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A Correlation between the Cytology and Histology of Serous Effusions at a Teaching Hospital in South-East Nigeria

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ABSTRACT

Background: Cytological evaluation of body fluids is an important diagnostic test for various malignant and benign conditions.

Objectives: Our study aims to analyse the various body fluids received in our department over a ten-year period. It also seeks to determine the accuracy and significance of specimen volumes of fluid cytology specimens in diagnosing malignancy in a resource-poor setting.

Methodology: This is a retrospective study carried out in the Department of Anatomic Pathology of a teaching hospital in South-East, Nigeria. Histopathologic tissue results, if available were also retrieved from the archives as gold standard.

Results: Within the period under review, 358 pleural fluid specimens, 358 ascitic fluid specimens and 2 pericardial effusion specimens were retrieved. Of all effusion cytology specimens, 126 (17.5%) were cytologically malignant. More specifically, 48 (13.4%) of pleural effusion specimens, and 78 (21.8%) of ascitic fluid specimens were malignant. The most common histologically diagnosed cancer observed in patients with malignant pleural effusion was breast cancer, while for ascitic fluid, it was ovarian cancer. For all cytologic diagnoses, the sensitivity was 37.0%, specificity 87.2%, PPV 84.4%, NPV 42.5% and accuracy 54.5%. With respect to the specimen volume of the histologically confirmed malignant cases received for cytological examination, 114 (52.1%) of the specimen were <10mls, only 36 (16.4%) were ≥20mls. Those cytologically positive for malignant cells had a median volume of 10.0ml, while those cytologically negative for malignant cells had a median volume of 8.8ml. In addition, there was an incremental increase in the percentage of cytologically malignant effusions with increased volume of specimen used for the analysis from 34.2% for specimens <10mls to 50% for specimen volumes ≥20mls. However, a *Chi-squared* test showed there is no statistically significant difference between these ($P = 0.213$).

Conclusion: The sensitivity of effusion cytology in this study is at the lower end of the spectrum. This may be related amongst other factors to the suboptimal specimen volume received for evaluation and lack of resources for cell block and immunocytochemistry.

***Key words**: Ascitic fluid, Pleural fluid, Cytology, Sensitivity, Specimen Volume*

INTRODUCTION

Cytological evaluation of body fluids is an important diagnostic test for various malignant and benign conditions. It is a rapid, simple, cost-effective and relatively patient compliant investigation.[1] Various disease processes, which include inflammatory, infectious and neoplastic (benign or malignant), primary or metastatic diseases can give rise to effusion. Identification of malignant cells in effusions has important implications for staging procedures and resulting therapeutic decisions.[2]

Our study seeks to analyse the various body fluids received in our department over a ten-year period with the aim of determining the sensitivity, specificity, positive predictive value, negative predictive value and significance of specimen volume of fluid cytology specimens in the diagnosis of malignancy within a resource-poor setting.

METHODOLOGY

This is a retrospective study carried out in the Department of Anatomic Pathology of Nnamdi Azikiwe University Teaching Hospital (NAUTH), Nnewi from 1st January 2009 to 31st December 2018. NAUTH is a federal government-owned tertiary hospital and the Anatomic Pathology laboratory of NAUTH is the largest tertiary histopathology laboratory in Anambra state and one of the largest in the entire South-East region of Nigeria. It receives specimens from Anambra state and neighbouring states in the South-East and South-South regions.

Patients' biodata, relevant clinical information and cytologic diagnosis of all effusion fluid specimens received for cytological examination during the study period were retrieved from the archives. The cytologic diagnoses were made by histopathologists/cytopathologists. Two Giemsa- stained and two Papanicolaou-stained slides of direct smears were prepared from sediment obtained by centrifuging the effusion fluid specimens at 2500 rpm for 5 minutes. Histopathologic tissue results were also retrieved from the archives as gold standard. The data were analysed using SPSS 23.0 software version for windows. A *Chi-squared* test was applied to test the relationship between the specimen volume and the malignancy diagnosis. A *p*- value of less than 0.05 was considered statistically significant.

RESULTS

Seven hundred and eighteen cases made up of 358 pleural fluid specimens, 358 ascitic fluid specimens and 2 pericardial effusion specimens were retrieved (Table 1). The ages of the patients range from one to ninety-five years with a median age of 48 years and interquartile range of 34-60 years. Four hundred and forty-two (61.6%) were female while 276 (38.4%) were males. However, the ascitic fluid specimens were predominantly from female patients (268 specimens; 74.9%), while 90 specimens (25.1%) were from male patients. However, for the pleural effusion specimens, there were 186 (52.0%) specimens from males and 172(48.0%) specimens from females.

Clinically, 342 (52.8%) of all effusions were considered to be of neoplastic origin, 188 (29.0%) inflammatory and 118 (18.2%) benign non-inflammatory (Table 1). Tuberculosis (146 cases; 22.5%) was the most common inflammatory aetiology accounting for 120 (39.7%) of pleural effusions, while liver cirrhosis (82 cases; 12.7%) was the most

common benign non-inflammatory cause accounting for 80 (23.1%) of ascitic fluids.

Only 126 (17.5%) of all effusion cytology specimens were cytologically malignant, 536 (74.7%) were benign, while 56 (7.8%) were unsatisfactory i.e. either acellular or consists only of degenerate cells owing to poor preservation. More specifically, 48 (13.4%) of pleural effusion and 78 (21.8%) of ascitic fluid were malignant. Effusions with an inflammatory picture accounted for 334 (46.5%) of all effusions, 218 (60.9%) of pleural fluids and 116 (32.5%) of ascitic fluids. Tuberculous inflammation was the only specific inflammatory entity cytologically diagnosed and accounted for 44 (6.1%) of all cytologic diagnosis. Furthermore, 32 (8.9%) of pleural effusions and 12 (3.4%) of peritoneal effusions were accounted for by tuberculosis.

One hundred and seventeen of the pleural effusion specimens had histologic diagnosis consisting of 81 malignant and 36 benign diagnoses (Table 1). Two hundred and nineteen of the ascitic fluid specimens had histologic diagnosis comprising 138 malignant and 81 benign diagnoses. Hence, histologic diagnosis was available for 336 cases (representing 46.8% of all fluid cytology specimen) and consisting of 219 histologically malignant cases and 117 histologically benign cases.

With respect to age distribution, both malignant and benign pleural effusions were most common in the 40-59 years' age group. The same finding was made for ascitic fluid. Interestingly, while, majority (16 cases; 50%) of tuberculous pleural effusion occurred in the 20-39-year age group, most of tuberculous ascites occurred in the 40-59-year age group (Table 2).

Malignant pleural effusion cytologic diagnosis had a sensitivity of 29.6%, specificity of 83.3%, positive predictive value (PPV) of 80.0%, negative predictive value (NPV) of 34.5% and accuracy of 46.2% (Table 3). Malignant ascitic fluid cytologic diagnosis had a sensitivity of 41.3%, specificity of 88.9%, PPV of 86.4% NPV of 47.1% and accuracy of 58.9%. Hence cytologic diagnoses for all malignant effusions had an overall sensitivity of 37%, specificity of 87.2%, PPV of 84.4%, NPV of 42.5% and accuracy of 54.5%.

With respect to the specimen volume of the histologically confirmed malignant cases received in the laboratory for cytological examination, 114 (52.1%) of the specimen were less than 10mls, only 36 (16.4%) were at least 20mls. Those cytologically positive for malignant cells had a median volume of 10.0ml, while those cytologically negative for malignant cells had a median volume of 8.8ml (Table 4). In addition, there was an incremental increase in the percentage of cytologically malignant effusions with increased volume of specimen used for the analysis from 34.2% for specimens less than 10mls to 50% for specimen volumes of at least 20mls. This, however, did not show any statistical difference (p= 0.213).

For the malignant cytologic diagnoses, the most common histologically identified malignancies were ovarian (45 cases; 55.6%) and breast (18 cases; 22.2%) (Table 5). The most common histologically diagnosed cancer observed in patients with malignant pleural effusion was breast cancer, while for ascitic fluid, it was ovarian cancer. In addition, 75 (92.6%) of malignant effusions were due to carcinomas, while only 6 (7.4%) were of mesenchymal origin (Table 6).

Table 1. Comparison of Age, Sex, Cytologic and Histologic diagnoses across specimen types

		Pleural fluid (%)	Ascitic fluid (%)	Pericardial fluid (%)	Total (%)
Total number of cytology cases		358 (49.9)	358 (49.9)	2 (0.2)	718 (100.0)
Age (years)	Mean	46.9 ± 18.9	47.0 ±18.0	-	46.9 ± 18.5
	Median	48.0	48.0	-	48.0
	Range	1 – 95	1 - 88	-	1 - 95
Sex	Male	186 (52.0)	90 (25.1)	0	276 (38.4)
	Female	172 (48.0)	268 (74.9)	2	442 (61.6)
Cytologic Diagnosis	Malignant	48 (13.4)	78 (21.8)	0	126 (17.5)
	Inflammatory	218 (60.9)	116 (32.5)	0	334 (46.5)
	Tuberculous inflammation	32 (8.9)	12 (3.4)	0	44 (6.1)
	Benign, non-inflammatory effusion	64 (17.9)	136 (38.0)	2	202 (28.1)
	Unsatisfactory	28 (7.8)	28 (7.8)	0	56 (7.8)
Available Clinical Diagnosis	Neoplastic	120 (39.7)	222 (64.2)	-	342 (52.8)
	Inflammatory	152 (50.3)	36 (10.4)	-	188 (29.0)
	Tuberculosis	120 (39.7)	26 (7.5)	-	146 (22.5)
	Pneumonia	22 (7.3)	-	-	22 (3.4)
	Rheumatoid Arthritis	2 (0.7)	-	-	2 (0.3)
	Benign Non-inflammatory	30 (9.9)	88 (25.4)	-	118 (18.2)
	Heart failure	26 (8.6)	2 (0.6)	-	28 (4.3)
	Liver Cirrhosis	2 (0.7)	80 (23.1)	-	82 (12.7)
	Chronic Kidney Disease	2 (0.7)	2 (0.6)	-	4 (0.6)
	Endometriosis	-	4 (1.2)	-	4 (0.6)
Available histologic diagnosis	Malignant	81 (69.2)	138 (63.0)	0	219 (65.2)
	Benign	36 (30.8)	81 (37.0)	0	117 (34.8)

Table 2. Comparison of frequency of the different categories across different age groups

Age		**<20 years**	**20-39 years**	**40-59 years**	**60+ years**	**Total**
Pleural fluid	Malignant (%)	2 (4.2)	8 (16.7)	22 (45.8)	16 (33.3)	48 (100.0)
	Inflammatory exudate (%)	18 (8.3)	64 (29.6)	78 (36.1)	56 (25.9)	216 (100.0)
	Tuberculosis (%)	0 (0.0)	16 (50.0)	8 (25.0)	8 (25.0)	32 (100.0)
	Benign, non-inflammatory effusion (%)	8 (12.5)	12 (18.8)	22 (34.4)	22 (34.4)	64 (100.0)
Ascitic fluid	Malignant (%)	8 (10.3)	12 (15.4)	30 (38.5)	28 (35.9)	78 (100.0)
	Inflammatory exudate (%)	8 (6.9)	40 (34.5)	38 (32.8)	30 (25.9)	116 (100.0)
	Tuberculosis (%)	2 (16.7)	2 (16.7)	6 (50.0)	2 (16.7)	12 (100.0)

Benign, non-inflammatory effusion (%)	8 (5.9)	40 (29.4)	56 (41.2)	32 (23.5)	136 (100.0)

Table 3. Cytologic Diagnosis and Histologic Diagnosis Cross tabulation

Specimen				Histologic Diagnosis: Malignant	Benign	Total
Pleural fluid	Cytologic Diagnosis	Malignant	Count	24	6	30
			% within Cytologic Diagnosis	80.0%	20.0%	100.0%
			% within Histologic Diagnosis	29.6%	16.7%	25.6%
		Benign	Count	57	30	87
			% within Cytologic Diagnosis	65.5%	34.5%	100.0%
			% within Histologic Diagnosis	70.4%	83.3%	74.4%
	Total		Count	81	36	117
			% within Cytologic Diagnosis	69.2%	30.8%	100.0%
			% within Histologic Diagnosis	100.0%	100.0%	100.0%
Ascitic fluid	Cytologic Diagnosis	Malignant	Count	57	9	66
			% within Cytologic Diagnosis	86.4%	13.6%	100.0%
			% within Histologic Diagnosis	41.3%	11.1%	30.1%
		Benign	Count	81	72	153
			% within Cytologic Diagnosis	52.9%	47.1%	100.0%
			% within Histologic Diagnosis	58.7%	88.9%	69.9%
	Total		Count	138	81	219
			% within Cytologic Diagnosis	63.0%	37.0%	100.0%
			% within Histologic Diagnosis	100.0%	100.0%	100.0%

Table 4. Specimen volume parameters for malignant and benign effusions

Volume	Positive for malignant cells (%)	Negative for malignant cells (%)	Total
Mean	13.9 +/- 18.2ml	9.3 +/- 6.1ml	-
Median	10.0ml	8.8ml	-
Range	1 - 90ml	1 - 25ml	-
< 10ml	39 (34.2)	75 (65.8)	114
≥ 10ml to < 20ml	24 (34.8)	45 (65.2)	69
≥ 20ml	18 (50.0)	18 (50.0)	36

DISCUSSION

The diagnostic accuracy of pleural fluid cytology for a malignant effusion has been reported to range from 40% to 87%, which is higher than that of pleural biopsy. [3] In this study, the pleural effusion cytologic diagnosis had an accuracy of 46.2%. This is within the above reported range from earlier studies.

The diagnostic specificity of ascitic fluid cytology in this study is 88.9% but identified only 41.3% of malignant ascites. The sensitivity of cytology in the diagnosis of malignant ascites ranges only between 50% and 70%.[4] A number of reasons may be responsible for this, such as the mechanism through which the effusion developed, type of malignancy, method of specimen processing and specimen volume.

Table 5. Frequency of different primaries in malignant effusions

Organ of Origin	Histologically malignant	Pleural fluid	Ascitic fluid	Cytologically malignant (%)
Ovary	90	3	42	45 (55.6)
Breast	36	18	--	18 (22.2)
Colon	12	--	3	3 (3.7)
Uterus	12	--	3	3 (3.7)
Stomach	9	--	3	3 (3.7)
Cervix	6	--	3	3 (3.7)
Skin	6	3	--	3 (3.7)
Pancreas	3	--	3	3 (3.7)
Lymphoid tissue	12	--	--	0 (0.0)
Lung	6	--	--	0 (0.0)
Prostate	6	--	--	0 (0.0)
Unknown primary	21	--	--	0 (0.0)
Total	219	24	57	81 (100.0)

Table 6. Types of malignancies in malignant effusions

Histogenesis	Histologically malignant	Cytologically malignant (%)
Epithelial	171	75 (92.6)
Mesenchymal	27	6 (7.4)
Lymphoid	12	0 (0.0)
Germ cell	9	0 (0.0)
Total	219	81 (100.0)

The presence or otherwise of malignant cells in an effusion may be determined by the mechanism through which the effusion developed in a patient with malignancy.[3] For instance, presence of tumour implants on serosal surfaces or direct tumour infiltration of the pleura and peritoneum are more likely to result in effusions that contain malignant cells[3]. The converse is the case for effusions due to impairment of lymphatic drainage of the pleura or peritoneum either by tumour infiltration of the lymph nodes or increased resistance to lymph flow into the vascular system.[3]

The type of malignancy also determines the sensitivity of fluid cytology. [5] Studies suggest a high rate of cancer cell detection in carcinomas than lymphomas. [5] While 75 of the 171 epithelial malignancies in our study were detected cytologically, none of the twelve lymphoid malignancies were detected cytologically. This would have adversely affected the sensitivity of effusion cytology in this study.

Another cause of negative cytology report is the method of specimen processing. A paraffin-embedded cell block collects more cellular components than the traditional method of processing fluid cytology specimens. In addition, the cell components are more concentrated, hence increasing its sensitivity and better demonstrating

architectural patterns. This could be of great assistance in making the correct diagnosis of the primary lesion. [4] This cell block technique is unavailable, not only in our institution, but also in nearly all hospitals in Nigeria. Hence, we used the traditional method. The traditional method of processing fluid cytology specimens is negatively affected by the time length of specimen storage, the thickness of the smear, solidification and dyeing quality.[4] These may inevitably lead to a missed diagnosis, misdiagnosis and low sensitivity as was seen in this study.

While there is on-site evaluation for specimen adequacy during aspiration cytology, effusions are routinely collected with no pathology staff on-site to determine whether diagnostic material is present. [6] In addition, there are no consensus regarding the minimum volume of fluid required to diagnose a malignant effusion. Only a few studies have been published regarding the volume required to diagnose malignant effusion.[3] Thomas *et al*. found 25–50 mL of fluid was adequate to diagnose malignant pleural effusion while Abouzgheib *et al* found that submission of >50 mL of pleural fluid did not increase diagnostic yield.[3,7,8] Swiderek *et al*. divided the pleural fluid in 10 ml, 60 ml, ≥150 ml and found 60ml adequate for diagnosing a malignant pleural effusion.[3,9] However, a recent retrospective analysis of 2450 cases by Rooper *et al*. supported the use of 75 ml as a minimum cut-off volume for pleural effusion specimen.[3,6]

On the other hand, though, Zhang *et al.* demonstrated that 200 ml was the optimal cut-off volume required for an accurate cytological diagnosis of malignancy in patients who provide greater than or equal to 1000 ml ascites, an earlier retrospective analysis by Rooper *et al.* showed that cytologic sensitivity increased from 56.7% for ascitic fluid specimens <80 ml to 75.4% for volumes ≥80 ml.[4,10] Rooper *et al.* concluded that although ascitic fluids should not be summarily rejected based on volume, a specimen volume of ≥80 mL minimizes the influence of specimen volume on diagnostic accuracy in ascitic fluid cytology specimens.[10]

Our study showed an incremental increase in the percentage of cytologically malignant effusions with increased volume of specimen used for the analysis. This may suggest that increase in specimen volume is associated with increase chances of identifying malignant cells in a malignant effusion specimen. It also instructive to note that only 16.4% of the histologically confirmed malignant cases had a specimen volume of at least 20mls. This obviously may have accounted for the relatively low sensitivity and negative predictive value of cytologic diagnosis observed in this study. Although that there were no statistical differences in the diagnostic rate between the three groups of specimen volume in our study, possibly due to the small sample size, these differences may be clinically relevant. Hence, it is hoped that clinicians in Nigeria will increase the volume of effusion specimens submitted for cytology at least to 25mls which is the minimum adequate volume from literature.[7]

This study shows that the most common specific cytologic diagnoses in both types of effusion are cancers and tuberculosis. Porcel *et al.* also reported that the aetiology of pleural effusion was, in order of frequency, cancer, heart failure, pneumonia and tuberculosis.[11] In their series, approximately half of all pleural effusion among young patients (<34 years) was caused by tuberculosis.[11] Similarly, in this study,

tuberculous pleural effusion was most common within the 20-39 years' age group.

Some researchers have suggested that gastrointestinal cancer is the most common cause of malignant ascites followed by ovarian, whereas ovarian cancer is considered the most common cause of malignant ascitic fluid in females.[2,4] In addition, a study from Spain revealed that lung cancer is responsible for at least a third of malignant pleural effusions.[11]

However, in our study, ovarian cancer is the most common in patients diagnosed with malignant ascites, probably because the female gender makes up 74.9% of all patients with peritoneal effusion in this study. Furthermore, the breast is the most common primary for malignant pleural effusion. This is probably explained by the fact that according to Nnewi Cancer Registry, breast cancer is the most common cancer, making up 23.4% of all new cancer cases.[12]

A limitation of this study is that only patients evaluated for the diagnosis of effusions were included. This could mean that some aetiologies of pleural and peritoneal effusions are poorly represented, such as heart failure (many patients presenting with characteristic symptoms are diagnosed with pleural effusion and do not undergo thoracocentesis). Second, our study reports results from a single institution with a limited sample size. After, extensive literature search, the authors did not find any study from Nigeria on volumes of effusion specimen submitted for cytology and its effect on diagnostic accuracy. This, therefore may be the first published work from Nigeria on this issue. Thus, our results need to be verified using larger samples from multi-centre studies. The study is retrospective in nature and is affected with the problems of inability to carry out some investigations (such as CT scan, magnetic resonance imaging and immunohistochemistry, which would have been useful in some cases). This accounted for a large number of malignant effusions with unknown primaries. Despite these limitations, we have been able to report the pattern and accuracy of effusion cytology in our setting.

CONCLUSION

Pleural effusion and ascites are common clinical problems confronting physicians in Nigeria. Malignancy and tuberculosis are the leading causes of effusions. The study also highlights the fact that the specimen volume of effusions received for cytologic evaluation is grossly below the minimum adequate volume from scientific literature. The sensitivity of effusion cytology in this study is at the lower end of the spectrum. This may be related to the suboptimal specimen volume receive for evaluation. Hence, there is need for training of clinicians on cytologic fluid specimen collection to improve on the volume of effusion specimens submitted for cytologic evaluation. There is also need for advocacy to government at all levels in Nigeria and other stakeholders to build capacity for cell block and immunocytochemistry through provision of better funding and facilities for cytology services.

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