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ORIGINAL ARTICLE

A prospective analysis of touch preparation cytology for intraoperative detection of mediastinal lymph node metastases

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Introduction Patients diagnosed with lung cancer may require immediate evaluation of mediastinal lymph nodes to determine treatment plan. Typically, frozen section (FS) analysis has been used, but this analysis can be time-consuming and uses more tissue than touch preparation (TP) cytologic analysis. TP accuracy has been studied in other organs, but no prospective studies comparing TP to FS have been performed on mediastinal lymph nodes in lung cancer. Our goal was to compare the accuracy of TP to FS in these cases.

Materials and methods After obtaining institutional review board approval, all patients undergoing mediastinal lymph node evaluation for a diagnosis of lung cancer were asked to participate. If consent was given, TP and FS analyses were performed on all mediastinal lymph node stations in all patients and compared to permanent hematoxylin and eosin analysis. Data were collected prospectively.

Results Twenty patients were enrolled. Mean age was 67.7 years. Fifty-five percent (11 of 20) of patients were men. The mean number of lymph node stations sampled in each patient was 3.4. In predicting the stage of the patient, TP had a sensitivity and specificity of 95% and 100%, respectively. FS had a lower sensitivity, 85%, and a specificity of 100%. On permanent analysis, metastatic foci ranged in size from 0.15 mm to 1.5 mm.

Conclusions TP was more sensitive than FS in detecting mediastinal lymph node metastases. The technical difficulty of obtaining full-thickness sections without creating significant artifact may contribute to the lower sensitivity of FS in detecting micrometastases.

Declaration of interests: None.

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Introduction

Lung cancer is the leading cause of cancer-related mortality in the Unites States annually. The American Cancer Society estimates that 154,050 deaths will occur from lung cancer in 2018.[1] Approximately the same number of people die from lung cancer each year than from colon, breast, prostate, and pancreatic cancers combined.[2]

Patients with lung cancer who require operation will occasionally require an intraoperative analysis of lymph nodes to determine the appropriate treatment plan. An intraoperative diagnosis of malignancy within a lymph node may preclude upfront lung resection and make neoadjuvant chemotherapy or radiation treatment more appropriate.[3] As such, the ability to obtain an accurate intraoperative diagnosis is imperative.

There are 2 main techniques that are used to diagnose cancer metastases to lymph nodes intraoperatively: touch preparation (TP) cytology and frozen section (FS). TP cytologic analysis uses an imprint of fresh lymph node tissue on a clean, grease-free glass slide.[4] FS analysis is performed by freezing tissue in a cryostat machine, cutting it with a microtome, and then staining it with hematoxylin and eosin (H&E) so it can be examined under a microscope.[5] FS can be time consuming and requires a larger tissue sample, however. Particularly in situations in which only a small amount of nodal tissue is accessible, FS risks depleting the specimen and leaving an inadequate amount of tissue available for permanent analysis.

There have been previous studies comparing TP with FS in the analysis of lymph nodes in other solid organ tumors,[6-8] but there has been minimal study of the accuracy of these methods in the analysis of lymph nodes in patients with lung cancer. Our goal was to determine the accuracy of TP and FS in the intraoperative detection of lymph node metastases in patients with lung cancer.

Material and methods

The study was a prospective trial that included all consecutive patients scheduled to undergo surgical sampling of mediastinal lymph nodes, either alone (mediastinoscopy) or as a part of a concomitant pulmonary resection. Institutional review board approval for the study was obtained. All patients who were included had a diagnosed lung cancer. Any patient who previously had a positive mediastinal lymph node detected by endobronchial ultrasound biopsy was excluded from the study. No patients received chemotherapy or radiation treatment prior to surgery. Two patients who underwent resection of a benign lung nodule were excluded from the study.

After informed consent was obtained, each patient in the study underwent mediastinal lymph node sampling alone or mediastinal lymph node sampling with concomitant pulmonary resection. Each mediastinal lymph node station that was harvested was sent for both TP and FS analysis. All TP and FS slides were prepared by the cytopathology fellow on service. After the TP slides were made, one slide was stained with H&E and another with Romanowsky (Diff-Quik) stain. The lymph node station was then also submitted for FS, followed by routine tissue processing for permanent H&E analysis. All TP and FS slides were reviewed by the same cytopathologist for every case in the study. All TP and FS slides were reviewed intraoperatively during the surgery, as opposed to being reviewed retrospectively for the purposes of this study. Both the cytopathologist and the surgical pathologist on service independently reviewed all permanent sections. Permanent section results were used as the gold standard when evaluating the accuracy of TP and FS.

Results

Twenty patients overall were included in the study. Table 1 displays patient demographics. There were 11 men and 9 women. Mean age was 67.7 years. Two patients underwent mediastinal lymph node sampling only, and the remaining 18 patients underwent pulmonary resection with concomitant mediastinal lymph node sampling. All patients who had pulmonary resection underwent a minimally invasive approach. There were no operative mortalities.

Overall, 68 lymph node stations were analyzed. Fifteen percent (10 of 68) of the lymph nodes in 7 patients were positive for malignancy on permanent analysis. On histopathological examination, metastatic foci ranged in size from 0.15 mm to 1.5 mm (Fig. 1).

When analyzing each lymph node station, TP had a higher but not statistically significant sensitivity than FS (98.5% versus 95.6%, $P = 0.31$). The specificity of TP and FS were each 100% (Table 2).

When determining the ability of intraoperative analysis to accurately stage a patient, TP had a higher but not statistically significant sensitivity than FS (95% versus 85%, $P = 0.81$). The specificity of TP and FS were each 100% (Table 3).

Discussion

This was a prospective study to determine the accuracy of TP and FS in the diagnosis of mediastinal lymph node metastases from lung cancer. Though our cohort was small,

Table 1 Patient data.

Male	55% (11/20)
Female	45% (9/20)
Age (mean)	67.7 years
Concomitant pulmonary resection	90% (18/20)
Mortality	0

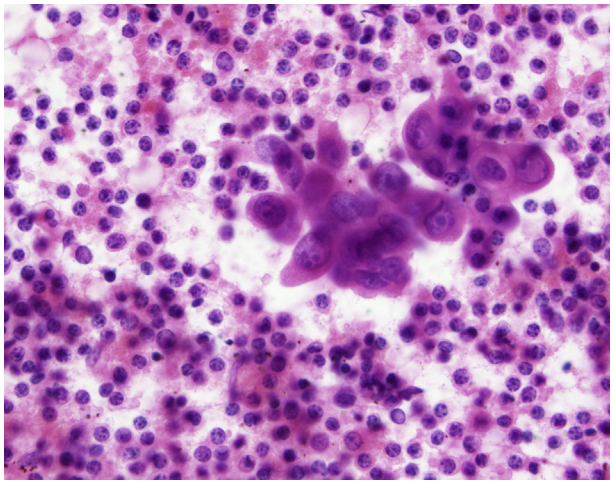

Figure 1 High-powered (400×) hematoxylin and eosin stained slide showing metastatic focus in mediastinal lymph node.

our goal was to assess whether analysis by TP was markedly inferior to FS. We analyzed each lymph node station in each patient using both methods, to ensure that the study would not risk an inaccurate diagnosis for any patient by using only 1 method.

In this study, the performance of TP and FS in the evaluation of mediastinal lymph nodes for lung cancer did not differ statistically. This similarity has been seen in other malignancies.[9] In one study the authors concluded that although FS had a slightly higher accuracy in the analysis of breast lesions, TP was acceptable and reliable given the lack of any significant difference between the 2 methods.[10] Additionally, multiple studies have shown that TP and FS are comparable in analyzing sentinel lymph nodes in patients with breast cancer.[11,12] There has only been limited investigations into the comparison of TP and FS in mediastinal lymph nodes, however. One study from 1994 looked at TP only compared with permanent analysis.[13] Th study showed a TP sensitivity of 96.6%. But that study did not perform TP and FS on each lymph node and only hypothesized that TP and FS would have relatively similar accuracy.

In our study there were some false-negative results from TP and FS, but no false-positive results. We expect that the fals- negative results occurred because the burden of disease in some of the lymph nodes was quite small, and a sampling error occurred during the TP and/or FS process.

Table 2 Statistical analysis of TP versus FS in each lymph node.

	TP, %	FS, %
Sensitivity	98.5	95.6
Specificity	100	100
Positive predictive value	100	100
Negative predictive value	98.4	95.3

Abbreviations: FS, frozen section; TP, touch preparation.

Table 3 Statistical analysis of TP versus FS in accurately staging a patient.

	TP, %	FS, %
Sensitivity	95	85
Specificity	100	100
Positive predictive value	100	100
Negative predictive value	92.9	81.3

Abbreviations: FS, frozen section; TP, touch preparation.

There are many potential advantages to using TP over FS if the methods are equivalent. TP can be performed more quickly and is more cost effective than FS. In our current environment, there has been an impetus placed on delivering excellent medical care while spending less money to do so. Every facet of medical delivery has been examined to identify ways to save money. When considering the reagents, time, and labor of the pathologist, a busy medical center could reap significant savings annually by using TP if it is as reliable as FS without any clinical risk to the patient. To have a clearer understanding of the potential cost benefit, we performed a cost analysis based on an average TP time of 5 minutes and a FS time of 15 minutes. Anesthesia and operating room costs for an additional 10 minutes of time are approximately $1,032.[14] A center that performs 125 lung resections each year would achieve an annual savings of $129,000. It is clear that not every lung resection requires intraoperative analysis of lymph nodes, but the potential cost reduction is quite clear if TP can be used instead of FS.

Furthermore, TP requires less tissue to be used in the process of producing slides. The ability to conserve tissue for future analyses is an important point. In some operations, the amount and accessibility of mediastinal lymph node tissue can be quite limited. Especially in patients with significant mediastinal fibrosis or patients who have undergone previous chest irradiation, there are times when there is only a limited amount of nodal tissue that can be harvested from a particular lymph node station. As the number of targeted therapies for lung cancer have been increasing, more immunostaining analyses and molecular studies may be required to determine appropriate adjuvant treatment. Being able to conserve tissue upfront for future analyses may help to shield patients from the inconvenience and potential morbidity of a repeat biopsy.

It appears that the performance of TP and FS are relatively similar, both in our study of patients with lung cancer and in other studies evaluating other types of cancer. TP may be slightly superior in the detection of micrometastases, but the significance of these micrometastases in lung cancer is unclear.[15] Most centers, in accordance with the National Comprehensive Cancer Network,[16] would recommend neoadjuvant chemoradiation treatment if an ipsilateral lymph node is found to harbor malignancy.[17-19] One of the

rationales behind this recommendation is the concept that trimodality treatment yields superior long-term survival compared with surgery alone.[20,21] In a previous era, up to 60% of patients who underwent a thoracotomy and lung resection were too incapacitated from surgery to receive a full adjuvant dose.[22] But with the advent of minimally invasive techniques for pulmonary resection, most patients recover quickly enough to be able to undergo a full adjuvant treatment plan. Though the majority of lung resections are still performed by thoracotomy,[23] the prevalence of minimally invasive approaches is increasing steadily.[24] This trend may allow TP to be used intraoperatively without the fear of undertreating a patient in whom micrometastasis is missed.

The significance of nodal micrometastases in lung cancer is a topic of shifting opinion. It is known that patients with multistation disease and bulky disease have worse outcomes than patients with nodal micrometastases.[25,26] But improved operative mortality and new targeted cancer therapies have improved survival in patients with nodal disease, particularly when the disease burden is low.[27]

There are some limitations of this study. Though this study is a prospective study, it is a small study. We designed this study to act as a pilot study. Because our plan was to evaluate every lymph node in every patient, we wanted to report our results in a small number of patients to show that the study was feasible and could yield important information even with 20 patients. We also wanted to determine whether TP was dramatically inferior to FS, but it appears that the 2 methods have relatively similar performance. We acknowledge that a dedicated cytopathologist may have more comfort with TP than a general surgical pathologist, however. Another limitation is that there were a variable number of lymph nodes harvested in our patients. All surgeries were performed by one attending surgeon, however, using the same systematic approach to lymph node dissection in all patients. Any variability in the lymph nodes harvested were related to the number of lymph nodes seen in the operative field.

In conclusion, it appears that TP and FS have similar accuracy in detecting lymph node metastases in patients with lung cancer. Future studies are needed to determine whether this equivalence is present with macrometastases and micrometastases, and whether preoperative imaging can predict whether one analytic method will be clearly superior to another. The ability to use TP more frequently without causing any risk to patients can be very cost effective and more efficient for medical centers.

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