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Comparative Evaluation of Conventional Cytology, Liquid-Based Cytology, and Cell Block Technique For Cytopathological Analysis of Pleural Aspirates

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Pleural effusion, cytopathology, cell block, diagnostic accuracy, malignant effusion.

Objective: To compare the diagnostic accuracy, practicality, and utility of conventional cytology (CC), liquid-based cytology (LBC), and cell block (CB) techniques in the cytopathological evaluation of pleural aspirates, aiming to determine the most effective method for diagnosing pleural effusion (PE), particularly in distinguishing between benign and malignant conditions.

Methods: A cross-sectional descriptive study involving sixty-eight patients aged over 18 years with non-traumatic pleural effusion confirmed by clinical evaluation and chest X-ray was conducted at Nnamdi Azikiwe University Teaching Hospital from January 31, 2020, to January 31, 2022. Pleural fluid samples were processed using three cytological techniques: CC, LBC, and CB. The slides were evaluated and categorized into five diagnostic categories: non-diagnostic, negative for malignancy, atypia of undetermined significance, suspicious for malignancy, and malignant. Statistical analysis was performed using SPSS version 22.

Results: The study included 40 males (58.8%) and 28 females (41.2%), with a mean age of 51.6 ± 17.12 years. Malignant effusions were observed in 23 patients (33.8%). The CB technique demonstrated superior performance with a sensitivity of 82.6%, specificity of 88.9%, and an accuracy of 1. In contrast, LBC showed a sensitivity of 65.2%

and specificity of 55.6%, while CC had the lowest sensitivity (17.4%) and specificity (51.1%). The CB method also achieved the highest negative predictive value (NPV = 1), outperforming LBC (NPV = 0.956) and CC (NPV = 0.852).

Conclusion: The CB technique was found to be the most reliable method for the cytopathological evaluation of pleural aspirates, exhibiting the highest sensitivity, specificity, and diagnostic accuracy. The study highlights the importance of selecting advanced cytological methods such as CB to enhance diagnostic precision in clinical practice, particularly in the differentiation of malignant from benign pleural effusions.

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INTRODUCTION:

Pleural effusion (PE) is the abnormal accumulation of fluid in the pleural cavity, resulting from various aetiopathogenetic processes, including malignancy, infection, and inflammation.[1] It is a common and significant clinical problem that affects millions worldwide, with a high mortality rate if left undiagnosed or misdiagnosed.[2] The analysis of pleural aspirates is crucial for accurate diagnosis, patient management, and predicting outcomes,[3] as it plays an important role in the initial work-up of the serous cavity effusion fluids, enabling the examination of cells in the pleural fluid, thus aiding in the differentiation of benign and malignant conditions.[4,5] The cytopathologic reporting of serous fluid includes 5 diagnostic categories having different malignancy risks: Non-diagnostic, negative for malignancy, Atypia of undetermined significance, Suspicious for malignancy and malignant categories.[6] Cytopathological evaluation of pleural aspirates is therefore critical in diagnosing malignant pleural effusion, a significant predictor of poor prognosis.[3]

PE cytology is a straightforward, crucial, swift, and easily accessible examination that, when fully utilized, will enhance the identification of pleural disorders, encompassing both malignant pleural conditions and benign origins.[7] The yield of aspirate cytology is frequently suboptimal and non-specific within our clinical context,[8] attributable to various challenges encountered in clinical practice, including the erroneous classification of cells as benign, malignant, or reactive mesothelial cells in serous effusions. Furthermore, the sensitivity of cytodiagnosis concerning effusions utilizing conventional method is typically influenced by the technique employed for fluid collection, the distribution of cells on microscopy slides, as well as the methods of laboratory processing.[9] Conventional cytology (CC) has been the traditional method for evaluating pleural aspirates, but recent advancements in cytopathology have introduced liquid-based cytology and cell block techniques as alternative approaches.[1] The choice of technique significantly affects diagnostic accuracy, and the limitations of conventional techniques have led to the development of alternative methods.[10,11] The selection of the most appropriate technique is critical for optimal patient care. This study aims to conduct a comprehensive comparative evaluation of three cytopathological techniques - conventional cytology, liquid-based cytology, and cell block preparation - in the analysis of pleural aspirates, with a focus on diagnostic accuracy, practicality, and utility in clinical practice.

Scope and Limitations

This study focuses on the comparative evaluation of three cytopathological

techniques in pleural aspirate analysis, with a scope limited to patients with non-traumatic pleural effusion. The study excludes patients with traumatic pleural effusion, as the cytopathological findings may differ significantly.

METHODOLOGY

This was a cross-sectional descriptive study conducted at Nnamdi Azikiwe University teaching hospital (NAUTH) from 31st January 2020 to 31st January 2022 on sixty-eight (68) patients >18 years of age presenting and admitted with non-traumatic pleural effusion, diagnosed by clinical evaluation and chest x-ray and, who underwent thoracentesis for diagnostic purposes. Written informed consent was duly obtained from the patients before commencement of the procedure. The study protocol was approved by the Ethics Committee of NAUTH, Nnewi with reference number NAUTH/CS/66/VOL.14/VER3/108/2021/078.

Thoracocentesis was done under a strict aseptic procedure, and 50mls pleural fluid collected for the cytology, cell block and chemistry, including aspirate glucose and protein. The fluid samples were sent to the pathology laboratory immediately for cytology, glucose and albumin.

For cytology smear preparation, 15ml of fresh Pleural fluid was centrifuged at 2,500 rpm for 20 minutes and the supernatant removed. The supernatant was poured off. From the sediment, one direct slide smear was prepared from the cell sediment for conventional cytology (CC) and submitted for Papanicolaou staining protocol. The remaining sediment was subjected to liquid-based cytology (LBC) technique to produce LBC slide; it had 15mL of CytoLyt solution (Hologic, Marlborough, MA, USA) added and centrifuged at 600 rpm for 20 minutes. The sediment from this was placed into a vial of Preservative solution, allowed to stand for another 15 minutes and then run on an automated ThinPrep® 2000 processor. The slide was fixed and submitted for Papanicolaou staining.[12]

For the cell block (CB), another 15ml of the pleural aspirate was centrifuged for 5 minutes at 6000 rpm. The supernatant was discarded, while agar solution was added to the sediment and refrigerated. The solid clot formed was fixed in 10% neutral buffered formalin solution and then processed into a formalin fixed paraffin-embedded (FFPE) block. Thin sections were cut from the FFPE block, and passed through the hematoxylin and eosin (H&E) staining protocol to produce a slide. The slides for the CC, LBC and CB were then mounted and evaluated using binocular diagnostic microscope (Leica DM1000 microscope). The observed features were used to categorize them into the five diagnostic categories, including non-diagnostic, negative for malignancy, Atypia of undetermined significance, Suspicious for malignancy and malignant categories.

Statistical Package for Social Sciences (SPSS) version 22, was used for data analysis. Descriptive variables were expressed as mean with standard deviation, frequencies and percentages, and shown in tables and figures. Chi-square was utilized to compare categorical variables and to test for statistical significance between categorical variables. $P < 0.05$ was considered statistically significant.

RESULTS

There were forty males (58.80%) and twenty-eight females (41.20%) aged between 21 and 84 years in this study, with a mean age of 51.60±17.12 years and modal age in the seventh decade of life. Most of the effusions were right-sided (n=41; 60.3%), commonly exudative (67.6%) with pleural aspirate protein >30g/l and most (80.9%) of the patients having a positive history of cigarette smoking. Malignant effusion was found in 23 (33.8%) of the patients. (see Table 1).

Table 1: Demographic data of the patients

VARIABLES	FREQUENCY	PERCENT (%)
Age Range		
21-30	11	16.2
31-40	9	13.2
41-50	12	17.6
51-60	11	16.2
61-70	17	25.0
71-80	6	8.8
>80	2	2.9
Mean = 51.60 ± 17.12		
Sex		**%**
Male	40	58.80
Female	28	41.20
Aspirate protein		
Exudate (>30g/l)	46	67.65
Transudate (<30g/l)	22	32.35
Aspirate sugar		
Greater than 3mmol	36	52.94
Less than 3	32	47.06
Sidedness		
Right	40	58.82
Left	28	41.18
Cigarette smoking		
Yes	55	80.88
No	13	19.12
Final diagnosis(Histology susp organ)		
Malignant	23	33.80
Negative for malignant cells	45	66.20

The percentage of malignant effusion was observed to relatively increase with age (table 2).

Table 2: Age distribution of the diagnoses

Age range	Negative for Malignancy (%)	Malignant (%)	Total
21-30 years	10 (90.9)	1 (9.0)	11
31-40 years	8 (88.9)	1 (11.1)	9
41-50 years	7 (58.3)	5 (41.7)	12
51-60 years	8 (72.7)	3 (27.3)	11
61-70 years	9 (52.9)	8 (47.1)	17
71-80 years	2 (33.3)	4 (66.7)	6
>80 years	1 (50.0)	1 (50.0)	2
Total	45	23	68

Table 3 shows the performance of the three cytological methods. The CC; LBC and CB methods have sensitivity and specificity of 17.4% and 51.1%; 65.2% and 55.6%; and 82.6% and 88.9% respectively. The positive predictive value for each of the 3 methods was 1, while the negative predictive values were 0.852, 0.956 and 1 for CC, LBC and CB respectively.

Table 3: Performance of the cytologic methods

			FINAL diagnosis (outcome)		Total	Performance Indices
			Neg for Mal	Malignant		
CC	Non-Diagn	Count	16	0	16	Sn = 17.4% Sp = 51.1% NPV = 0.852 PPV = 1 Accuracy = 0.871
		% within FINAL diagnosis	35.6%	0.0%	23.5%	
	Neg for Mal	Count	23	4	27	
		% within FINAL diagnosis	**51.1%**	17.4%	39.7%	
	AUS	Count	5	9	14	
		% within FINAL diagnosis	11.1%	39.1%	20.6%	
	Susp for Mal	Count	1	6	7	
		% within FINAL diagnosis	2.2%	26.1%	10.3%	
	Malignant	Count	0	4	4	
		% within FINAL diagnosis	0.0%	**17.4%**	5.9%	
Total		Count	45	23	68	
		% within FINAL diagnosis	100.0%	100.0%	100.0%	
LBC	Non-Diagn	Count	16	0	16	Sn = 65.2% Sp = 55.6% NPV = 0.956 PPV = 1 Accuracy = 0.952
		% within FINAL diagnosis	35.6%	0.0%	23.5%	
	Neg for Mal	Count	25	2	27	
		% within FINAL diagnosis	**55.6%**	8.7%	39.7%	
	AUS	Count	3	1	4	
		% within FINAL diagnosis	6.7%	4.3%	5.9%	
	Susp for Mal	Count	1	5	6	
		% within FINAL diagnosis	2.2%	21.7%	8.8%	
	Malignant	Count	0	15	15	
		% within FINAL diagnosis	0.0%	**65.2%**	22.1%	
Total		Count	45	23	68	
		% within FINAL diagnosis	100.0%	100.0%	100.0%	
CB	Non-	Count	3	1	4	

	Diagn	% within FINAL diagnosis	6.7%	4.3%	5.9%	Sn = 82.6% Sp = 88.9% NPV = 1 PPV = 1 Accuracy = 1
	Neg for Mal	Count	40	0	40	
		% within FINAL diagnosis	**88.9%**	0.0%	58.8%	
	AUS	Count	2	0	2	
		% within FINAL diagnosis	4.4%	0.0%	2.9%	
	Susp for Mal	Count	0	3	3	
		% within FINAL diagnosis	0.0%	13.0%	4.4%	
	Malignant	Count	0	19	19	
		% within FINAL diagnosis	0.0%	**82.6%**	27.9%	
Total		Count	45	23	68	
		% within FINAL diagnosis	100.0%	100.0%	100.0%	

CC= conventional cytology; LBC= Liquid-Based Cytology; CB= Cell Block; AUS= Atypia of Undetermined Significance; Neg for Mal- Negative for malignant cells; Susp for Mal- Suspicious for malignancy; Non-Diagn= Non-Diagnostic; PPV= positive predictive value; NPV= negative predictive values; Sn= sensitivity; Sp= specificity

Figure 1 shows the comparative performance of the cytologic methods. The CB method out-performs LBC and CC using the various performance indices. Diagnostic accuracy was better with LBC and CB.

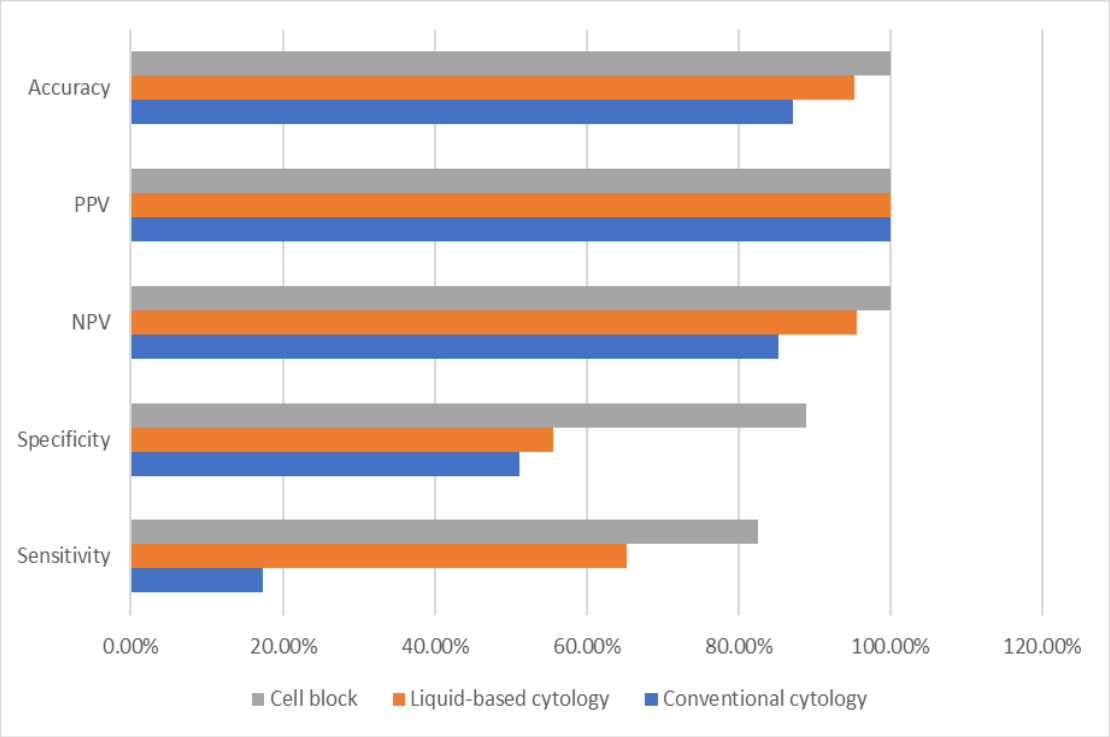

Figure 1: Comparing the performance of conventional cytology, liquid-based cytology and cell block

DISCUSSION

This study included sixty-eight pleural fluid aspirate specimens from patients aged between 21 and 84 years (mean = 51.60±17.12 years). Similar, but wider age range of 7-89 years was reported by Acharya et al in their studies,[13] whereas the patients in the study by Rani et al were aged between 20 and 61 years.[12] Pleural effusion can therefore occur within any age group, though commoner in the seventh decade of life as seen in our study and that of Acharya et al.[13] In agreement with other studies, the patients studied were largely (58.80%) males with a male: female ratio of 1.43:1.[8,12,14-15]

PE in our study were largely right-sided (n=41; 60.3%) and commonly exudative (67.6%) with most (80.9%) of the patients having a history of cigarette smoking. Malignant effusion was found in 13 (19.12%) of the patients. The reason for the sidedness of PE is not clear, but cigarette smoking is known to be a major contributor to most diseases including malignant and non-malignant lesions.[16] These effusions were largely benign, with only 33.8% (n=23) being malignant. Most studies have reported predominance of benign effusions.[12,17-1]However, categorizing PE as benign and malignant often presents a diagnostic dilemma. [13]

Cytologic evaluation of effusion samples is an important minimally invasive and rapid method of resolving this dilemma, being able to not only diagnose but also stage and prognosticate the underlying disease processes.[19] According to the current guideline of PE management, it is recommended in the early/initial work-up to categorize the effusion as benign or malignant .[5,20]

CC has been traditionally employed to evaluate pleural aspirates. However, there has been advancements in cytopathology with the introduction of LBC and CB techniques.[1] The choice of cytopathological technique for the analysis of pleural effusions can significantly impact diagnostic accuracy and patient management. The findings from Table 4 on the performance of the three cytological methods —CC, LBC and CB technique— provided. valuable insights into their effectiveness in evaluating pleural fluid aspirates. The sensitivity and specificity metrics indicated varying performance levels among the methods. The CC method showed a sensitivity of 17.4% and specificity of 51.1%, which suggests a limited ability to accurately identify true positive cases. In contrast, LBC demonstrated improved sensitivity (65.2%) and moderate specificity (55.6%), indicating a better capacity for detecting malignancies but still falling short in specificity. Notably, the CB method outperformed the other two, with a sensitivity of 82.6% and a high specificity of 88.9%, suggesting it is the most reliable method among those evaluated. The positive predictive values (PPV) of 1 across all methods indicate that when a diagnosis is made, it is accurate. However, the negative predictive values (NPV) varied significantly, with CB achieving a perfect NPV of 1, implying that a negative result is highly reliable. In contrast, CC and LBC had NPVs of 0.852 and 0.956, respectively, suggesting that there is still a non-negligible chance of false negatives with these methods.

In contrast to these performance findings of our study, Nemade et al., reported that Liquid-based cytology is advantageous over conventional techniques in cytomorphology of body fluids, but not better in sensitivity and specificity.[21] Similarly, Buch et al., stated that LBC smears were qualitatively superior to CC but the overall diagnostic accuracy was comparable in both techniques.[22] However, the findings in our study align with previous studies that have compared these cytological techniques. For instance, a study by Joshi et al., reported that the cytospin and cell block method provide high cellularity, better architectural patterns, morphological features and an additional yield of malignant cells, and thereby, increases the

sensitivity of the cytodiagnosis when compared to conventional smear method.[23] Saha et al, stated that Morphological features were better identified by the cell block method when compared to LBC.[24] Other similar comparative studies had also shown that CB techniques provide superior cellular preservation and diagnostic yield, devoid of artefacts compared to conventional methods, supporting the observed high sensitivity and specificity of the CB method in our findings.[15, 25]

In summary, while all three cytological methods have their merits, the cell block technique appears to be the most effective for cytopathological analysis of pleural aspirates, as indicated by its superior sensitivity and specificity. These findings underscore the importance of method selection in clinical practice to enhance diagnostic accuracy.

CONCLUSION

The cytological methods, though all offering diagnostic value, the CB technique stands out as the most reliable for assessing pleural fluid aspirates because of its high sensitivity and specificity. These qualities enhance diagnostic accuracy and patient management. The selection of cytological method has a significant impact on clinical outcomes, highlighting the importance of utilizing advanced techniques such as CB for achieving optimal diagnostic performance.

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