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Literature Review: Comparison of the Quality of Papanicolaou, Giemsa, and May-Grunwald Giemsa Staining in Pleural Effusion Specimens

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ABSTRACT

Pleural effusion is a pathological condition characterized by fluid accumulation in the pleural cavity, which can occur due to infection, malignancy, or systemic disorders such as congestive heart failure. Cytological examination is an effective diagnostic method for detecting abnormal cells and malignancy in pleural effusion by reviewing the quality of staining results. This study aims to analyze and compare the staining quality of *Papanicolaou, Giemsa, and May Grunwald Giemsa* in pleural effusion cytology examination. The literature review results included 8 journals that met the inclusion criteria. These journals were obtained from the *Google Scholar, PubMed,* and *Publish or Perish* databases using the PICO search method and then selected based on inclusion and exclusion criteria using the PRISMA *flow* diagram. The results of the *literature review* showed that the Papanicolaou method provided clearer contrast between the nucleus and cytoplasm with a good background, *Giemsa* displayed cell morphology but the background was less clean, while *May-Grunwald Giemsa* showed stable staining results and good cell detail.

INTRODUCTION

Pleural effusion is a condition characterized by the accumulation of fluid in the pleural cavity and chest wall, caused by infection, malignancy, or systemic disorders such as congestive heart failure (Hayuningrum, 2020). This condition includes 75% of pleural effusion cases caused by cancer, including lung, breast, ovarian, and lymphoma cancers (Pahlawi & Zahra, 2023). The prevalence of this diagnosis reaches 320 cases per 100,000 population each year (Rozak & Clara, 2022). Therefore, it is essential to diagnose pleural effusion through anamnesis, physical examination, and analysis of pleural fluid using cytology techniques.

Cytological examination of pleural effusion fluid serves to detect abnormal cells, nuclear structures, cell cytoplasm, and malignancy. Cytological staining with *Papanicolaou, Giemsa,* and *May-Grunwald Giemsa* has its own principles, composition, characteristics, advantages, and limitations. *Papanicolaou* staining, which consists of hematoxylin as a nuclear stain and Orange G and Eosin Azure as counterstains, is often used for smear samples because it can show nuclear details optimally but can produce nuclei that are too pale if there is hematoxylin contamination (Putri, 2022). *Giemsa* staining uses eosin, which is acidic, azure A and B as neutral stains, and methylen blue, which is basic, as the base color. It is capable of displaying the morphology of the nucleus and cytoplasm of cells in cytological examination diagnoses but has flammable ingredients (Dila et al., 2023); (Sabattini et al., 2018). Meanwhile, *May-Grunwald Giemsa* staining combines methylene blue, azure, and eosin at an optimal pH of 6.5–6.8, excels in visualizing blood cell morphology and inflammatory processes but tends to get dirty and damaged quickly (Ariyanti et al., 2017).

To date, there has been no consensus on the optimal staining method for pleural effusion specimens, particularly in terms of nuclear color contrast, background clarity, and cell morphology clarity. Therefore, this study was conducted to review and compare *Papanicolaou, Giemsa,* and *May-Grunwald Giemsa* staining based on the latest literature review relevant to the inclusion. This study strongly supports the development of more accurate staining methods, the most effective staining techniques, and understanding the advantages and limitations of each staining method in the processing of pleural effusion specimens.

METHOD

This journal uses a literature review research design by collecting and analyzing various scientific journals sourced from *Google Scholar, PubMed, and Publish or Perish.* The selected literature consisted only of full-text journals published within the last ten years, namely from 2015 to 2024, with keywords based on PICO, namely: *Papanicolaou, Giemsa, May-Grunwald Giemsa*, and Pleural Effusion. A total of 2,294 journals were reviewed, and 8 journals were found to meet the inclusion criteria, namely comparisons of staining in pleural effusion specimens. All journals that met the inclusion criteria were collected and summarized in a table and analyzed using an expository method in the form of descriptive and analytical presentation of facts.

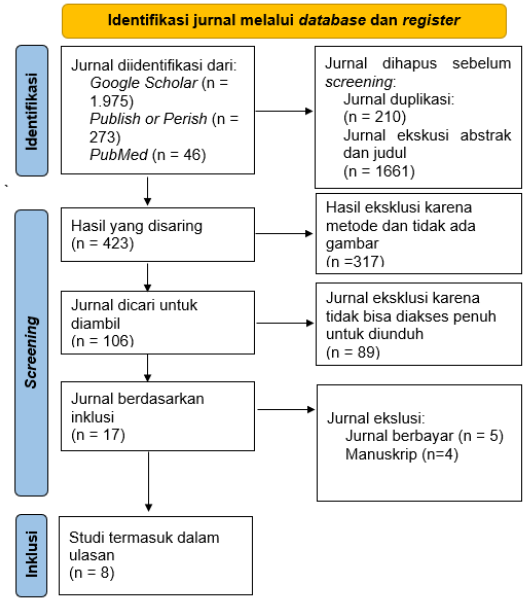

Figure 1. Journal Selection Results Based on the PRISMA Flow Diagram

RESULTS AND DISCUSSION

Data regarding coloring characteristics in this *literature review* are presented in Tables 1 to 4.

Table 1. Summary of Journal Characteristics

No.	Researcher/Year	Country	Method	Type of Staining	Staining Results	Result Category
1.	Dila, 2023	Indonesia	Descriptive with a *cross-sectional* design	*Papanicolaou* and *Giemsa*	*Papanicolaou* found cells with clear nuclei and cytoplasm, with a clean background. Meanwhile, *Giemsa* found clear nuclei, less clear cytoplasm, and a dirty background.	Good (*Papanicolaou*), Not so good (*Giemsa*)
2.	Tarigan, 2022	Indonesia	Descriptive	*Giemsa*	Cell shape is unclear, cytoplasm contains many bubbles, nucleus color intensity is reduced due to loss.	Good results accounted for 56.25%, while poor results accounted for 43.75%.

3.	Sitorus et al, 2024	Indonesia	Descriptive and chi-square test	*Papanicolaou* and *Giemsa*	*Papanicolaou* shows cell shape, clear contrast between cell nucleus and cytoplasm, clean background. *Giemsa* shows clear cell nucleus, unclear cytoplasm, dirty background.	Good (*Papanicolaou*), Not so good (*Giemsa*)
4.	Woo et al., 2022	Malaysia	Descriptive	*May-Grunwald Giemsa*	No staining details mentioned.	Unknown
5.	Kinoshita et al., 2015	Jepang	Descriptive	*May-Grunwald Giemsa*	Clean background, reactive mesothelial cells visible.	Unknown
6.	Biswas et al., 2016	India	Descriptive	*Giemsa*	No staining details mentioned.	Unknown
7.	Kaur et al., 2016	India	Descriptive	*May-Grunwald Giemsa* and *Papanicolaou*	*Papanicolaou* and *May-Grunwald Giemsa* staining details are not mentioned.	Unknown
8.	Utami et al., 2024	Indonesia	Descriptive	*Giemsa*	Thick preparations consist of cell clusters, while thin preparations allow cells to be clearly observed.	Good

Table 2. Results of Journal Grouping Based on Centrifugation Time and Speed, and Fixation Type in Pleural Effusion Specimen Staining

No.	Researcher/Year	Time (Minutes)	Centrifugation Speed (rpm)	Wet Fixation	Dry Fixation	Result Description
1.	Dila, 2023	5	3000	√		Good (*Giemsa*), Not so good (*Papanicolaou*)
2.	Tarigan, 2022	10	Unknown	√		Baik
3.	Sitorus et al., 2024	5	500	√		Good (*Giemsa*), Not so good (*Papanicolaou*)
4.	Kinoshita et al., 2015	5	3000		√	Unknown
5.	Utami et al., 2024	15	4500		√	Good

Table 3. Results of Journal Grouping Based on Pleural Effusion Smear

No.	Researcher/Year	Smear		Result Description
		Conventional	*Liquid Based Cytology* (LBC)	
1.	Dila, 2023	√		Good
2.	Tarigan et al., 2023	√		Good
3.	Sitorus et al., 2024	√		Good
4.	Kinoshita et al., 2015	√	√	Good
5.	Biswas et al., 2016	√		Good
6.	Kaur et al., 2015		√	Not Listed
7.	Utami et al., 2024	√		Good

Table 4. Results of Journal Grouping Based on Cell Types Found

No.	Researcher/Year	Cell Structure
1.	Dila., 2023	Cytoplasm and cell nucleus are visible in *Giemsa* and *Papanicolaou* staining
2.	Tarigan., 2022	Cytoplasm and cell nucleus are visible in *Giemsa* staining.
3.	Sitorus., 2024	Cytoplasm and cell nucleus are visible in *Giemsa* and *Papanicolaou* staining.
4.	Woo et al., 2022	Plasma cells and mesothelium with a clear background in *May-Grunwald Giemsa* staining.
5.	Kinoshita et al., 2015	Clinical samples of pleural effusion show larger reactive mesothelial cells in *May-Grunwald Giemsa* staining.

6.	Biswas et al., 2016	The staining results show that pleural fluid smears are more dominant in lymphocyte morphology and there are no mesothelial cells.
7.	Kaur et al., 2016	There are scattered tumor cells, loose cohesive tumor cell clusters showing moderate to severe nuclear pleomorphism in *May-Grunwald Giemsa* staining. Meanwhile, *Papanicolaou* staining of the SurePathVR Liquid Based Cytology smear showed tumor cells and lymphocytes.
8.	Utami et al., 2024	Figures 1 and 3 show cell accumulation due to the smear being too thick. Figures 2 and 4 show thin smears that are easier to observe under a microscope.

The results of the study obtained 8 selected journals with Papanicolaou, Giemsa, and May Grunwald Giemsa staining. These journals were selected through a rigorous selection process from 2,294 journals found through the PubMed, Publish or Perish, and Google Scholar databases. The selection process was based on inclusion and exclusion using the PRISMA flow diagram. This study was grouped into three stages: the pre-analytical stage in Table 1, the analytical stage in Table 2, and the post-analytical stage in Table 3. The results in Tables 1, 2, and 3 are marked as "unknown" because these journals did not mention good or poor criteria, but only mentioned the overall staining results.

Pleural effusion in cytological analysis requires centrifugation to separate cellular elements from the supernatant so that cell morphology can be optimally analyzed under a microscope (Tarigan, 2023). Centrifugation that is too short can cause cells to not settle completely, uneven cell distribution, and unrepresentative preparations. Meanwhile, centrifugation that is too long in pleural effusion causes damage to morphology, artifacts, and inaccurate cytological interpretation (Hettich, 2014). Based on the pre-analytical stage results in Table 2, there are differences in the use of centrifugation at speeds of 500-4500 rpm for 5-15 minutes. Ideally, pleural effusion specimens should be centrifuged at 3000 rpm for 15 minutes to ensure accurate and representative results (Porcel, 2013).

The type of fixation used is selected based on the target diagnosis and the distance traveled to collect the specimen (Tarigan, 2023). Wet fixation using 96% alcohol is more optimal for Papanicolaou staining because it can preserve cell morphological details without heating for protein denaturation. Papanicolaou staining with wet fixation by Dila et al. (2023) and Sitorus et al. (2024) produced good quality results, as shown in Table 2. According to Leite et al. (2018), fixation using methanol is optimal when used for dry fixation for 1-2 minutes, but if soaked for 15 minutes, the process changes to wet fixation. Therefore, the studies by Dila et al. (2023), Tarigan (2023), and Sitorus et al. (2024) on Giemsa staining, which was originally performed with dry fixation, were classified as wet fixation with poor staining quality.

Dry fixation using methanol is suitable for Giemsa staining because it is more time-efficient, faster, and economical (Sitorus et al., 2024). Based on the results of the study in Table 2 by Utami et al. (2024), dry fixation with a single dip and drying using a hair dryer produces good staining quality on thin preparations. Dry fixation using spray in May-Grunwald Giemsa staining by Kaur et al. (2017) produced larger-looking cells compared to wet fixation in Papanicolaou staining. Dry fixation with a drying aid can accelerate protein denaturation by air, so that cells can

bind dyes and maintain morphology for blood cell and infection diagnosis (Tarigan, 2023).

There are reasons for using specimen processing techniques with Liquid Based Cytology or conventional methods at the pre-analytical stage, as shown in Table 3. Research by Kinoshita et al. (2015) states that conventional techniques are more common because they are more time-efficient, produce larger cell sizes, have clear cell morphology color intensity, and are fast. Research in Japan by Kinoshita et al. (2015) shows that Liquid Based Cytology (LBC) produces cytomorphology similar to conventional preparations. In addition, the Liquid-Based Cytology (LBC) technique has better sample preservation, uniform fixation, more contrasting staining, a clearer background, less clumping, and less cell blurring compared to conventional smears. There are no significant differences between the two types of smear methods, except that Liquid-Based Cytology has superior sensitivity compared to conventional smear methods.

The analytical stage of pleural effusion sample processing, which involves a comparison of Papanicolaou, Giemsa, and May-Grunwald Giemsa staining, is presented in Table 1. Research by Dila et al. (2023) and Sitorus et al. (2024) explains the differences in the quality of results from Giemsa and Papanicolaou staining. These studies show that Papanicolaou staining produces good results in displaying contrast between the nucleus, cytoplasm, and a clean background. Research by Kaur et al. (2017) states that this staining provides detailed cell information, such as the detection of tumor cells and lymphocytes using the Liquid-Based Cytology method.

Giemsa staining is often used in pleural effusion staining because it is very practical and efficient. Research by Tarigan (2023) in Table 1 shows that the staining quality is good at 56.25%, while the results are less than satisfactory at 43.75%. However, the test results still show many bubbles in the cytoplasm and a loss of color intensity. Research by Biswas et al. (2016) on Giemsa staining using the conventional smear method found lymphocytes. In contrast, Giemsa staining by Dila et al. (2023) and Sitorus et al. (2024) was considered suboptimal due to a dirty background and unclear cytoplasm contrast. This is assumed to be due to several factors, such as technical errors, improper staining process, poor quality of the diluting buffer, and poor quality of reagent filtration. According to Tjokrosonto (2017) in Tarigan (2023), a dirty background in the preparation can be caused by insufficient cleaning in the final washing stage, resulting in residual dye remaining attached.

May-Grunwald Giemsa staining by Kinoshita 2015 showed the ability to detect mesothelial cells, similar to the study by (Woo et al., 2022) which showed mesothelial cells and plasma cells. This staining has greater cell and cellularity results compared to Papanicolaou staining. However, the results of May Grunwald Giemsa staining are not consistent because they depend on the fixation and smear techniques used. The cells shown from conventional preparations and May-Grunwald Giemsa Liquid Based Cytology (LBC) methods tend to be similar in cell size and ratio and can show moderate to severe cell nucleus appearance.

The post-analytical stage involves reading cell morphology as shown in Table 4. Papanicolaou staining is superior in clearly displaying cell morphology and background. Giemsa staining is more suitable for staining blood cells, including lymphocytes, while May Grunwald Giemsa staining is effective in displaying plasma and mesothelial cells. Looking at the explanation in Table 4, Papanicolaou staining with wet fixation is the most effective method for examining cell morphology from pleural effusion specimens. Giemsa staining with dry

fixation is more suitable for lymphocytes and blood cells, while May-

Grunwald Giemsa staining is effective in displaying plasma and mesothelial cells.

Research by Susilowati (2022) and Astuti (2017) in Dila et al. (2023) states that composition, principles, hydration, and dehydration affect the final staining results. Similarly, the use of xylol in the clearing stage of Papanicolaou staining plays a role in removing residual alcohol within the cells. These stages are not present in Giemsa or May-Grunwald Giemsa staining, so the staining results tend to be less clear than Papanicolaou staining. Based on this study, there are differences between Papanicolaou, Giemsa, and May-Grunwald Giemsa staining in terms of the contrast between the nucleus and cytoplasm of cells, the morphology found, the cell type, and the background quality in pleural effusion specimens.

CONCLUSIONS AND RECOMMENDATIONS

The most effective staining method for staining the morphology of pleural effusion epithelial cells is Papanicolaou staining with wet fixation. The best staining method for blood cells in pleural effusion smear examinations is Giemsa staining with dry fixation. Meanwhile, May-Grunwald Giemsa staining with dry fixation is more optimal for staining mesothelial cells and blood cells in pleural effusion specimens. The optimal centrifugation speed for pleural effusion samples is 3000 rpm for 15 minutes. The recommended preparation techniques are the conventional smear method for early detection of cells and Liquid-Based Cytology to provide useful supportive cytodiagnostic information, such as in the malignancy of a pleural effusion specimen or other cytology samples.

Based on the research conducted, the type of fixation and preparation method can be considered. The choice of staining is adjusted to the needs of cell detection. Further experimental research is recommended.

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