity, sensitivity, and the negative and positive predictive values were 93.1%, 95.6%, 99.5%, and 99.1%, respectively. Insufficient tumor adhesion on the slides in two cases caused false-negativity.[12] In our study, hypocellular smears, insufficient sampling and obtaining only the reactive lymphoid elements were the reasons of three false-negative evaluations of the investigated specimens. Rakha et al. investigated 155 consecutive patients with pulmonary lesions who underwent intraoperative frozen section, and 110 of those cases also had touch print cytology available for analysis.[13] Touch print cytology was contributory to or diagnostic for frozen diagnosis in 97 (88%) cases, and was not contributory in 13 cases, mostly due to inadequate or low cells on print slides. Touch print cytology was diagnostic in 10 (9.1%) cases in granulomatous lesions with or without necrosis. In five cases (including four with tuberculosis), touch print cytology was the sole diagnostic modality since frozen could not be completed.[13] In our study including 8 specimens with granulomatous inflammation, 7 and 8 specimens were sufficiently

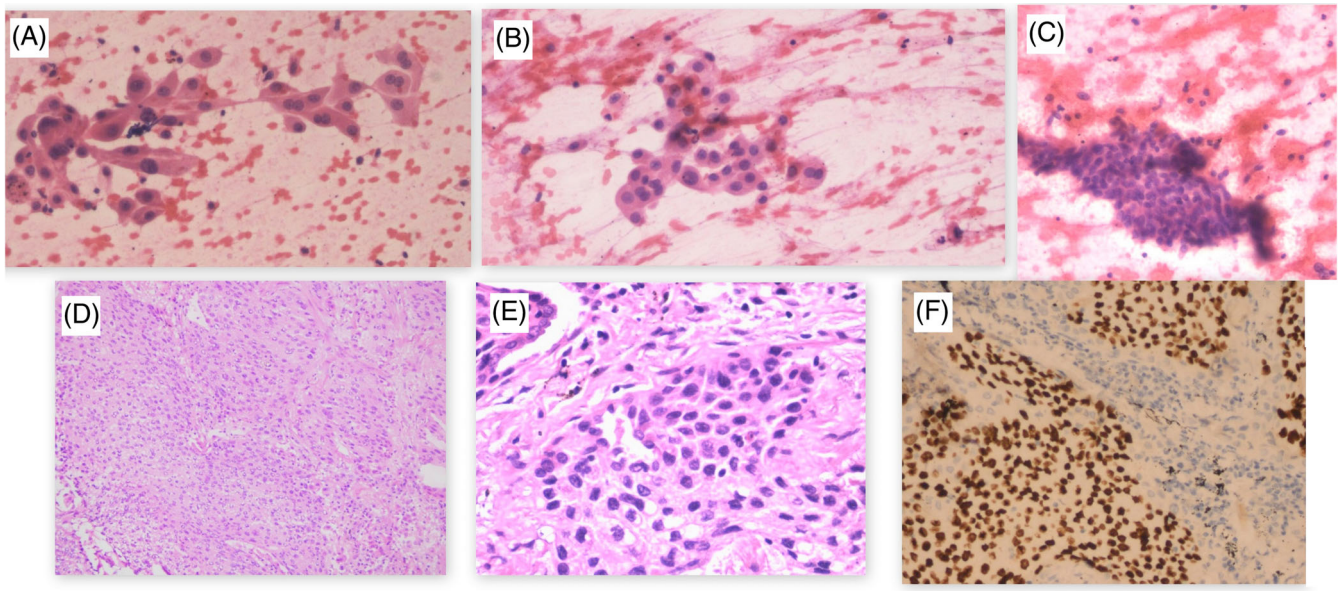

FIGURE 2 A case which was evaluated as adenocarcinoma in the intraoperative cytology and which was evaluated as non-small-cell carcinoma—unable to be subtyped—in frozen section. (A–C) In smear preparation, cells with eosinophilic cytoplasm and vesiculated nuclei with prominent nucleoli were observed. In cytology, adenocarcinoma was considered in the forefront due to lack of manifest cytoplasmic keratinization. (all stained with H&E, ×200 magnification). (D) Solid epithelial tumor islands which cannot be subtyped in frozen sections (stained with H&E, ×200 magnification). (E) Paraffin section of the case diagnosed as nonkeratinizing squamous cell carcinoma (stained with H&E, ×200 magnification. (F) p40 positivity (×200 magnification) [Color figure can be viewed at wileyonlinelibrary.com]

sampled for cytology and frozen, respectively. Among these, all available cytological and frozen investigations correctly diagnosed the granulomatous inflammation (Table 4).

Biancosino et al. estimated the relevance of intraoperative cytology by investigating a total of 532 surgically obtained specimens out of the 518 resected lung tumors from 360 patients. The specificity and sensitivity of fast intraoperative cytology were 99% and 82%, respectively, with positive and negative predictive values of 99% and 86%, respectively.[14] Our data on the positive and negative predictive values of cytology (100% and 96.7%, respectively) revealed that our results were more successful regarding the negative predictive value. This difference occurred due to a high number of false negatives in the Biancosino's study. To evaluate the diagnostic yields of scratch-imprint cytology, Sugiyama et al. compared intraoperative diagnoses of lung lesions between frozen section histology and scratch-imprint cytology.[15] Their results were inferior to our results especially regarding the negative predictive values and diagnostic accuracy due to their higher number of false negative cases. The majority of lesions for which the scratch-imprint cytology provided indeterminate results (21/26; 80.8%) later determined as malignant, the inconsistency was associated with the few number of obtained malignant cells.[15] In opposite, 15 of the 18 (83.3%) lesions for which the scratch-imprint cytology specimens were insufficient turned out to be pathologically benign.[15] Such lesions were generally nonepithelial lesions, with the majority constituted of granulomas with central caseous coagulative necrosis. Regarding the false-negative results of scratch-imprint cytology, it was realized that either it was difficult to obtain the target cells or the neoplasms in such cases exerted mild cytological atypia.[15] Changes in vital malignant cells or prominent tumor necrosis due to preoperative chemotherapy could also influence the frozen section results. In our study, frozen seemed to very slightly differ from cytological investigation in correctly defining malignant cases (79.2% and 81.19% true positives, respectively). The same was relevant in defining benign cases, where cytology and frozen defined 96.7% and 100% of benign cases, respectively. Further, kappa statistics between cytology and frozen revealed a very high interrater reliability (Cohen's Kappa value: 0.911; $p = .001$; $p < .01$) suggesting that cytology may supplant frozen analyses in many instances.

4.1 | Pathological subtype

According to Rakha et al., touch print cytology provided valuable guidance for tumor subtyping, but its sensitivity was low in diagnosing mucinous neoplasms and it was also less specific in determining bronchial resection margins.[13] Yeh et al. found that the frozen section could obtain information on the existence of aggressive histopathological patterns such as solid and micropapillary with low sensitivity but high specificity.[5] In our study, both frozen and cytology correctly identified all ($n = 4$) small cell carcinomas (Table 3). Further, diagnoses of NSCLC (90.16%) and large cell neuroendocrine carcinomas (100%) were successfully accomplished by cytology in our study (Table 3). Strâmbu et al. evaluated 311 tissue fragments obtained from lung lesions, lymph nodes, mediastinal and pleural masses.[3] Their diagnostic efficacies in cytological investigations were considerably lower than the reported data in our current study and the reason for this

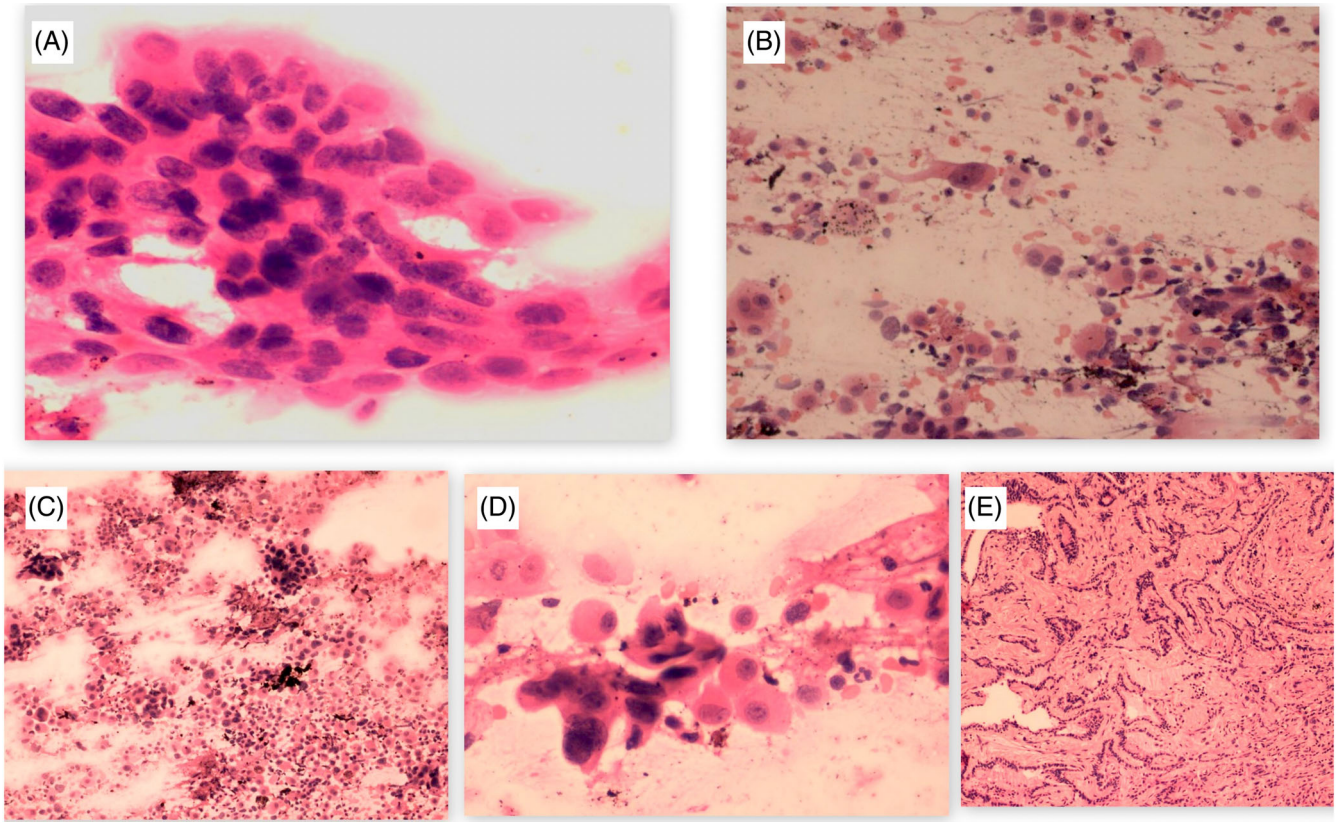

FIGURE 3 Adenocarcinoma case which cytological features mimicking squamous cell carcinoma. (A–D) Cytological smear preparation. (A) Cells with intense eosinophilic cytoplasm and coarse chromatin which resemble to squamous cell carcinoma; (B) tadpole-resembling cells (marked with arrow), (A–D all stained with H&E, A: ×400, B: ×200, C: ×100, D: ×400 magnification). (E) Acinar-pattern adenocarcinoma in paraffin section (stained with H&E, ×200 magnification) [Color figure can be viewed at wileyonlinelibrary.com]

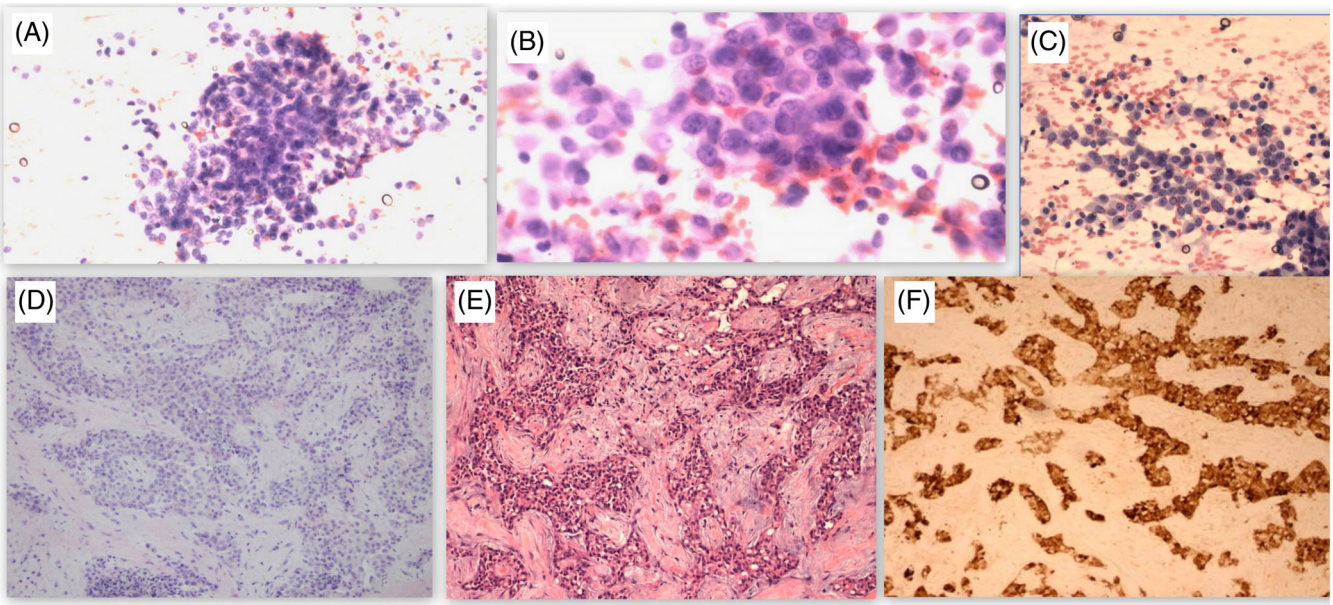

FIGURE 4 Epithelioid malignant mesothelioma case which diagnosis was deferred to paraffin sections due to the necessity of differential diagnosis from well-differentiated adenocarcinoma. (A–C) In cytological smear preparations, cell groups with monotonous cytological features and epithelioid morphology were observed (stained with H&E, A: ×200, B: ×400, C: ×200 magnification). (D) Infiltrative pattern indicating epithelioid mesothelioma in frozen sections (stained with H&E, ×200 magnification). (E) Paraffin section (stained with H&E, ×200 magnification). (F) Calretinin positivity (×100 magnification) [Color figure can be viewed at wileyonlinelibrary.com]

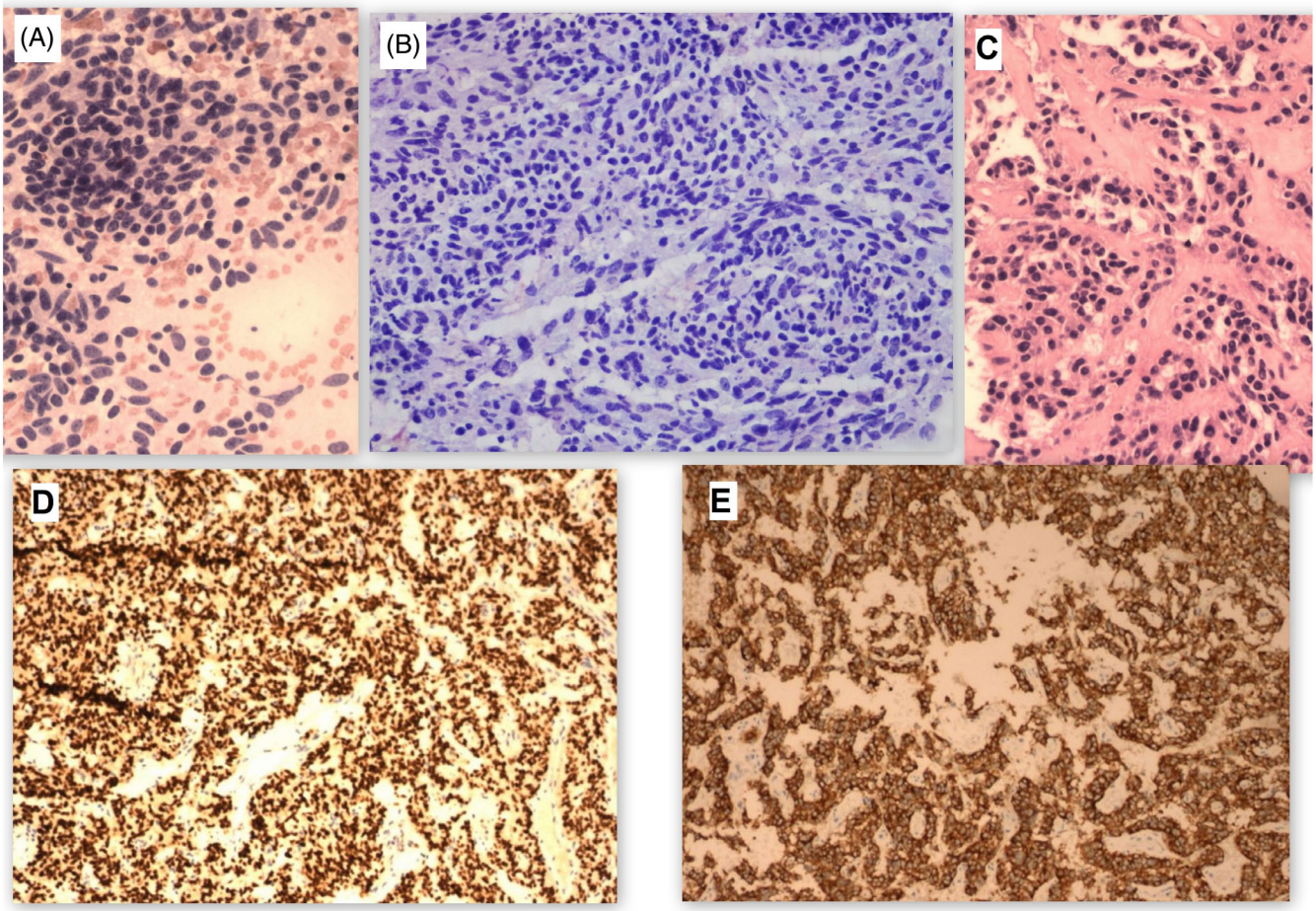

FIGURE 5 A typical carcinoid tumor case, which was diagnosed as round-spindle cell tumor in cytology and frozen and which definitive diagnosis was deferred to paraffin investigation. (A) Cells with monotonous round-spindle nuclei, fine chromatin, and narrow cytoplasms were observed in cytology (stained with H&E, ×200 magnification). (B) Frozen section (stained with H&E, ×200 magnification). (C) Paraffin section (H&E, ×200 magnification). (D) TTF1 positivity (thyroid transcription factor-1, ×100 magnification). (E) Synaptophysin positivity (×100 magnification) [Color figure can be viewed at wileyonlinelibrary.com]

discrepancy remains elusive. Again, our results are superior to those reported by Strâmbu et al in defining tumor subtypes.[3] However, as shown in Figures 2 and 3, we also had cases which correct subtyping was only possible with paraffin sections. According to results of Strâmbu et al, tissue print cytology corresponded in 76% of lymph node specimens with the pathology. In our study, 69 of 72 reactive lymph nodes (95.83%) were correctly identified (Table 4).

4.2 | Deferrals and errors

Akyildiz discussed frozen section deferrals and errors in 25 cases which included 14 (56%) lesions of pulmonary parenchyma and 11 (44%) of pleura.[4] Akyildiz classified these 25 cases into five groups which caused difficulties in frozen section diagnosis: 1—Benign pulmonary parenchymal lesions. 2—Distinction of adenocarcinoma and nonmucinous bronchioloalveolar carcinoma (adenocarcinoma with lepidic pattern) from type II pneumocyte hyperplasia. 3—Distinction benign conditions from malignancies such as chronic pleuritis and pleural mesothelial proliferation. 4—Differentiation between pleural adenocarcinoma and malignant mesothelioma. 5- Distinction granulomatous inflammatory necrosis from tumor necrosis. In our current study, the most frequent deferral reason to paraffin diagnosis from cytology investigation was the difficulty in distinguishing primary lung carcinoma from metastasis followed by difficulty in discriminating carcinoma and mesothelioma (Table 5). The most frequent deferral reasons from frozen investigation to paraffin diagnosis were similar to cytology evaluations (Table 6). In case of metachronous or synchronous multiple NSCLC, the distinction of independent multiple primary tumors from metastases is crucially important as it will determine staging and the treatment strategy.[16] In the study of Marchevsky et al., the equivocal frozen section diagnoses included two carcinoid tumors defined as "atypical carcinoma" and "spindle cell lesion, sclerosing hemangioma versus carcinoid," two bronchioloalveolar carcinomas (BAC) defined as "atypical hyperplasia, favor bronchioloalveolar carcinomas" and two bronchioloalveolar carcinomas diagnosed as "alveolar hyperplasia."[10] In our study, both cytology and frozen missed to diagnose 1 out of 5 carcinoid tumors (20%) (Figure 6), while

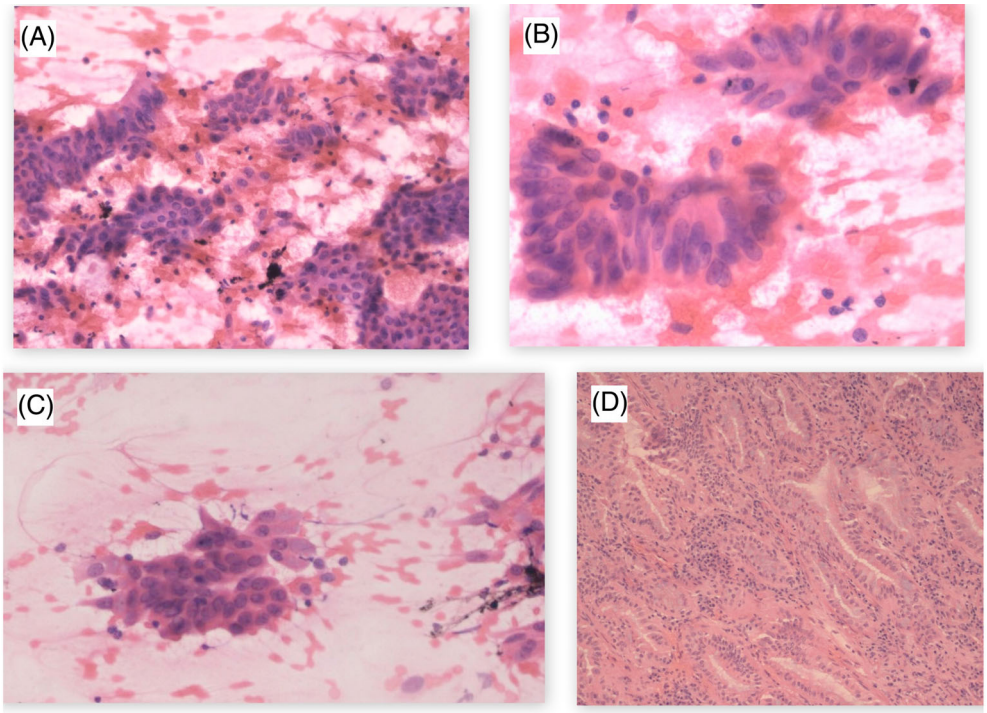

FIGURE 6 Invasive mucinous adenocarcinoma case which cannot be distinguished as malignancy versus reactive changes. (A–C) Columnar cells with well-differentiated cytological features containing intracytoplasmic mucin (all stained with H&E, A: ×200, B: ×400, C: ×200 magnification). (D) Invasive mucinous adenocarcinoma in paraffin section (stained with H&E, ×100 magnification) [Color figure can be viewed at wileyonlinelibrary.com]

TABLE 3 True positive rates in intraoperative cytology and frozen section diagnosis of malignant cases according to paraffin diagnosis

Paraffin diagnosis	Number of cases	Number and ratios of cytologic true positive specimens	Number and ratios of frozen true positive specimens
Non-small-cell lung cancer	60	54 (90%)	55 (91.6%)
Large cell neuroendocrine carcinoma	5	5 (100%)	5 (100%)
Large cell neuroendocrine carcinoma and adenocarcinoma[a]	1	1 (100%)	1 (100%)
Small cell carcinoma	4	4 (100%)	4 (100%)
Typical/atypical carcinoid tumor	5	4 (80%)	4 (80%)
Metastatic tumors	18	10 (55.56%)	11 (61.1%)
Sarcomatoid carcinoma	1	0 (0%)	0 (0%)
Malignant mesothelioma	1	0 (0%)	1 (100%)
Thymoma	2	1 (50%)	1 (50%)
Atypical spindle cell proliferation	1	0 (0%)	0 (0%)
Isolated tumor cells within lymph node	1	1 (100%)	0 (0%)
Carcinoma with unknown primary	2	0 (0%)	0 (0%)
Total	101	80 (79.2%)	82 (81.19%)

[a]Neuroendocrine component was not encountered during the intraoperative diagnosis. Postoperative diagnosis confirmed a combined tumor. Due to the existence of adenocarcinoma both in cytology and frozen, it was considered as positive for both.

both missed to diagnose 1 atypical spindle cell proliferation (0%) (Table 3).

4.3 | Differentiation of metastasis and primary tumors

Paraffin diagnosis revealed lung metastasis in 18 of the cases with one or more primary tumors. The general difficulty in the differentiation of primary versus metastatic tumors was experienced more frequently regarding poorly differentiated carcinomas. In one of three cases with clear cell renal cell carcinoma metastasis, both frozen and cytology were insufficient to differentiate between primary tumor and metastasis. In this case, the tumor was in the morphology of a poorly differentiated carcinoma and due to the presence of pleomorphic tumor cells, differential diagnosis could not be made, and the final result was left to paraffin. In general, no problems were experienced in metastatic colon tumors. The prominent columnar morphology and

TABLE 4 Sufficiency and true negative ratios in intraoperative cytological and frozen section diagnoses in nonneoplastic/benign cases according to paraffin diagnosis

Paraffin diagnosis	Total number of cases	Number of specimens with sufficient cytologic sampling	Cytologic real negative number/ratios	Number of specimens with sufficient frozen sampling	Frozen section true negative number/ratios
Reactive inflammatory changes	10	9	7 (77.78%)	10 (100%)	10 (100%)
Lymph node—reactive lymph node hyperplasia	72	69	68 (98.55%)	72 (100%)	72 (100%)
Granulomatous inflammation	8	7	7 (100%)	8 (100%)	8 (100%)
Others	6	6	6 (100%)	6 (100%)	6 (100%)
Total	96	91	88 (96.7%)	96 (100%)	96 (100%)

TABLE 5 Cases with deferral to paraffin diagnosis after cytological evaluation

Clinical prediagnosis	Number of cases	Paraffin diagnoses
Carcinoma versus malignant mesothelioma	4	1 Epitheloid malignant mesothelioma 1 Clear cell carcinoma 1 Sarcomatoid carcinoma 1 Adenocarcinoma with enteric differentiation
Primary lung carcinoma versus metastatic carcinoma	11	2 Primary lung adenocarcinoma 1 Primary squamous cell carcinoma 7 Metastatic carcinoma (1 renal clear cell carcinoma, 3 invasive breast carcinoma, 2 colon carcinoma, 1 pancreatic ductal adenocarcinoma) 1 Primary carcinoma/metastasis (enteric immunophenotypic adenocarcinoma)
Solitary pulmonary nodule—malignancy?	2	1 Typical carcinoid tumor 1 Invasive mucinous adenocarcinoma
Pleural thickening—malignancy?	3	2 Reactive mesothelial proliferation 1 Atypical spindle cell infiltration
Hilar mass—mediastinal lymph node—malignancy?	1	Reactive lymphoid hyperplasia
Total	21	

TABLE 6 Cases with deferral to paraffin diagnosis after frozen section evaluation

Clinical prediagnosis	Number of cases	Paraffin diagnosis
Carcinoma versus malignant mesothelioma	3	1 Clear cell carcinoma 1 Sarcomatoid carcinoma 1 Adenocarcinoma with enteric differentiation
Primary lung carcinoma versus metastatic carcinoma	11	2 Primary lung adenocarcinoma 1 Primary squamous cell carcinoma 7 Metastatic carcinoma (1 renal clear cell carcinoma, 3 invasive breast carcinoma, 1 colon carcinoma, 1 pancreatic ductal adenocarcinoma, 1 ovarian cancer) 1 Primary/metastasis? (enteric immunophenotypic adenocarcinoma)
Solitary pulmonary nodule—malignancy?	1	Typical carcinoid tumor
Pleural thickening—malignancy?	1	Atypical spindle cell infiltration
Mediastinal mass—malignancy?	1	Thymoma type B2
Total	17	

the presence of cytoplasmic mucin in glandular structures can be more easily recognized in cytology and frozen sections. One of the six colon cancer cases had a history of two primary (breast and colon carcinoma) tumors in addition to the lung lesion; and cytomorphologically and histomorphologically, poorly differentiated carcinoma was observed and the final result was left to paraffin. In another case, cytological intraoperative diagnosis was not decided, but the enteric histomorphology of this case could be evaluated well in frozen sections. In one case with ovarian cancer metastasis, papillary structures in frozen sections could not be evaluated clearly due to freezing artifacts. However, papillary groups with high-grade nuclear features in cytological smears were more easily recognized and a decision was made in the direction of metastasis. In three cases with metastatic

breast carcinoma, definitive differential diagnosis could not be made either by frozen and cytology. In some of our cases with triple negative breast carcinoma metastasis and urothelial carcinoma metastasis with squamous differentiation; even immunohistochemistry did not stain supportively and indecisive results are obtained in paraffin sections. In such cases, it is important to compare the morphology and the immune profile of the primary tumor with the tumor suspicious for metastasis, and to carefully evaluate the sections in terms of the presence of carcinoma in situ especially if it will be differentiated from squamous cell carcinoma.

4.4 | Multiple rare primary neoplasias of differing tissue origin which need to be considered

In our study, one of the interesting features was the detection of several multiple primary tumors of differing tissue origin and in several instances, these coincidental tumors were of rare types. One patient had both round cell malignant tumor of the nasopharynx and large cell neuroendocrine lung carcinoma. It is recently demonstrated that pulmonary neuroendocrine carcinoma is associated with a considerable incidence of developing second primary cancers.[17] According to the National Cancer Institute's SEER (Surveillance, Epidemiology, and End Results) database, the ratio of observed/expected number of second primary cancers in lung high grade neuroendocrine carcinomas was 1.53, with the most common cancers reported in the oral cavity and pharynx.[17] Nonetheless, round cell malignant tumors are considerably rare, making their cooccurrence with lung cancer more unique. One patient with hepatocellular carcinoma also had colloid lung adenocarcinoma. According to the SEER database, among patients with a primary hepatocellular carcinoma, lung cancer was among the second primary neoplasias which risk was increased.[18] Again, colloid type lung cancers are among the rare types of lung malignancies. One patient with colon cancer had a squamous cell carcinoma of the lung. According to SEER database, lung and bronchus cancers constituted 11.6% and 13.6% of second cancers in male and female colon cancer patients, respectively.[19] One patient with noninvasive papillary urothelial carcinoma of the bladder had small cell lung cancer. According to SEER database, the most common first primary malignancy in dual primary cancer patients with lung cancers as a second primary malignancy was prostate cancer, followed by breast cancer and bladder cancer.[20] But, here it shall be also underlined the rarity of the small cell lung carcinomas. One young male patient with both colon adenocarcinoma and bladder carcinoma was diagnosed with thymoma type B2. A detailed analysis of the literature revealed only one recent previous report regarding the cooccurrence of these three types of different tumors.[21] We hypothesize that this unique case may have occurred due to mutation(s) associated with microsatellite instability.

5 | CONCLUSIONS

In lesions that are difficult to obtain in mediastinoscopic samples, if a small amount of tissue is sent and our cytological definition is sufficient to guide the operation or patient management, we prefer not to study frozen to preserve tissue for immunohistochemical and molecular examinations. Therefore, we think that cytological experience is very important in thoracic intraoperative consultation. Nonetheless, obtaining frozen sections and simultaneous cytological samples until gaining sufficient experience can also be accepted as a method in which pathologists can feel safe.

AUTHOR CONTRIBUTIONS

Zuhal Kuş Silav involved in the concept, designation, and definition of intellectual content of the study and performed clinical pathological analyses. Cansu Sönmez performed literature search and data analysis including statistics. Bülent Aydemir, Mehmet Yıldırım, and Tamer Okay collected clinical samples and involved in manuscript preparation. Fügen Vardar Aker performed manuscript editing and review.

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CONFLICT OF INTEREST

The authors declare no conflicts of interest.

DATA AVAILABILITY STATEMENT

The data that support the findings of this study are available on request from the corresponding author. The data are not publicly available due to privacy or ethical restrictions.

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