Research Article

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Rapid and Reliable Detection of Malignant Cells in Serous Fluids Using Clinical Microscopy

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Abstract

The incidence of cancer in Thailand is rising and is now the leading cause of death. Serous fluid, found in cavities between organs, can be analyzed for malignant cells in advanced cancer stages. While Clinical Microscopy Laboratories offer rapid initial screening for malignant cells within two hours, definitive diagnosis in cytology labs typically takes up to a week. This study aims to compare the detection of malignant cells in serous fluid by clinical microscopy and cytology laboratories. A retrospective analysis of 778 serous fluid samples from 2022-2023 was conducted. These included 517 pleural effusions (67%), 221 peritoneal effusions (28%), and 40 pericardial effusions (5%). Simultaneous examinations were performed in both laboratories, showing strong concordance (Kappa = 0.76), with 120 positive and 601 negative concordant results. Sensitivity was 77.9%, and specificity was 96.3%. The findings suggest that clinical microscopy provides an effective and timely screening method for malignant cells in serous fluids, facilitating early cancer detection, treatment planning, and monitoring of patient outcomes. This approach can significantly improve the management of suspected cancer cases.

Keywords: Malignant cells, Serous fluid, Clinical microscopy laboratory, Cytology laboratory, Early cancer detection

What was Known

- Cytology labs are the gold standard for detecting malignant cells.
- Clinical microscopy offers rapid screening with limited evidence.
- Cytology delays may hinder early cancer detection.

What's New and Next

- Clinical microscopy shows high concordance with cytology for detecting malignant cells.
- Rapid screening with clinical microscopy can enable earlier cancer detection.
- Future research should refine detection methods to improve accuracy and reliability further.

Introduction

Cancer remains the leading cause of mortality in Thailand, with its incidence steadily increasing over the past two decades. In 2020, 9,842 new patients were treated in hospitals, of which 2,890 were newly diagnosed with cancer, accounting for 29.4%. The majority of cases were observed in individuals aged 50–60 years[1]. Early cancer detection is essential as it enables timely treatment, improves patient outcomes, and reduces mortality rates.

Serous fluid is a clear, pale-yellow fluid present in body cavities such as the pleural, pericardial, and peritoneal cavities. Its primary function is to provide lubrication and reduce friction between organs. Serous fluid examination is a valuable diagnostic tool for various diseases, particularly cancers. Malignant cells from nearby organs, such as the breast, ovaries, lungs, or gastrointestinal tract, can enter serous fluid. Detecting these cells is crucial for diagnosing cancers in these systems. Hematologic malignancies, including lymphoma and leukemia, may also involve serous fluid[2].

In clinical microscopy, serous fluid analysis by medical laboratory technicians is a critical step in diagnosing diseases, including cancer. This process involves examining physical characteristics, performing a white blood cell (WBC) differential count using automated analyzers, and identifying abnormal cells, such as immature blood cells and metastatic cancer cells, under a microscope[3]. This comprehensive analysis helps detect abnormal conditions in the body.

The cytology laboratory employs specialized techniques for cancer cell detection in serous fluid. Samples are processed using cytospin centrifugation to prepare monolayer slides, stained with Papanicolaou stain to highlight cellular structures, and analyzed by pathologists. Results are classified into four categories: negative for malignancy, atypical cells, suspected malignancy, and positive for malignancy. These classifications aid in determining the risk of malignancy (ROM) with varying degrees of certainty[4-6].

While cytology laboratory analysis provides detailed results, it typically requires 7–10 days to report findings. Conversely, clinical microscopy offers preliminary results within 2 hours, making it a valuable screening tool. This study aims to compare the consistency of cancer cell detection results between clinical microscopy and cytology laboratories to assess the validity, precision, and reliability of clinical microscopy in diagnosing malignancy.

Materials and Methods

Method of Study

This is a descriptive study. Retrospective data on serous fluids were collected and submitted to Surat Thani Hospital for analysis. The Hospital Information System (HIS; HomC) and Laboratory Information System (LIS; HCLAB) from Surat Thani Hospital database consist of two main parts: (1) clinical microscopy results, including cell count and differential diagnosis data, and (2) cytologic diagnostic test results.

Population and Sample

All serous fluid samples sent to Surat Thani Hospital for testing between October 2022 and September 2023 were approved by the Human Research Ethics Committee (Surat Thani Hospital Research Project Number REC 66-0108).

Inclusion and Exclusion Criteria

A graphical flow diagram illustrating the inclusion and exclusion process and the analysis timeline is provided to clarify the methodology (Figure 1).

1. **Sample Collection**: Serous fluid samples received at Surat Thani Hospital between October 2022 and September 2023.

2. **Inclusion Criteria**: Samples were included if adequate volume was available and if the clinical microscopy and cytology data were both accessible for analysis.

3. **Exclusion Criteria**: Samples were excluded if they were hemolyzed, clotted, or insufficient in volume, or if either clinical microscopy or cytology results were unavailable.

4. **Analysis Timeline**: Eligible samples underwent clinical microscopy within two hours of receipt, followed by cytological evaluation using Papanicolaou staining within 24 hours.

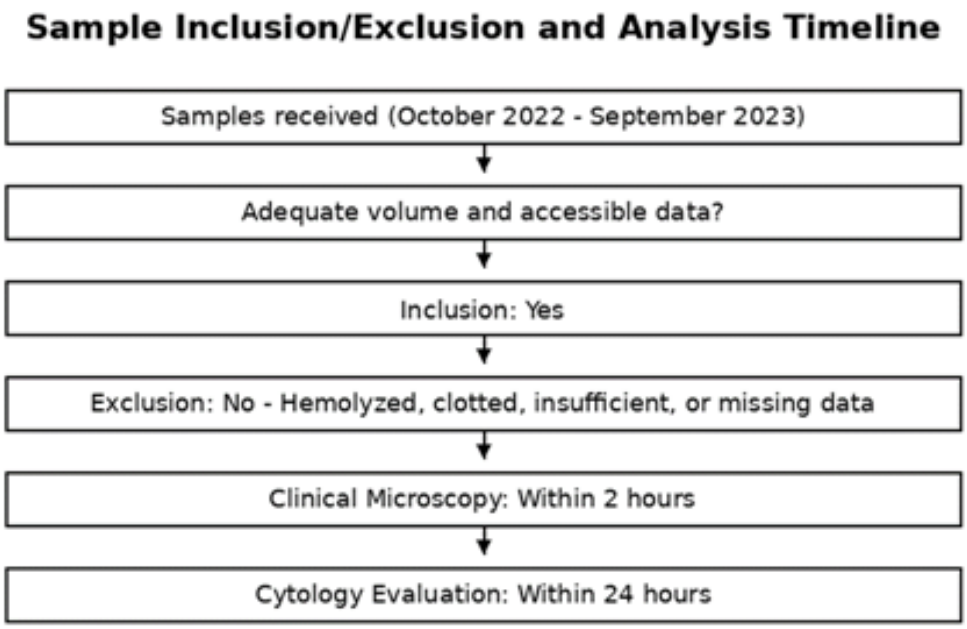

Figure1 A graphical flow diagram illustrating the inclusion and exclusion process and the analysis timeline is provided to clarify the methodology

Analysis of Serous Fluid: Clinical Microscopy Laboratory

All serous fluid samples were analyzed within two hours of receipt. This included physical examination and preparation of a smear by simple centrifugation. The number of cells was counted by a medical technician using a Sysmex automatic analyzer.

Analysis of Serous Fluid: Cytology Laboratory

The smear was prepared using a centrifuge specifically for the preparation of aqueous smears (cytospin centrifugation) and stained with Papanicolaou (Papanicolaou staining) to identify the shape and structure within the cells. Results reported by the pathologists were categorized into four groups: negative for malignancy, atypical cells present, suspicious for malignancy, and positive for malignancy.

Differentiation of Normal and Abnormal Cells

Normal and abnormal cells were differentiated based on morphological characteristics observed under microscopy. Normal cells displayed consistent size, uniform chromatin distribution, and regular nuclear contours. In contrast, abnormal cells exhibited features such as nuclear enlargement, irregular nuclear membranes, hyperchromasia, and increased nuclear-to-cytoplasmic ratios. These criteria guided classification into benign, atypical, suspicious, or malignant categories.

Data Analysis

Data analysis was conducted using SPSS version 16.0. The agreement of cancer cell detection results between the clinical microscopy and cytology laboratories was assessed using the Kappa statistic to measure inter-laboratory reliability. Sensitivity, specificity, positive predictive value (PPV), and negative predictive value (NPV) were calculated along with their 95% confidence intervals. These metrics provided a comprehensive evaluation of diagnostic performance and inter-laboratory consistency.

Results

Sample Analysis

A total of 778 serous fluid samples were submitted for analysis, including 517 pleural fluid samples, 221 ascitic fluid samples, and 40 pericardial fluid samples. The analysis of cancer cell detection by the clinical microscopy and cytology laboratories found 154 samples (19.8%) with positive findings for malignancy. Of these:

- 114 pleural fluid samples (74.0%),
- 31 peritoneal fluid samples (20.2%), and
- 9 pericardial fluid samples (5.8%). The clinical microscopy laboratory reported positive abnormal cells in 143 samples, with the distribution being:
- 102 pleural fluid samples (71.3%),
- 31 peritoneal fluid samples (21.7%), and
- 10 pericardial fluid samples (7.0%).

Consistency Between the Two Laboratories

The agreement analysis between the clinical microscopy and cytology laboratories revealed:

- 120 samples had concordantly positive results between both laboratories, accounting for 15.4% of the total cases.
- 601 samples had concordantly negative results, accounting for 77.2%.
- 34 samples (4.4%) were negative in clinical microscopy but positive in the cytology laboratory.
- 23 samples (3.0%) were positive in the clinical microscopy laboratory but negative in cytology.

These findings suggest a reasonable level of agreement between the two diagnostic approaches.

Statistical Analysis

Statistical analysis was conducted to evaluate inter-laboratory agreement and detection performance metrics:

- **Kappa Statistic:** A Kappa value of 0.76 was observed, indicating substantial agreement between the two laboratories. This supports the feasibility of using the clinical microscopy laboratory as an initial screening tool for the detection of malignant cells in serous fluid.
- **Sensitivity:** 77.9%
- **Specificity:** 96.3%
- **Positive Predictive Value (PPV):** 83.9%
- **Negative Predictive Value (NPV):** 94.6%

These statistical values highlight the diagnostic reliability and accuracy of the clinical microscopy laboratory findings, as shown in **Table 1.**

Table 1 Consistency Metrics and Performance for Cancer Cell Detection

Metric	Value (%)
Kappa	**0.76**
Sensitivity	**77.9**
Specificity	**96.3**
Positive Predictive Value (PPV)	**83.9**
Negative Predictive Value (NPV)	**94.6**

Sensitivity, Specificity, PPV, NPV between Clinical Microscopy and Cytology Lab

Comparison of Discrepant Results

The analysis of discrepancies between the two laboratories revealed:

- 34 cases were cytology-positive but microscopy-negative. These were primarily from pleural fluid (25 cases) and peritoneal fluid (9 cases).
- 23 cases were microscopy-positive but cytology-negative. These were primarily from pleural fluid (13 cases), peritoneal fluid (9 cases), and pericardial fluid (1 case).

The findings highlight that discrepancies may occur because the two methods have unique diagnostic strengths. Clinical microscopy focuses on cell counts and initial screening, while cytology incorporates advanced methods like cytological staining and detailed cellular analysis.

The table 2 illustrates the number of discrepancies between clinical microscopy and cytology results across the different fluid types:

Table 2 Comparison of Detection Results Between the Two Laboratories

Fluid Type	Cytology-Positive & Microscopy Negative	Cytology-Negative & Microscopy Positive
Pleural Fluid	25 cases	13 cases
Peritoneal Fluid	9 cases	9 cases
Pericardial Fluid	1 case	0 cases
Total	34 cases	23 cases

Explanation of Table 2:

This table summarizes the number of cases where there were discrepancies between the clinical microscopy laboratory and cytology laboratory results.

- **Cytology-Positive but Microscopy-Negative:** This represents cases where the cytology laboratory identified malignant cells, but the initial clinical microscopy did not detect them. Most of these cases were found in pleural fluid (25 cases) and **peritoneal fluid (9 cases)**.
- **Microscopy-Positive but Cytology-Negative:** These represent cases where clinical microscopy detected abnormal cells, but cytology did not confirm malignancy. These cases were predominantly found in pleural fluid (13 cases) and peritoneal fluid (9 cases).

This table and its explanation underscore that while both approaches are complementary, they also have distinct limitations. Discrepancies could potentially be reduced with improved techniques, cross-validation, and additional testing.

Discussion

The findings from this study underscore the potential of clinical microscopy as a reliable, cost-effective, and rapid screening tool for detecting malignant cells in serous fluids. Substantial agreement (Kappa = 0.76) was observed between clinical microscopy and cytology laboratories, indicating that clinical microscopy can serve as a valuable initial screening tool for cancer detection in serous fluid analysis, particularly in resource-constrained settings.

Comparison with Prior Studies

The results align with earlier evidence demonstrating the diagnostic value of pleural fluid cytology. Porcel et al. identified pleural fluid as a key diagnostic indicator for malignancies such as lung and breast cancer[7,8]. This study expands on their findings, emphasizing that clinical microscopy reduces delays by providing results within two hours, compared to the 7–10 days often required for cytology results[9].

Training laboratory staff significantly enhances diagnostic accuracy. Jerz et al. reported that sensitivity for detecting cancer improved from 23% to 60% after targeted training programs (10). Additionally, the use of automated cytological tools like cytospin centrifugation and automated analyzers improves diagnostic consistency by reducing observer bias[11,12].

Emerging Diagnostic Tools

The integration of flow cytometry and fluorescence microscopy offers promising advancements in cancer diagnostics. These tools enhance sensitivity and specificity by employing fluorescent markers and immunophenotyping [13,14]. Machine learning algorithms and digital imaging also hold potential for reducing inter-observer variability and improving diagnostic precision, as highlighted by Kulkarni et al.[15]. The application of digital pathology solutions, particularly in low-resource settings, further underscores their role in improving diagnostic workflows while maintaining cost-effectiveness[16,17].

Kulkarni et al. further emphasize the transformative potential of artificial intelligence in cytopathology, noting its ability to bridge diagnostic gaps by providing consistent and reliable interpretations, even in under-resourced settings[16,17]. This integration ensures accuracy in early cancer detection and improves laboratory throughput.

Limitations and Challenges

While promising, clinical microscopy has limitations. False negatives, often associated with low-cellularity samples (<2,000 cells/μL), highlight the need for adequate sample volumes[11,12]. False positives, although less frequent, suggest variability due to manual microscopy and potential gaps in training. Johnston et al. emphasize the importance of reassessing cytological approaches, particularly in cases with high variability[18]. Additionally, addressing time-to-diagnosis metrics, as Wright et al. note, can further improve overall diagnostic efficiency[19].

Previous studies highlight that improved training programs can address these issues, enhancing diagnostic reliability[10,20]. Moreover, integrating rapid cytological testing workflows, as discussed by Nguyen et al., ensures that even resource-limited laboratories maintain diagnostic precision while addressing capacity constraints[21-25].

Nguyen et al. emphasize the role of adopting rapid cytological testing workflows to ensure timely and accurate cancer diagnostics even in under-resourced settings, contributing to equity in public health access[(21-25)]. These rapid testing approaches reduce time-to-diagnosis while improving the quality of findings, particularly in rural or low-resource areas.

Public Health Implications

The rapid diagnostic capability of clinical microscopy has significant implications for public health. In low-resource settings, where access to cytology is limited, clinical microscopy provides timely analysis, facilitating early detection and treatment[15,16]. Chandra et al. underscore the importance of such rapid diagnostic tools in addressing healthcare disparities[17].

Combining traditional methods with emerging technologies, such as digital pathology and machine learning, can improve access and accuracy in under-resourced populations. These advancements bridge diagnostic gaps, ensuring equitable healthcare outcomes (21-25). Nguyen highlights the importance of enhancing workflows through the adoption of rapid cytological testing in public health settings[23, 25].

Conclusion

Conclusion and Recommendations

The study confirms the utility of clinical microscopy as an effective, rapid, and affordable diagnostic tool for early cancer detection. The incorporation of technological innovations like flow cytometry and machine learning is recommended to further improve diagnostic accuracy.

Expanding training programs and conducting multi-center studies will enhance diagnostic consistency and validate findings across diverse settings.

Recommendations for Future Research and Practice

Future research should include multi-center studies to validate these findings and explore variability across different healthcare settings. Emphasis should also be placed on adopting advanced diagnostic technologies such as flow cytometry and fluorescence microscopy to improve sensitivity and specificity. Continuous professional development for laboratory staff will further enhance diagnostic accuracy and consistency.

Impact on Hospital Workflow

The integration of clinical microscopy into hospital workflows significantly accelerates the diagnostic process, enabling prompt therapeutic decisions. This approach reduces dependence on time-intensive cytological analysis, streamlines patient management, and ultimately contributes to improved public health outcomes.

Ethical Approval Statement

This research has been approved by the Human Research Ethics Committee (Surat Thani Hospital Research project number REC 66-0108)

Author Contributions

SJ, PT and MS designed the study and conducted the conceptualization with data collection. MS analyzed, interpreted the data, and contributed to the drafting and revising of the manuscript.t. SJ, PT, TW and ST produced the original Thai abstract and data. All authors have read and agreed to the published version of the manuscript.

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Conflicts of Interest

The authors declare no conflicts of interest.

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