Role of Immunohistochemistry in Determining Origin of Metastatic Tumors in Pleural and Peritoneal Effusions

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Role of Immunohistochemistry in Determining Origin of Metastatic Tumors in Pleural and Peritoneal Effusions

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Abstract:-

- ***Aim of Study***

Effusion cytology is a test used to determine the etiology of a disease. Pleural, ascitic, pericardial, synovial, and cerebrospinal fluid are commonly analyzed samples. When a malignancy is detected in effusions, the place of origin cannot be determined merely by cytologic appearance. In the era of advanced technology, cytomorphology alone is insufficient, and hence, immunocytochemistry is the most widely used modality in cytology effusion. Application of judicious immunopanel can help determine origin of metastatic tumor, hence aiding the clinician in initiation of treatment and prompt management of wider spread disease.

- ***Material and Methods***

It was a single center study carried out at Chugtais Institute of pathology for a period of one year. All malignant pleural and peritoneal effusions from both genders were included in the study. Concomitant naturally formed clots were fixed in 10% neutral buffered formalin and processed as cell blocks. Cell blocks were prepared using the complex streptavadin-biotin peroxidase technique. Immunohistochemistry was applied to 104 cases with external positive controls. CK7, CK20, Wt1, GATA3, Napsin A, CDX2, LCA, PAX8 & TTF1 were applied to determine primary site of origin.

- ***Results***

Most common cause of malignant peritoneal effusion was due to ovarian malignancies in females and adenocarcinoma in males while, in case of pleural effusion, it was breast carcinoma in females and lung carcinoma in males.

- ***Conclusion***

Cellblock combined with a judicious immunohistochemical panel according to gender and most common metastatic tumors can be an accurate and affordable method to determine the primary site of cancer. Our study results signifies the necessity of utilizing a panel of markers to prevent misidentification of the primary sites of metastatic carcinoma in effusions.)

Keywords:- *Component Cell Block, Immohistochemistry, Effusion Cytology, Primary Site.*

I. INTRODUCTION

Effusion cytology is a test used to determine the etiology of a disease. Pleural, ascitic, pericardial, synovial, and cerebrospinal fluid are commonly analyzed samples. In 1867, Lucke and Klebs were the first to observe malignant cells in ascitic fluid [1,2]. A cytological examination of fluids is valuable for cancer diagnosis, disease staging, and prognosis. It is a comprehensive diagnostic tool that identifies the cause of effusion, disease progression, and prognosis. Using cell blocks (CB) can significantly improve the sensitivity of this test. Malignant serous effusions almost always indicate metastatic disease. Therefore, in cases with metastases from an unknown primary origin, evaluating the serous effusions for malignant cells is standard for diagnosing and planning further treatment [3]. When a malignancy is detected in effusions, the place of origin cannot be determined merely by cytologic appearance. In the era of advanced technology, cytomorphology alone is insufficient, and hence, immunocytochemistry is the most widely used modality in cytology effusion. Although other methods are also described in the literature, immunocytochemistry is still the method of choice in anatomic pathology laboratories due to its lower cost, ease of use, readily available reagents, and excellent accuracy in most cases [4]. Malignancy and the primary site can be diagnosed with 81% accuracy using a combination of traditional smear and cell block techniques for reporting effusions [5].

Our study aimed to determine the most common types of malignancies and the primary site of origin in pleural and peritoneal effusions to help the clinician promptly diagnose and ensure timely treatment. For this purpose, the cell block approach was used on samples of pleural and peritoneal effusions from women and men with carcinoma of unknown primary site. An immunocytochemical panel was applied to evaluate the expression of markers indicative of the primary site.

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II. MATERIAL AND METHODS

This cross-sectional descriptive study was conducted for 12 months, from 1st February 2021 to 31st January 2022. It was approved by the institutional review board of the Chughtai Institute of Pathology (Reference letter number CIP/IRB/1041). All malignant pleural and peritoneal effusions from both genders were included in the study. Concomitant naturally formed clots were fixed in 10% neutral buffered formalin and processed as cell blocks. Cell blocks were prepared using the complex streptavadin-biotin peroxidase technique. Cell blocks with low cellularity (less than 50 cells) and cases with a final diagnosis showing reactive atypia and degenerated cells with atypia were excluded from the study. IHC was applied to 104 cases with external positive controls. CK7, CK20, Wt1, GATA3, Napsin A, CDX2, LCA, and PAX8 were manufactured by DAKO (Agilent, Santa Clara, USA). Only TTF1 was obtained from GeneAb. The frequency of numerical and categorical variables was determined using the SPSS-26 version.

III. RESULTS

The study included 104 positive fluids with 56 pleural and 48 malignant peritoneal effusions. Of 56 cases of malignant pleural effusion, 21 were males (37.5%), and 35 were females (62.5%). Of 48 cases of malignant peritoneal effusions, 2(4.1%) were male, and 46(95.8%) were female. Among both genders, 40(38.4%) patients were less than 45, and 64(61.5%) patients were above 45. Out of 56 positive pleural fluids, 16(28.5%) cases were metastatic from the lung in males. Among females, 14(25%) cases were metastatic from the lung, and 5(8.9%) were Lymphoproliferative disorders. However, 21(37.5%) cases in females were metastatic from breast. Among peritoneal fluids, 32(66.6%) cases were metastatic from the gynecological tract, and 10(17.8) cases were metastasis from GIT in the female population. A total of 4(8.3%) cases were classified as lymphoproliferative disorder in females. 2 cases were metastatic in the male gender of gastrointestinal in origin. Females with malignant pleural and peritoneal effusion most commonly experienced metastasis originating from the breast (37.5%) and ovaries (66.6%), respectively. The most common metastatic sites in males were the lung (28.5%) and Gastrointestinal tract (4.1%), respectively.[Table 1 & 2]

Table 1 The Most Common Metastatic Sites

Characteristics	**Number (n, %)**	
Sex Male Female	 23 (22.1%) 81 (77.8%)	
Age <45 >45	 40 (38.4%) 64 (61.5%)	
Effusion type Pleural effusion Peritoneal effusions	Male 21 (37.5%) 2 (4.1%)	Female 35 (62.5%) 46 (95.6%)
Primary site Lung Gynecological tract Breast primary Gastrointestinal primary Lymphoid	Male 16(28.5%) 0 (0%) 0 (0%) 2(4.1%) 5(8.9%)	Female 14(25%) 32(66.6%) 21(37.5%) 10(17.8%) 4(8.3%)

Table 2: Most Common Metastatic Site in Male and Female

Most common metastatic site in pleural effusions in females	Breast 21 (37.5%)
Most common metastatic site in pleural effusions In males	Lung 16 (28.5%)
Most common metastatic site in peritoneal effusions in females	Ovarian 32 (66.6%)
Most common metastatic site in peritoneal effusions in males	Gastrointestinal 2 (4.1%)

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IV. DISCUSSION

Analysis of effusions for the presence or absence of malignancy is critical from a clinical point of view. The origin and nature of malignancy help the clinician plan the treatment mode. It is essential to determine the tumor site if there is no known malignancy and in the case of patients with a known malignancy, whether it is a recurrence or a second new tumor [4]. The average survival rate of patients with malignant effusions is from 4 to 7 months and is primarily dependent on the pathologic stage and aggressiveness of malignancy [6]. Lung cancer, breast cancer, and lymphoma are the most common causes of malignant effusions as they drastically change the TNM staging status from T4 to M1a [7]. The most typical causes of malignant pleural effusions in males are lung cancer, lymphoma, and gastrointestinal tumors. Breast cancer is the leading cause of malignant pleural effusions in females. Malignant peritoneal effusions in males are frequently caused by lymphoma/leukemia, gastrointestinal tumors, and pancreatic carcinomas. At the same time, in women, they are due to cancers of the gynecologic tract, breast carcinomas, and gastric carcinomas [8]. Cell blocks from malignant effusion show characteristic morphological features; however, it sometimes becomes difficult to distinguish between reactive and malignant mesothelial populations. IHC can be a valuable adjunct to determining the origin of cells [10]. In routine pathology practice, four primary sites, the breast, lung, gastrointestinal tract, and female genital tract must be ruled out first. The most frequently used markers for the primary site of origin are TTF1 and Napsin A for the lung, GATA 3, ER, and Mammoglobin for the breast, PAX 8 and WT1 for the ovary, CK7, CK 20, and CDX2 for the Gastrointestinal tract [9]. [Figure 1 and 2]

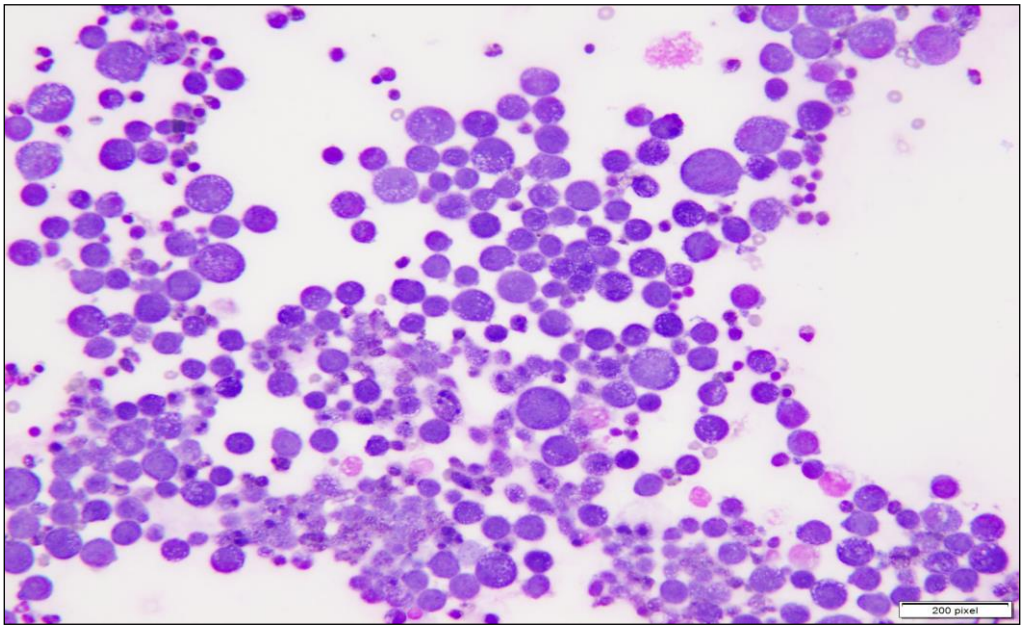

Fig 1a: Diff Quick Stained Slides of Pleural Effusion in a Female Patient at 40x Showing Two Cell Population of Cells with High N/C Ratio and Nuclear Pleomorphism.

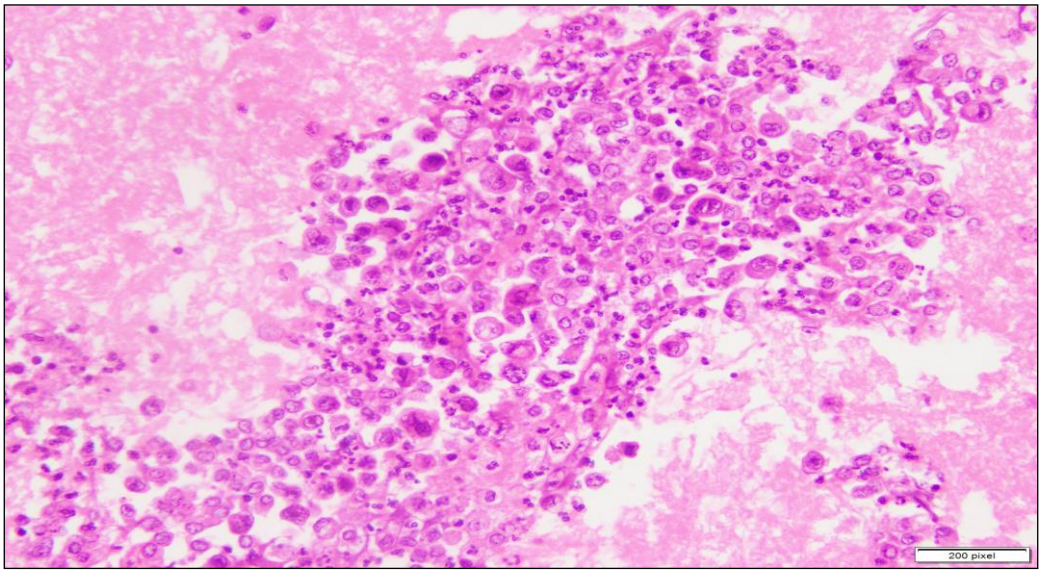

Fig 1b: Cell Block Showing Glandular Structures

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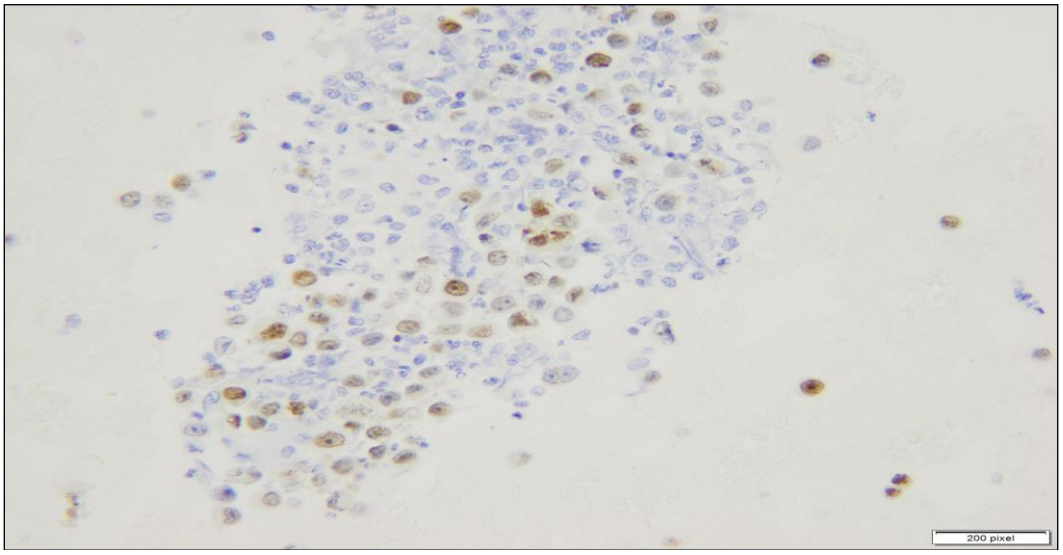

Fig 1c: GATA3 Positive Cells in Cell Block

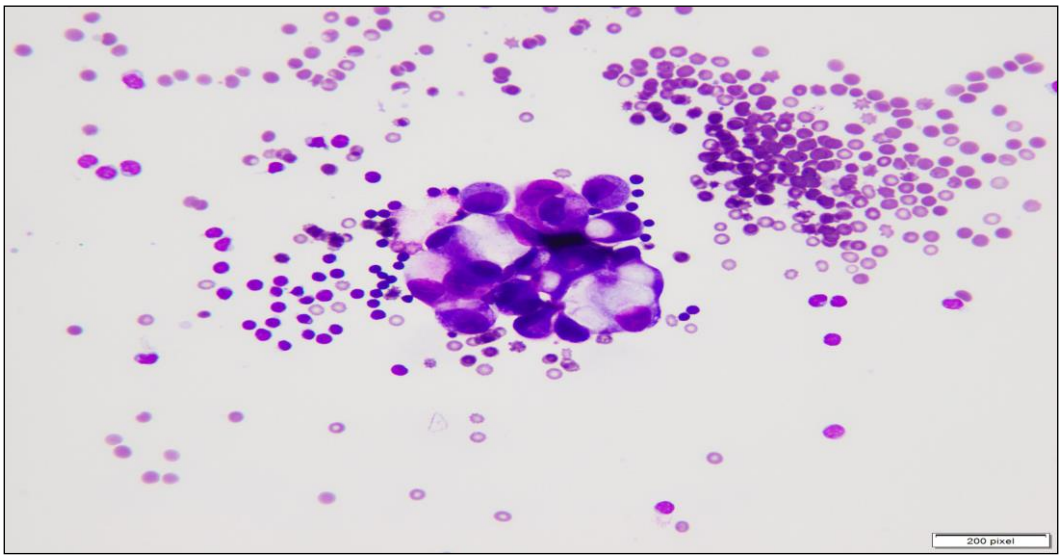

Fig 2a: Diff Quick Stained Slides of Pleural Effusion in a Male Patient at 40x Showing Group of Cells with Multivacoulation, High N/C Ratio and Pleomorphism

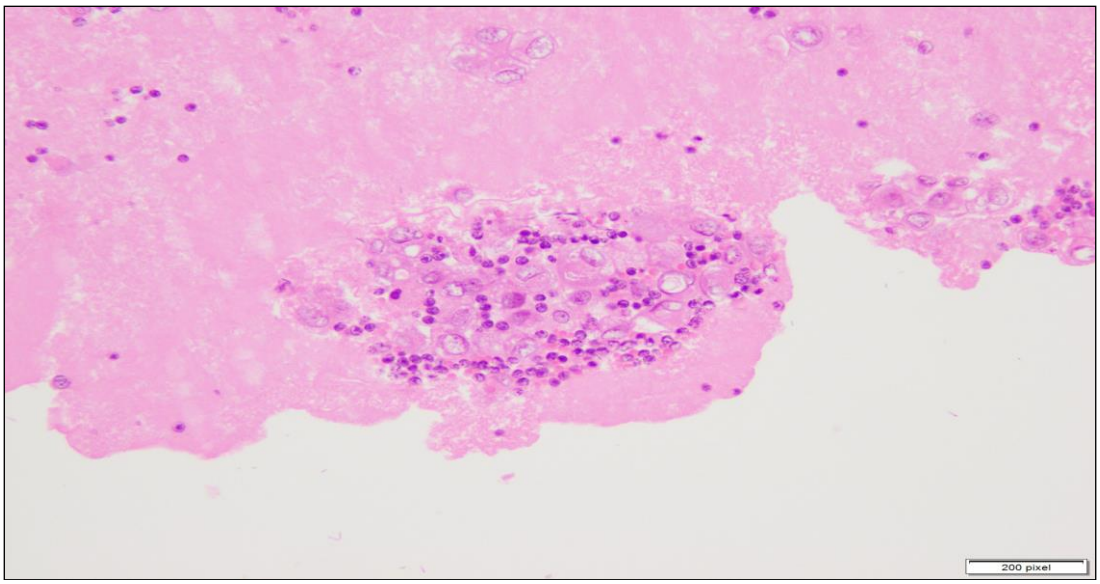

Fig 2b: Cell Block Showing Groups of Atypical Cells

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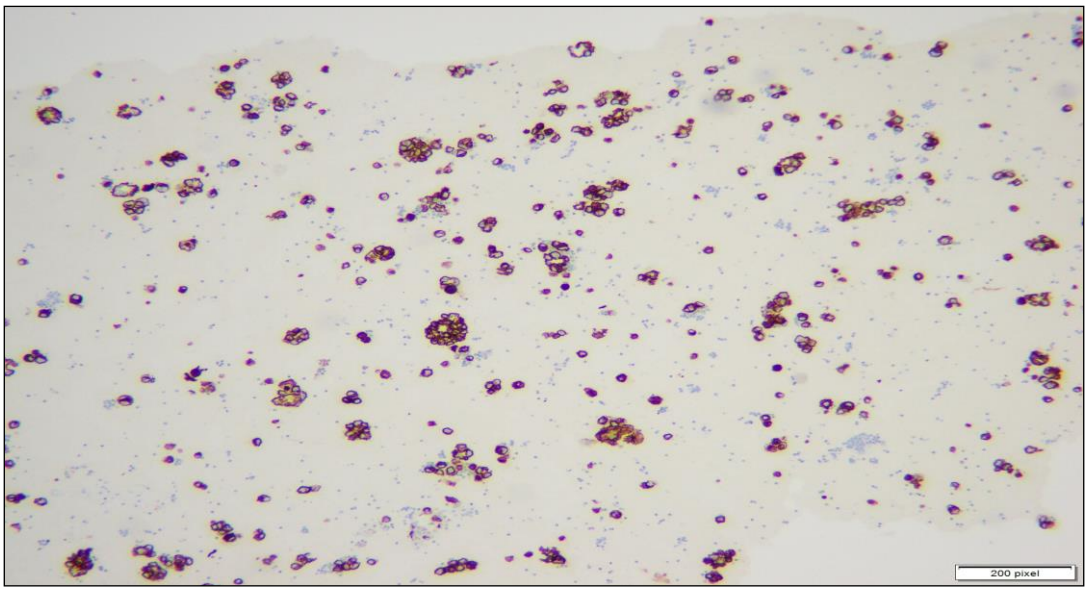

Fig 2c: CK7 Positive in Malignant Cells Sparing Background Mesothelial Cells

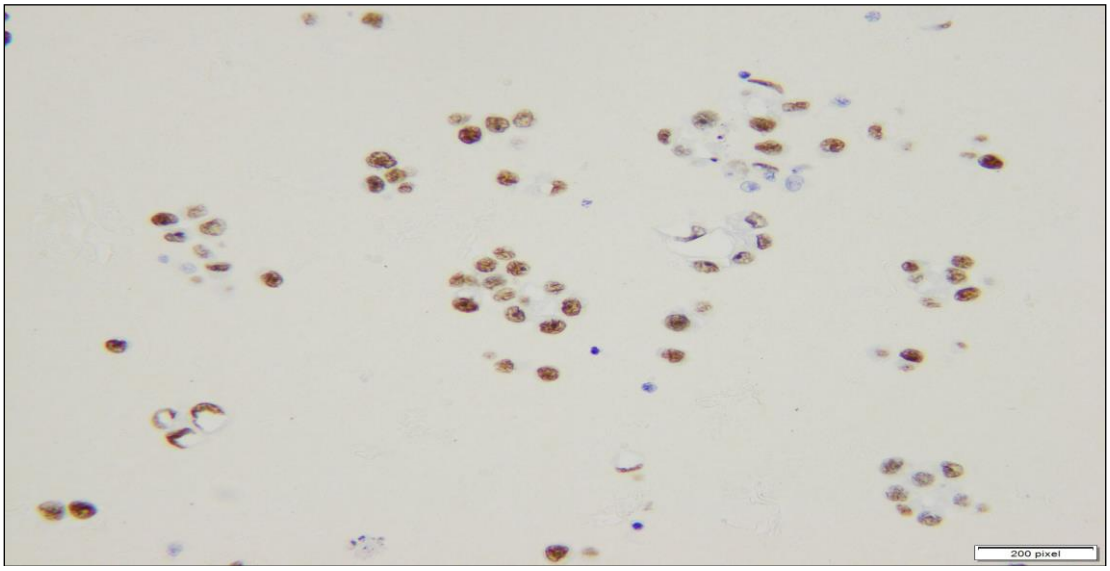

Fig 2d: TTF1 Positive Malignant Cells

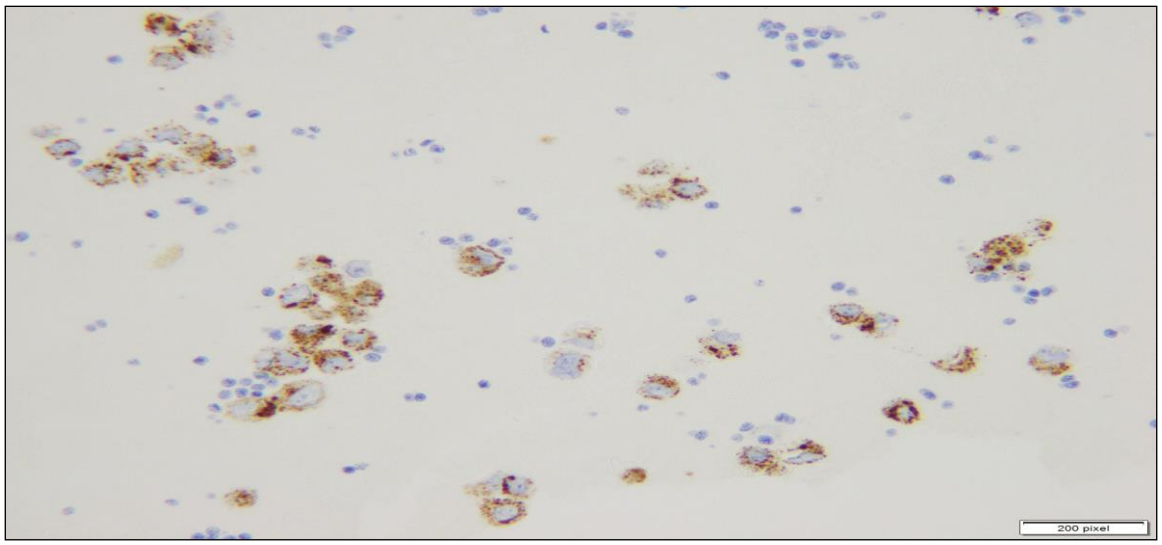

Fig 2e: Napsin A Positive Atypical Cells

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In our study, the most common type of metastatic malignancy in peritoneal effusion in females turned out to be ovarian in origin, which is in concordance with the analysis carried out by Patel TS et al. that depicted the ovary to be the most common site in malignant ascitic fluids followed by gastric malignancies. [10].

A 5-year retrospective study on an international level concluded that in females, the most common cause of malignant pleural cytology is breast, lung, and non-Hodgkins concordant with our study [11].

In 2020, a single-center institutional review of 30085 specimens showed that the most common etiology of metastasis is lung in males and breast in females, leading to pleural effusions and Mullerian tumors in malignant ascites, in keeping with our study. In contrast, hematolymphoid was the most common site in males in peritoneal effusions [12]. However, In a study by Hsu et al. l, lung adenocarcinoma was identified as the most common metastatic malignancy in both genders [13]

In a study of nearly 215 malignant peritoneal effusions by DiBonito et al., gastric carcinomas were the most frequent site of origin in males and ovarian in females. This is similar to our study, which shows that the gastrointestinal tract in males and the breast are the primary sites of origin in females [14].

Overall, in pleural effusions, various studies like Ramzy, koss & Irani et al. reported lung adenocarcinoma as the most common, which is concordant with our study (15,16,17)

Cellblock combined with a judicious immunohistochemical panel according to gender and most common metastatic tumors can be an accurate and affordable method to determine the primary site of cancer. It helps to determine the stage and initiate treatment without any delay. Hence, our study results underscore the necessity of utilizing a panel of markers to prevent misidentification of the primary sites of metastatic carcinoma in effusions.

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