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# New Teaching Modality for Cytotechnology and Cytopathology Students Utilizing IHC Panel/Flow Charts to Identify Primary Carcinoma Based on Cytologic, Histologic, and Immunohistochemical Assessment of Metastatic Tumors in Cytologic Specimens

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*Abstract:* **Background: Patients with cancer cannot be treated until a primary tumor is identified. Often, the only means to determine the primary tumor is to run panels of immunohistochemical (IHC) stains on available slides and cell-block sections. The aim of this study is to analyze the effectiveness of a panel of IHC stain pathways tested by the Cytotechnology Master's students to guide them to identify primary carcinoma based on cytologic, histologic and IHC assessment of metastatic tumors in cytologic specimens.**

**Methods: This study will use eight cases with tumors of unidentified primary carcinoma. All differential diagnoses will be listed for cytology final results stating only malignancy with an unidentified primary tumor. All cases used in this study applied in new teaching modality pathways. The Cyto-Histo-correlation pathway was analyzed. The case studies supporting surgical confirmation were compiled and a flowchart /panel designed.**

**Results: The resulting flowchart of IHC panels, which were tested with the Cytotechnology Master's students shows final results and became a new teaching modality. Each student pre-tested and post-tested final diagnosis demonstrates the accurate identification of a primary tumor based on the IHC structured flowchart/panel.**

**Conclusion: The comparison of pre-testing results of students' final interpretations and post-testing results using the new pathway were suggesting the use of a created panel as a teaching modality of IHC stains. The panel of IHC stains was effective during the post-testing interpretations by the cytologists and their evaluation of the cytology specimens.**

*Keywords:* **tumor markers, immunohistochemical (IHC), Fine Needle Aspiration (FNA), malignant sites, metastatic effusion, tumor marker limitations.**

## 1. INTRODUCTION

Cancer is one of the most common diseases worldwide due to many different reasons. However, treatments for cancer based on cytological and pathological interpretations have led to better quality of life and prognosis for cancer patients[11]. Chemotherapy and antitumor vaccines have been developed based on studying immunological aspects of the ontogenesis

of cancer cells[11]. Cancer is a tumor disease caused by an inability to manufacture enough immune responses to induce healthy blood circulation. People with cancer have an issue with their immune defense system[10]. The immune system of cancer patients who are diagnosed with metastatic carcinoma does not properly control blood circulation from and into other organs. Failure of the defense mechanism of one hosted cell can lead to a cancer factory cell and overgrowth of tissue. Tumor markers are used to identify different cancer types using special stains to represent primary cancer cells that are not defined by cytology. Elevations of tumor markers are signaling cancerous cells from benign cells in the body fluids, and tissue organs. A tumor marker is utilized in cancer patient treatment to disclose upcoming cancer growth. A large amount of tumor markers organs tissue detected cancer occurrences and differentiate the primary carcinoma in patients with metastatic cancer [10].

The purpose of this study was to design a curriculum for cytotechnology students to learn and understand the logical flow of immunohistochemical stains required in identifying primary tumor origins in cytology specimens. The study analyzed the effectiveness of a panel of IHC stain pathways tested by the Cytotechnology Master's students in identifying primary carcinomas based on cytologic, histologic, and IHC assessment of metastatic tumors in cytological specimens. Should further cytologists consider a new IHC panel of pathways be testing method of teaching IHC? Developed pathways on IHC stains and research articles suggest so.

## 2. MATERIALS AND METHODS

### A. Prepare cases:

In this study, eight to ten patients with tumors of unidentified primary carcinoma were found using a Natural Language Search of patients from the Pathology department at Philadelphia, Pennsylvania University Hospital. An initial search included the keywords 'Fine Needle Aspiration (FNA)' and 'malignant' for the years 2015 and 2016. A filtering search applied to the results of the initial search, and using the keywords 'lung', 'liver', 'effusion', 'pleural fluid', 'peritoneal mass', 'pelvic wash', and 'lymph node'. For each patient in this study, the cytology slides and reports reused with the microscopic morphology findings. The glass slides were retrieved from the files and screened to include the criteria in the new modality teaching technique. The basic tumor criteria described all differential morphology diagnoses reported for cytology. Final results stated only malignancy without an identified primary tumor.

### B. Immunohistochemical stains panel approach:

Eight patients selected from the ten cases consisted in this study to be tested by students. The following Immunohistochemical stains: BER-EP4, Calretinin, TTF-1, Napsin-A, CK19, CK7, CK20, PAX8, CK5/6, P40, CDX2 were included in the pathway teaching modality. IHC sections reviewed under the microscope for the eight positive cases. The final tumor primary identified and tumor-specific cytologic criteria described to initiate a new flow chart for each patient. For each, the patient's history was reported without any identities. Then, a panel of Cyto-Histo-correlation created including the cytologic morphology to the case as positive for malignancy, cell-block slide to confirm malignancy. Tumor markers for patient #1 were BER-EP4/Calretinin, TTF-1/Napsin-A, CDX/CK20/ and CK7. Patients # 2 had P16, P40, and CK5/6, while patient # 3 undergo PER-EP4/Calretinin, CDX2/CK20, and PAX-8. Patients #4 indicated by TTf-1/Napsin-A only. Patient # 5 had TTF-1/Napsin-A, and CK7/CDX2/CK20. For patient # 6 the tumor markers were TTF-1/Napsin-A; however, patient # 7 tumor markers were PER_EP4. Last patient's #10 immunohistochemical stains were CK7, and CDX7/ CK20/ CK19. All cases used in this experiment had diagnostic confirmation by surgical specimens in the form of glass slide sections of the primary tumor organ or tissue to confirm the final diagnosis of cytology. The final tumor primary identified and tumor-specific cytologic criteria described in the new panel/flow chart.

Seven of the Cytotechnology Master's students were pre-tested by screening assigned cases and report their final interpretations. Then, the same students were reassigned to the cases during a post-testing using a PowerPoint lecture, and a multi-head microscope. A form of correlation of Cyto-Histo-Chemical collections constructed by a flowchart of IHC stains pathways for students during their post-testing. The accuracy of the flow chart/panel modality created to students ability to read the results, and the accurate identification of a tumor primary based on the IHC panel/pathway in the post-testing experiment.

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## 3. RESULTS

However, ten patients studied, slides, IHC, and supporting surgical confirmation compiled and a flow chart designed. The new teaching flowchart/panel modality for cytotechnology & cytopathology students of the Cyto-Histo-Chemical correlation created to utilize special stains for differential diagnostics of neoplasms. The basic immunochemical panels/flowcharts led to identifying primary carcinomas in lung, liver, effusion, pleural fluid, peritoneal mass, pelvic wash, and lymph node based on an assessment of new teaching modality. Adaptation of the most recently developed special stains for diagnosis of malignancies generated a new diagnostic pathway modality process for interpretation of the non-GYN cases utilizing the combination of the cytologic, histologic & tumor markers identification findings. According to figure.1, following the pathway guided students to make accurate diagnosis of the primary carcinomas. Students start to continue to the next step in the panel with a positive sign and shift in the pathway with the negative signs.

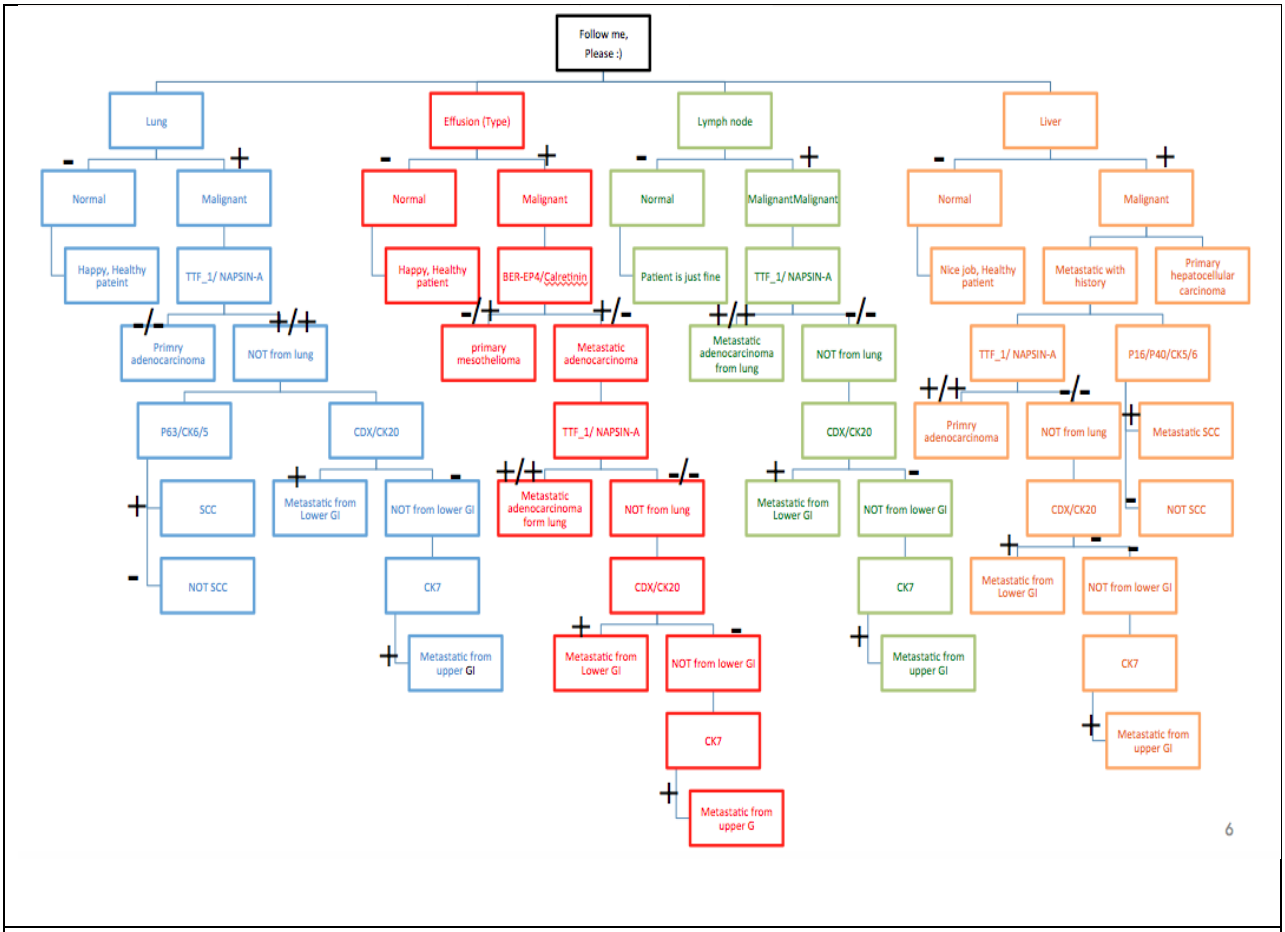


**Figure. 1: adaptation of the most recent developed special stains for diagnosis of malignancies generated a new educational tool based on collection of cyto-histo-special stains slides from the Pathology Department of TJUH**

- **Statistical analysis:**

Development of the educational tool based on collection of cyto-histo-special stains slides from the Pathology Department gave a better understanding of the clinical importance of immunodiagnostic panels. The clinical immunodiagnostic panels as it presented in figure.1 educated the Cytotechnology Master's students on most recent findings on molecular markers for differential diagnosis of primary cancers and demonstrated the specifics of special stains detection in cytologic and surgical specimens. According to table.1, patients' history and age were provided to conduct any associated reason for the current tumor. Column two in the table guide the cytologists to follow the stain needed for diagnosis. The results in column three are restating the pathways of the teaching panel to confirm final interpretations statistically of the primary tumor.

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**Table 1: data in this table shows eight cases with positive malignancy. The first column presents the case number, the history associated with patients, and age. The seecond column is identifying the type of IHC stain that was run for the case while column three is classifying the primary tumor origins in cytology specimens.**

| Patient's history: | Tumor markers used: | Results of final diagnosis: |
|---|---|---|
| Patient 1:<br>Patient ID: 52-year-old<br>Site: pleural fluid, right (thin prep, cell block)<br>Medical history: small bowel obstruction | Cytology and cell block: | Positive for malignancy |
| | PER-EP4/Calretinin (+/-): | Malignant for adenocarcinoma, not mesothelioma. |
| | TTF-1/Napsin-A (-): | Metastatic carcinoma not from the lung. |
| | CDX/CK20 (-): | Carcinoma is not from lower GI (colon) |
| | CK7 (+): | Carcinoma is metastatic from upper GI. |
| | Surgical section: | Confirm the cytological finding with no limitations. |
| Patient 2:<br>Patient ID: 64-year-old<br>Site: liver, right lobe (thin prep, cell block)<br>Medical history: pancreatic adenocarcinoma and multiple liver masses | Cytology and cell block: | Positive for malignancy |
| | P16 (+), P40 (+): | Metastatic Squamous cell carcinoma |
| | CK5/6 (+): | Confirmation of Squamous cell carcinoma |
| | Surgical section: | Confirm the cytological finding with no limitations. |
| Patient 3:<br>Patient ID: 75-year-old<br>Site: peritoneal mass, left (thin prep, cell block)<br>Medical history: peritoneal masses in ultrasound | Cytology and cell block: | Suspicious for malignancy |
| | BER-EP4/Calretinin (+/-): | Indicated adenocarcinoma rather than mesothelioma. |
| | CDX2/CK20 (-): | Adenocarcinoma not from the lower GI. |
| | PAX-8 (+): | Metastatic adenocarcinoma from bladder |
| | Surgical section: | Confirm the cytological finding with no limitations. |
| Patient 4:<br>Patient ID: 60-year-old<br>Site: lung masses, and pleural effusion (thin prep, cell block)<br>Medical history: smoker patient. | Cytology and cell block: | Positive for malignancy favor lung cancer |
| | TTF-1, Napsin-A (+/+) | Confirmation of primary adenocarcinoma of lung |
| | Surgical section: | Confirm the cytological finding with no limitations. |
| Patient 5:<br>Patient ID: 42-year-old, male<br>Site: liver, left lobe (thin prep, cell block)<br>Medical history: pancreatic adenocarcinoma and liver masses | Cytology and cell block: | Suspicious for malignancy |
| | TTF-1/Napsin A (-/-): | Carcinoma not from lung |
| | CK7, CDX2, CK20 (+/+/+): | Upper GI pancreatic-biliary. |
| | Surgical section: | Confirm the cytological finding with no limitations. |
| Patient 6:<br>Patient ID: 68-year-old male<br>Site: lymph node, 4R (thin prep, cell block)<br>Medical history: Lung masses and lymphadenopathy | Cytology and cell block: | Positive for malignancy |
| | TTf-1/Napsin-A (+/+): | Primary adenocarcinoma of the lung not metastatic. |
| | Surgical section: | Confirm the cytological finding with no limitations. |

| | | |
|---|---|---|
| Patient 7:<br>Patient ID: 49-year-old female<br>Site: pelvic wash (thin prep, cell block)<br>Medical history: no history | Cytology and cell block: | Positive for malignancy |
| | BER-EP4 (+): | Adenocarcinoma not mesothelioma. |
| | Surgical section: | Confirm the cytological finding with no limitations. |
| Patient 8:<br>Patient ID: 60-year-old<br>Site: liver, sigment 7 (diff quick, cell block)<br>Medical history: pancreatic adenocarcinoma, and liver masses | Cytology and cell block: | Suspicious for malignancy |
| | CDX2/CK20 (-/-): | Cancer is not from lower GI |
| | CK7 (+): | Stained positively to show an epithelial lesion |
| | CK19 (+): | Cancer is from upper GI. |
| | Surgical section: | Confirm the cytological finding with no limitations. |

## 4. DISCUSSION

The Cytotechnologist microscopically examined thousands of cells on Fine Needle Aspiration (FNA) samples and applies physical criteria to patient specimens to determine infection, inflammation, disease processes, pre-cancer, and cancer[2]. In the human body, cancer cells from different organs and tissues can look similar, and specimen results are often equivocal—having as many as two to five differential diagnoses[4]. These patients cannot be treated until a tumor primary is identified[1]. Determining the origin of the tumor is essential for optimal prognostic and therapeutic purposes[3]. Often, the only means to determine the primary tumor is to run panels of immunohistochemical (IHC) stains on available slides and cell block sections. This is a complicated process of both inclusion and elimination of tumor types based on IHC results[4], and the scope of many Cytotechnology programs often cannot include in-depth instruction regarding IHC positivity and negativity and tumor types. The design of a curriculum for Cytotechnology students to learn and understand the logical flow of IHC stains required identifying primary tumor origins in cytology specimens. The results of the teaching module to students were smoothly understandable and easy to follow. Cytotechnology students in the experiment were able to identify the primary tumor markers in eight cases that were given to each of them.

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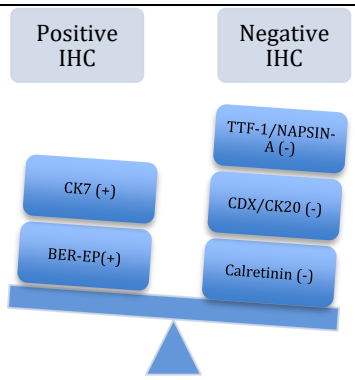
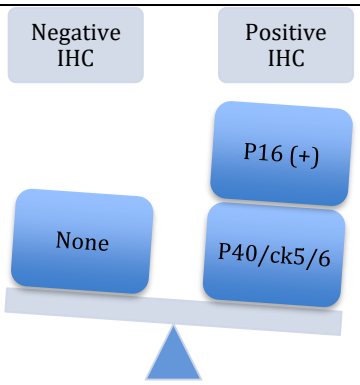
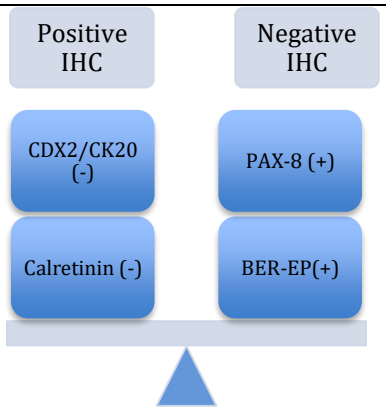
## APPENDICES – A

| | |
|---|---|
| Representative case # CN:<br>• Patient ID: 52 year-old<br>• Site: pleural fluid, right (thin prep, cell block)<br>• Medical history: small bowel obstruction |  |
| **Representative case CN:**<br>• Patient ID: 64 year-old<br>• Site: liver, right lobe (thin prep, cell block)<br>• Medical history: Squamous cells carcinoma and multiple liver masses |  |
| **Representative case CN:**<br>• Patient ID: 75 year-old<br>• Site: peritoneal mass, left (thin prep, cell block)<br>• Medical history: peritoneal masses in ultrasound |  |

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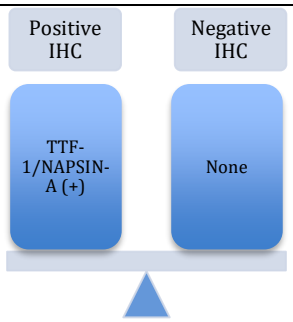
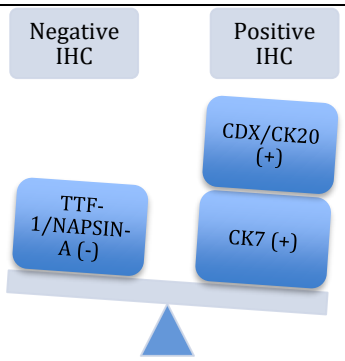
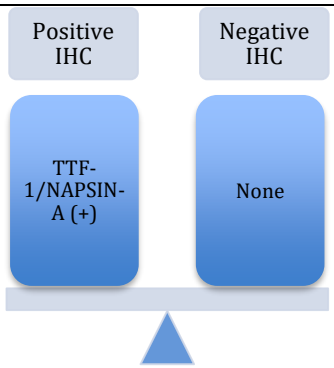
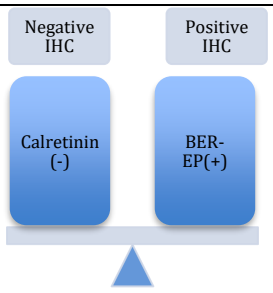
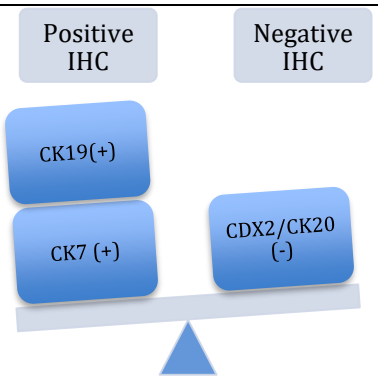
| | |
|---|---|
| **Representative case CN:**<br>• Patient ID: 60 year-old<br>• Site: lung masses, and pleural effusion (thin prep, cell block)<br>• Medical history: smoker patient. |  |
| **Representative case CN:**<br>• Patient ID: 42 year-old, male<br>• Site: liver, left lobe (thin prep, cell block)<br>• Medical history: pancreatic adenocarcinoma and liver masses |  |
| **Representative case CN :**<br>• Patient ID: 68 year-old male<br>• Site: lymph node, 4R (thin prep, cell block)<br>• Medical history: Lung masses and lymphadenopathy |  |
| **Representative case CN:**<br>• Patient ID: 49 year-old female<br>• Site: pelvic wash (thin prep, cell block)<br>• Medical history: no history |  |
| **Representative case :**<br>• Patient ID: 60 year-old<br>• Site: liver, sigment 7 (diff quick, cell block)<br>• Medical history: pancreatic adenocarcinoma, and liver masses |  |