Diagnostic challenges of cytology in the diagnosis of pleural effusion in Lung Adenocarcinoma

***Fatma Ayman Elbadry, Taiseer Refaat Ebrahim, Aziza E. Abde lrahman, Mohamed Ali Alabiad**
Pathology Department, Faculty of Medicine, Zagazig university, Egypt
***Corresponding author:** Fatma Ayman Elbadry
Email: fatmaayman200.patho@gmail.com , **Mobile:** 01286584010

Abstract

Malignant mesothelioma (MM) is a fatal tumor with high mortality rate worldwide. Moreover, lung cancer is a lethal tumor that should be diagnosed early for better prognosis. Despite new treatments, the 5-year survival is less than 12%. Over the past 4 decades, there has been a marked increase in lung adenocarcinoma. It's mandatory to search for new diagnostic markers that could facilitate early diagnosis and differentiating lung adenocarcinoma from mesothelioma in pleural effusion cytology.

KeyWords: Cytology, lung adenocarcinoma, pleural effusion

DOINUMBER:10.48047/NQ.2022.20.19.NQ99400 **NEUROQUANTOLOGY2022;20(19):4349-4359**

Introduction:

In patients with a malignant pleural effusion, lung adenocarcinoma is the most prevalent primary lung cancer. It's classified as non-small cell lung cancer (NSCLC) and is closely linked to smoking. Even though its incidence and mortality have decreased, it continues to be the leading cause of cancer-related deaths globally **(1)**.

In 2018, 2,094,000 new cases of lung cancer were reported worldwide, making it the cancer with the highest incidence, this is according to the most recent GLOBOCAN data. Lung cancer is predicted to have1,369,000 cases, making it the second most prevalent cancer in men, after prostate cancer, and the second most prevalent cancer in women, after breast cancer, with 725,000 cases. The age-standardized cumulative lifetime risk of diagnosis of lung cancer is 3.8% among men while women is 1.77% **(2)**.

North America, Europe, and East Asia have the greatest rates, with China accounting for more than one third of all new cases. The rates are significantly lower in South Asia and Africa **(3)**.

According to the Egyptian National Cancer Registry Program, lung cancer is rated as the fourth most frequent malignant tumor among men representing 5–7% of all malignant tumors **(4)**.

In comparison to other forms of lung cancer, lung adenocarcinoma rates began to increase in the 1960s. This is partially a result of the introduction of filter cigarettes. Filters are used to eliminate the larger tobacco smoke particles, which lessens the amount of smoke that settles in the larger airways. To get the same amount of nicotine, however, smokers must breath more deeply, which increases particle buildup in the tiny airways where adenocarcinoma frequently develops **(3)**.

Adenocarcinoma is the commonest lung cancer subtype **(5)**, it accounts for half of all lung cancer diagnoses, and its occurrence is on the rise**(6).** This is assumed to be attributable to a rise in the number of women who smoke, who are more prone to acquire adenocarcinoma, as well as changes in the design and composition of cigarettes over the previous 50 years. The latter has led to smokers inhaling more deeply, which is likely to enhance carcinogen exposure in peripheral airway cells, where adenocarcinoma develops**(7)**.

Risk factors:

Non-modifiable risk factors:

- ***Age:***

In the US, both men and women received a diagnosis of lung cancer on average at the age of 70. According to estimates, 53% of instances affect people aged 55 to 74, while 37% affect people over 75. In the US, lung cancer is the primary cause of death for men over 40 and women over 59 **(2)**.

Younger persons can also develop lung cancer; in the US, 10% of instances include patients under the age of 55. Younger patients tend to be female, non-smokers, and present with more advanced adeno-carcinoma, according to studies of NSCLC in persons aged 20 to 46 years. This suggests that heritable mutations play a larger role in the disease course than environmental mutagens**(2)**.

- ***Gender:***

Men are more than twice as likely as women to be diagnosed with lung cancer and face death from it globally. Men are more prone to smoke tobacco, which is the main cause of the gender gap. In the developed world, lung cancer rates are falling for men while rising for women, because as women were later to historically adopt, and then cease, tobacco smoking **(8)**. Cigarette smoking and lung cancerrelative risk estimates for the major histological types from a pooled analysis of case – control studies.

Modifiable risk factors:

- ***Smoking:***

Tobacco use is the major cause of lung cancer death, accounting for 70 percent of lung cancer deaths in men and over 55 percent of lung cancer deaths in women **(9)**.

At least 73 recognized carcinogens, such as benzo [α] pyrene**(3, 10),**NNK,1,3-butadiene and a radioactive isotope of polonium,polonium-210 **(10)** are present in cigarette smoke.

According to new research, smoking filtered cigarettes reduces tar absorption while increasing nitrosamine consumption. It could be one of the key variables in the pathologic transformation of squamous cell carcinoma to adenocarcinoma**(11)**.

Nonsmokers who have cohabited with a smoker had a 24% increased chance of developing lung cancer **(12)**.

- ***Air pollution:***

It was showed that the prolonged exposure to outdoor polluted air produced by factories , vehicles and indoor air pollution as cooking fumes, or formaldehyde from decoration is one of the important environmental risk factors for lung cancer **(13)**.

According to preliminary data, burning wood, charcoal, dung, or agricultural residue for cooking and heating increases the risk of lung cancer by polluting indoor air **(14)**.

It is estimated that 1-2% of lung cancer cases are caused by outdoor air pollution **(3)**. According to a previous study, the principal carcinogenic agents in automobile exhaust are mono-nitrogen oxides such as nitric oxide and nitrogen dioxide. And according to the findings, There was a dose-response association between gaseous nitric oxide concentrations and lung cancer **(11)**.

- ***Diet:***

After decades of research on diet and lung cancer, many specific micronutrients thought to have anticarcinogenic activity, such as retinol and beta carotene, have been found. Most of the micronutrients are common in fruits and vegetables. Intake of more fresh fruits and vegetables may decrease the risk of lung cancer**(11)**.

- ***Alcoholism:***

Heavy drinking increases the risk of many chronic diseases. Alcoholics are those with an alcohol use disorder (AUD), characterized by excessive consumption of alcohol, alcohol abuse behavior and/or dependence. In addition, they often have micronutrient deficiencies due to poor diet, which increases their risk for lung cancer and other chronic diseases. Heavy drinking was reported to be a possible risk factor for lung cancer, although smokers also tend to be drinkers **(15).**

- ***Occupational exposure:***

Occupational exposures play a crucial part in the genesis of lung cancer, and lung cancer risk is higher among workers in a variety of sectors and occupations **(16)**.

Twelve occupational exposure factors have been identified by the International Agency for Research on Cancer as carcinogenic to the human lung (aluminum production, arsenic, asbestos, bis-chloromethyl ether, beryllium, cadmium, hexavalent chromium, coke and coal gasification fumes, crystalline silica, nickel, radon, and soot) **(11)**.

The breakdown of radioactive radium, which is itself a decay product of uranium found in the Earth's crust, produces radon gas, an odourless and colourless gas. The radioactive materials ionise genetic material, leading to alterations that can occasionally result in cancer. In the USA, radon is the second-most frequent cause of lung cancer**(17)**.

Lung adenocarcinomas in pleural effusion cytology:

Cytologic features used to diagnose adenocarcinoma included single or small clusters of cells with enlarged nuclei, cytoplasmic vacuoles, and occasional signet ring cell morphology **(18)**.

There may be a hollow sphere of cells. On smear preparation, the empty space is seen in the central part of the cluster, and two different layers of cells are also identified. In the cell block section, the hollow sphere is seen as a central clear space with surrounding cells resembling a glandular appearance **(19)**.

There is great heterogeneity in the cytomorphology of lung adenocarcinomas, depending on the predominant histologic pattern. The malignant cells can be arranged in flat sheets with or without acinar formations, densely packed solid masses, true papillae with fibrovascular cores, or small, tight micropapillary balls. These patterns

are generally better appreciated on cell block preparations. Scattered isolated tumor cells are also frequently present **(20)**.

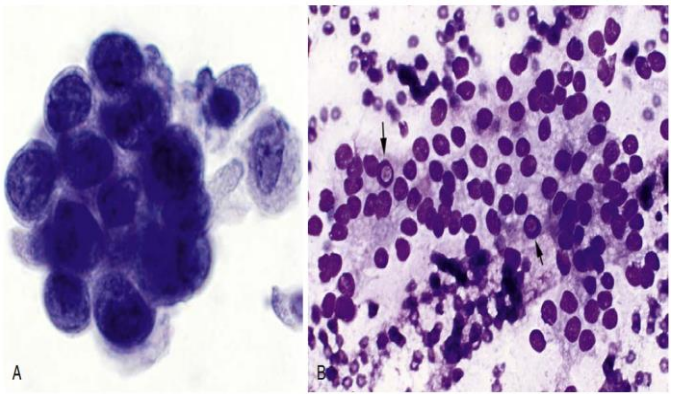

Figure 1: Adenocarcinoma (Lepidic predominant). (A) Nonmucinous. These cells are hyperchromatic and have a high nuclear-to-cytoplasmic ratio, with scant microvacuolated cytoplasm. No clues suggest a lepidic predominant pattern (bronchoalveolar lavage, Papanicolaou stain). (B) Mucinous, the flat honeycomb-like sheet of these uniform tumor cells and intranuclearpseudoinclusions (arrows). (smear, Romanowsky stain) **(20)**.

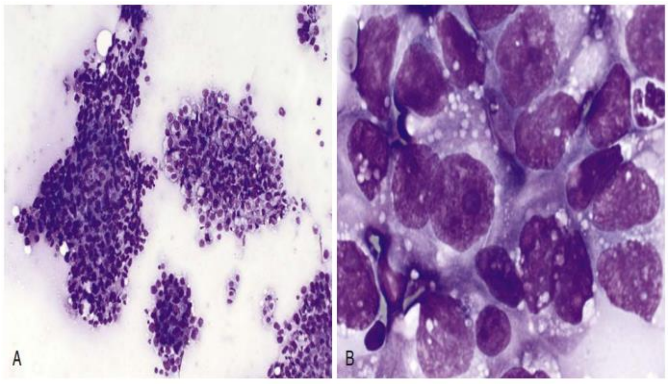

Figure 2: Adenocarcinoma (Solid predominant), (A) These malignant cells are arranged in large, densely packed, solid clusters (Romanowsky stain). (B) High magnification of this tumor reveals marked nuclear enlargement, with irregular nuclear contours and prominent nucleoli. Note the microvacuolated cytoplasm, a characteristic feature of adenocarcinomas as compared with squamous cancers (Romanowsky stain)**(20)**.

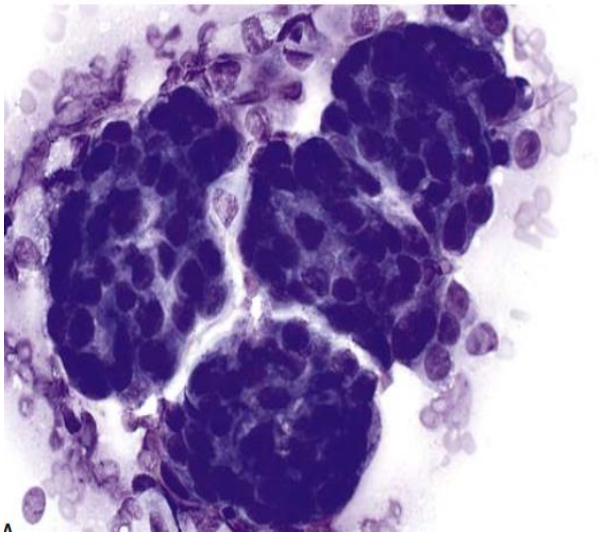

Figure 3: Adenocarcinoma (Micropapillary predominant). (A) The cells are arranged in tight balls without a fibrovascular core (smear, Romanowsky stain**) (20).**

The cells may be present in single layers admixed with many mesothelial cells. Discrete recognizable malignant cells are also seen. Cell cannibalism is frequently noted in malignant effusion. There may be frequent bi-and multinucleation. The individual cells are relatively large, with moderately pleomorphic nuclei and prominent nucleoli. The cytoplasm of the cells shows single to multiple large vacuoles. The vacuoles often push the nucleus to the periphery of the cell. Vacuoles may not contain mucin. However, they often contain glycogen and are Periodic acid–Schiff (PAS) positive**(19)**.

Other NSCLC in pleural effusion cytology:

Lung squamous cell carcinoma in pleural effusion cytology specimens:

The cytomorphology of classical well-differentiated squamous cell carcinoma is characterized by dense cytoplasm, distinct cell borders, and occasional keratinization, it is usually singly lying polygonal, spindle, or tadpole-shaped squamous cells with orangeophilic cytoplasm in (PAP smears) indicating keratinization. The background usually shows inflammatory exudate,

necrosis, and nuclear debris as a result of central cavitation of these tumours, The tumour cells in contrast to ADC cells show hard edges, dense cytoplasm, and hyperchromatic nuclei with clumped pyknotic nuclear chromatin**(21)**.

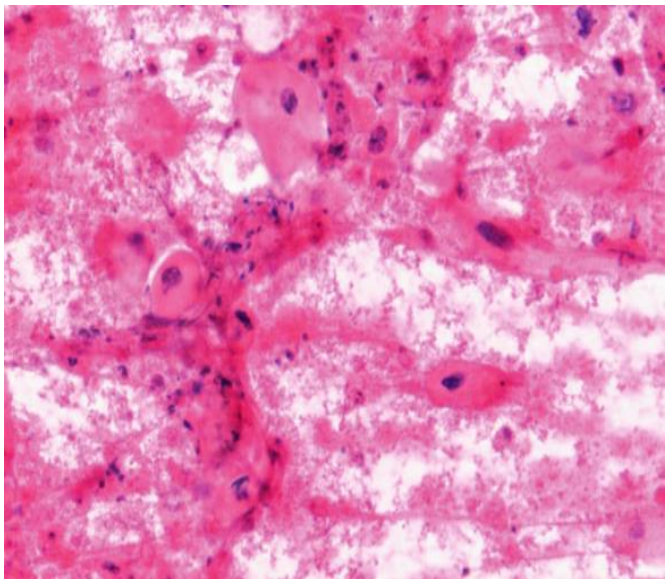

Figure 4: Well-differentiated squamous cell carcinoma. Smear is composed of mainly dispersed, often elongated or spindle-shaped cells with dense cytoplasm and keratinization. Nuclei are often pyknotic or hyperchromatic with angulated contours (H&E) **(22).**

Large cell carcinoma in pleural effusion cytology specimens

Large cell carcinoma is an undifferentiated non-small cell malignancy that accounts for about 9% of all lung cancers. It is a diagnosis of exclusion based on the absence of squamous, glandular, or small cell differentiation**(23).**

The majority of large cell carcinoma cases are located in the peripheral part of the lung**(19)**.

Cytologically, a diagnosis of malignancy is rarely difficult. Large cell carcinoma cells are often arranged in large, syncytial-like sheets of crowded, overlapped cells. There is no apparent cytoplasmic differentiation. The nuclei are large and either round or markedly irregular, with irregularly distributed, coarse chromatin. Nucleoli are usually quite prominent**(24)**.

Large cell carcinoma usually shows CK7 positivity; the squamous cell markers CK5 and p63 are usually negative and TTF1 is commonly negative **(22)**.

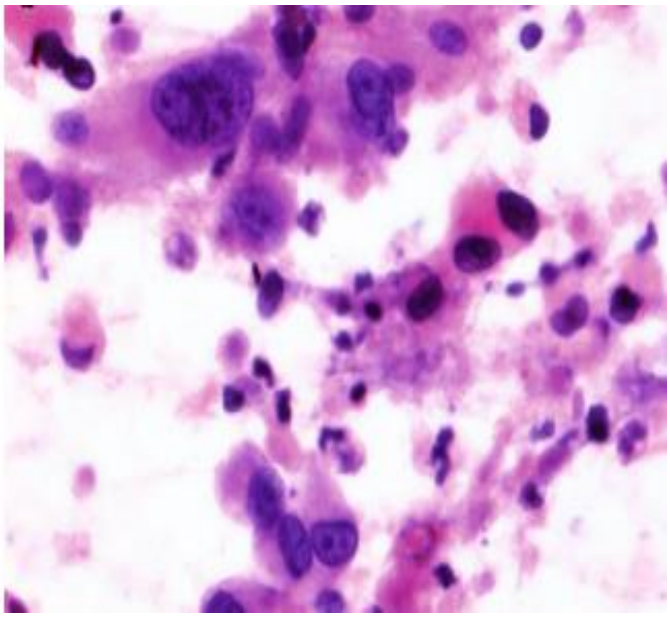

Figure 5: Large cell carcinoma. Loose aggregates of pleomorphic cells with markedly enlarged nuclei, hyperchromatic nuclei with irregular chromatin, and nucleoli (H&E) **(22)**.

Small cell lung cancer (SCLC) in pleural effusion cytology:

Small cell carcinoma in metastatic pleural effusion reveals small to medium-sized cells with very scant cytoplasm and nuclei showing coarsely granular chromatin with occasional small molding nucleoli, the cytoplasm is sparse, but in some types it might be more abundant, which might create a diagnostic problem in the differentiation from other lung carcinomas **(22).**

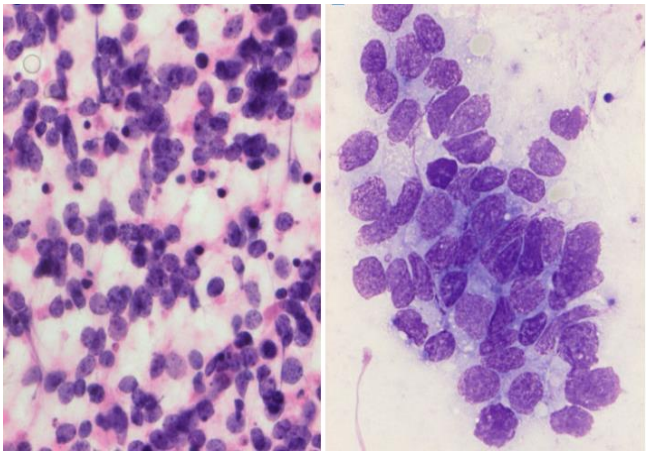

Figure 6: Small cell carcinoma. Small cells with high N/C ratio apoptosis, "salt and pepper" chromatin texture, and nuclear moulding (H&E) **(22)**.

Cells of small cell carcinomas could be easily detected using immunocytochemistry where it showed positive for neuron-specific enolase (NSE), chromogranin, and synaptophysin**(19)**.

Diagnostic challenges of cytology in the diagnosis of pleural effusion

A cytological smear has a 60% diagnosis rate, It will assist with pleural effusion diagnosis as well as determining the stage of cancer and assessing the severity of the condition **(25, 26).**

Conventional cytological smears, on the other hand, can present diagnostic challenges because they have a lower diagnostic yield, especially with poorly differentiated malignancies **(27).**

Diagnostic challenges with benign effusion:

Reactive mesothelial proliferations mimic mesothelioma (or metastatic carcinoma) because they exhibit high cellularity, numerous mitotic figures, cytologic atypia, necrosis and formation of papillary groups **(28)**.

The wide range of sensitivity (high false-negative rate) is likely attributable to sampling rather than assessment, but one must admit that there is a significant overlap in atypical features and immunoreactivity between benign reactive and malignant mesothelial cell proliferations. Several cytologic features (scalloped borders of cell clumps; intercellular windows with lighter, dense cytoplasm edges; and low nuclear to cytoplasmic ratios) are common between reactive and malignant epithelioid mesothelial cells **(28)**.

Mesothelial cells that are actively dividing in response to injury or stimulus are often called reactive mesothelial cells. Reactive mesothelial cells show a spectrum of changes that can resemble malignancy. These changes may include a coarsened chromatin pattern, enlarged nucleoli and mitotic figures. In these cases, careful analysis of cytomorphology, together with clinical details and immunocytochemistry, should resolve the diagnostic **dilemma(29)**.

Two of the most common difficulties encountered in serous fluid cytology are differentiating mesothelial cells from macrophages and bland adenocarcinoma cells. The former usually have more bean-shaped nuclei with a smooth small nucleus, and their cytoplasm shows less dense microvacuolation. The latter can be particularly difficult when a background reactive mesothelial cell population cannot be appreciated. In both of these circumstances, it may be necessary to resort to immunocytochemistry to clarify the nature of the cells **(30)**.

Immunocytochemistry can be used to identify mesothelial cells, which usually stain positive with the immunocytochemical markers calretinin, thrombomodulin, and CK 5/6. Benign mesothelial cells also stain positive with desmin. However, they generally do not stain with epithelial membrane

antigen (EMA) or, if they do, it will be weak staining **(29)**.

Diagnostic challenges with malignant mesothelioma in pleural effusion cytology

As a rule, the epithelioid type is diagnosed by cytology. Some advocate the use of cytology for diagnosis, while others state it has limited use as they believe morphological confirmation of tissue invasion is essential for robust diagnosis. This is somewhat surprising since many other malignant tumors are diagnosed on cytological grounds without identifying tissue invasion **(30)**.

On the other hand, part of the difficulty in diagnosis is that mesothelioma cells are often bland in fluid samples and they are more likely to be overlooked than metastatic adenocarcinoma cells **(30)**.

Often, serous cavity fluids in patients with MM will contain a large number of malignant cells with well-developed cytomorphologic characteristics. However, diagnostic issues arise when the amount of lesional cells is low or when cytomorphology overlaps significantly with metastatic adenocarcinoma (a common scenario)**(31)**.

The diagnosis of mesothelioma in cytological preparations is challenging even for experienced cytologists, as in some cases malignant mesothelial cells may look very similar to reactive mesothelial cells. This similarity often leads to a false negative diagnosis. The next diagnostic challenge after identifying malignant cells is establishing their mesothelial origin, as distinguishing malignant mesothelioma and metastatic malignancy is important for therapeutic purposes. We routinely employ immunocytochemistry to resolve these diagnostic dilemmas, and we will look at this later in this chapter **(29)**.

In mesotheliomas, the malignant cells look like native mesothelial cells. If the cells are sufficiently well differentiated to be recognized as mesothelial, it becomes difficult to call them malignant based on morphology. On the other hand, atypia in reactive proliferations may alert the pathologist to the diagnosis of malignant mesothelioma in an appropriate clinical setting. An important clue to the diagnosis of malignant mesothelioma is the presence of “more and bigger cells in more and bigger clusters.” High cellularity with many large aggregatessuggests malignancy, especially in pleural effusions. Benign effusions show fewer mesothelial cells and smaller, less complex groups. Cell-in-cell arrangements are more common in malignant proliferations. Macronucleoli favor malignancy **(32)**.

Malignant mesothelial cells may simulate reactive mesothelial cells. The mesothelioma cells are in large groups, with moderate nuclear pleomorphism and often varies **(19)**.

Another challenge for the cytologist is to differentiate adenocarcinoma from mesothelioma. Cells from both mesothelioma and adenocarcinoma may show almost similar morphology. In this situation, the cytologist should depend on (1) clinical history; (2) radiological features; and (3) ancillary tests, such as ICC **(19)**.

Diagnostic challenges with metastatic lung carcinoma in pleural cytology:

Adenocarcinoma challenges

When mesothelial cells come together in groups, slit-like spaces called “windows” are seen

between adjacent cells. They are the reflection of the long slender microvilli on their surface. The presence of "windows" in a group of cells is a clue regarding their mesothelial origin. However, it should be noted that "windows" can also be seen between adenocarcinoma cells. Two different studies showed that "windows" are detected in 13 and 44% of adenocarcinoma cases, and in most cases, slit-like spaces between neoplastic cells represented mucin secretion**(33, 34)**.

Mesothelial groups typically have flower-like, knobby contours. In contrast, adenocarcinoma cells form groups with common borders, such as cell balls and papillae. Knobby-contoured cell clusters are a feature of mesothelial cells both seen in reactive proliferation and in malignant mesotheliomas. However, not infrequently (36.9%), they may also be present in adenocarcinomas (Fig.38). On the other hand, in some cases of mesothelial hyperplasia, papillary structures may develop, creating a pitfall in the differential diagnosis **(32)**.

Squamous cell carcinoma challenges

Conventional morphological criteria for squamous cell carcinoma are keratinization and intercellular bridges; however, these features become obscure in poorly differentiated tumours **(35)**.

Morphologically clear-cut glandular and squamous differentiation is indeed recognized in only 50%–60% of NSCLC cases. The remaining cases require ancillary testing in mucin stains and/or immunophenotyping by lineage-specific markers such as thyroid transcription factor-1 (TTF-1), Napsin A, p40 and cytokeratin 5/6 (CK5/6) **(21)**.

Large cell carcinoma challenges

It is a diagnosis of exclusion based on the absence of squamous, glandular, or small cell components, and should not be used for small biopsy or cytology specimens; rather, it is restricted to resection specimens after the tumor is thoroughly sampled**(23)**.

Because of the poorly differentiated nature and variable cytology of tumors that ultimately are diagnosed as large cell carcinoma on resection, the cytologic differential diagnosis is broad. It includes a wide variety of poorly differentiated malignant tumors such as sarcomas, metastatic carcinomas from other sites, melanoma, and high-grade lymphoma**(20)**.

The term*large cell carcinoma* is only used in the absence of positivity for TTF-1, p63/p40, neuroendocrine markers, and mucin staining **(20)**.

Immunostaining, such as vimentin, is helpful for the confirmation of sarcoma. Malignant melanoma cells are positive for HMB45 **(19)**.

Small cell carcinoma challenges:

In most small cell carcinomas, the cytoplasm is sparse, but in some types, it might be more abundant, which might create a diagnostic problem in terms of differentiating these small cell carcinoma subtypes from other lung carcinomas. In addition, combined tumors of small cell carcinoma with components of adenocarcinoma or squamous carcinoma may cause difficulties in the examination of FNA smears **(22)**.

Immunocytochemistry for CD56, TTF1, and synaptophysin may be helpful as the majority of small cell carcinomas are positive for CD56 and

TTF1 with around 50 % positive for synaptophysin**(30)**.

Despite the potential of small cell carcinoma to rapidly degenerate in fluids, it tends to retain its reactivity to immunomarkers**(32)**.

Neuroendocrine markers: The combination of chromogranin, synaptophysin, and CD56 represents the best balance between sensitivity and specificity **(32)**.

References:

1. **Li C., and Lu H. (2018).** Adenosquamous carcinoma of the lung. OncoTargets and therapy, 11, 4829.
2. **Thandra K. C., Barsouk A., Saginala K., Aluru J. S., and Barsouk A. (2021).** Epidemiology of lung cancer. Contemporary Oncology/Współczesna Onkologia, 25(1), 45-52.
3. **Mustafa M., Azizi J., Iiizam E. L., Azizan N., Sharifa S., and Abbas S. A. (2016).** Lung Cancer: Risk Factors, Management, And Prognosis. IOSR Journal of Dental and Medical Sciences, 15, 94-101. doi: 10.9790/0853-15100494101
4. **Eid S. S. M., Ahmed A. R. H., Abdelgawad M. I. K., Mohamed A. E.-S., Mohamed A. O., Mohamed D. O., . . . Saad-Eldeen H. M. (2020).** Mutation of EGFR in Non-small Cell Lung Cancer, a Regional Study in Upper Egypt. Cancer Research Journal, 8(1), 1.
5. **Bray F., Ferlay J., Soerjomataram I., Siegel R. L., Torre L. A., and Jemal A. (2018).** Global cancer statistics 2018: GLOBOCAN estimates of incidence and mortality worldwide for 36 cancers in 185 countries. CA Cancer J Clin, 68(6), 394-424. doi: 10.3322/caac.21492
6. **Barta J. A., Powell C. A., and Wisnivesky J. P. (2019).** Global epidemiology of lung cancer. Annals of global health, 85(1).
7. **Succony L., Rassl D., Barker A., McCaughan F., and Rintoul R. (2021).** Adenocarcinoma Spectrum Lesions of the Lung: Detection, Pathology and Treatment Strategies. Cancer Treatment Reviews, 102237.
8. **Pesch B., Kendzia B., Gustavsson P., Jöckel K. H., Johnen G., Pohlabeln H., . . . Brüske I. (2012).** Cigarette smoking and lung cancer—relative risk estimates for the major histological types from a pooled analysis of case–control studies. International journal of cancer, 131(5), 1210-1219.
9. **O'Keeffe L. M., and Taylor G. (2018).** Smoking as a risk factor for lung cancer in women and men: a systematic review and meta-analysis. 8(10), e021611. doi: 10.1136/bmjopen-2018-021611
10. **Hecht S. S. (2012).** Lung carcinogenesis by tobacco smoke. International journal of cancer, 131(12), 2724-2732.
11. **Mao Y., Yang D., He J., and Krasna M. J. (2016).** Epidemiology of Lung Cancer. Surg Oncol Clin N Am, 25(3), 439-445. doi: 10.1016/j.soc.2016.02.001
12. **Ridge C. A., McErlean A. M., and Ginsberg M. S. (2013).** Epidemiology of lung cancer. Paper presented at the Seminars in interventional radiology.
13. **Raaschou-Nielsen O., Bak H., Sørensen M., Jensen S. S., Ketzel M., Hvidberg M., . . . Loft S. (2010).** Air pollution from traffic and risk for lung cancer in three Danish cohorts. Cancer Epidemiol Biomarkers Prev, 19(5), 1284-1291. doi: 10.1158/1055-9965.epi-10-0036

14. **LIM W. Y., and Seow A. (2012).** Biomass fuels and lung cancer. Respirology, 17(1), 20-31.
15. **Repp B., Jamieson P., Sun C., and Takata Y. (2022).** Alcoholism and Risk of Lung Cancer in Observational Studies: A Systematic Review. Current Developments in Nutrition, 6(Supplement_1), 944-944. doi: 10.1093/cdn/nzac067.064
16. **Cogliano V. J., Baan R., Straif K., Grosse Y., Lauby-Secretan B., El Ghissassi F., . . . Wild C. P. (2011).** Preventable exposures associated with human cancers. J Natl Cancer Inst, 103(24), 1827-1839. doi: 10.1093/jnci/djr483
17. **Choi H., and Mazzone P. (2014).** Radon and lung cancer: assessing and mitigating the risk. Cleve Clin J Med, 81(9), 567-575.
18. **Dorry M., Davidson K., Dash R., Jug R., Clarke J. M., Nixon A. B., and Mahmood K. (2021).** Pleural effusions associated with squamous cell lung carcinoma have a low diagnostic yield and a poor prognosis. Translational Lung Cancer Research, 10(6), 2500-2508.
19. **Dey P. (Ed.). (2022).** Diagnostic Cytology: Jaypee Brothers Medical Publishers (P) Ltd
20. **Edmund S. Cibas M., and Barbara S. Ducatman M. (Eds.). (2021).** CYTOLOGY: DIAGNOSTIC PRINCIPLES AND CLINICAL CORRELATES, FIFTH EDITION: Elsevier.
21. **Jain D., and Bubendorf L. (2022).** Can cytology reliably subtype non-small cell lung carcinomas? Cytopathology, 33(1), 8-13.
22. **Domanski H. A. (2013).** Atlas of fine needle aspiration cytology: Springer Science & Business Media.
23. R**ossi G., Mengoli M. C., Cavazza A., Nicoli D., Barbareschi M., Cantaloni C., . . . Pelosi G. (2014).** Large cell carcinoma of the lung: clinically oriented classification integrating immunohistochemistry and molecular biology. Virchows Archiv, 464(1), 61-68. doi: 10.1007/s00428-013-1501-6
24. **French C. A. (2009).** Chapter 2 - Respiratory Tract. In E. S. Cibas & B. S. Ducatman (Eds.), Cytology (Third Edition) (pp. 65-103). Philadelphia: W.B. Saunders.
25. **Biswas B., Sharma S. K., Negi R. S., Gupta N., Jaswal V. M. S., and Niranjan N. (2016).** Pleural effusion: Role of pleural fluid cytology, adenosine deaminase level, and pleural biopsy in diagnosis. Journal of Cytology/Indian Academy of Cytologists, 33(3), 159.
26. **Rivera M. P., Mehta A. C., and Wahidi M. M. (2013).** Establishing the diagnosis of lung cancer: Diagnosis and management of lung cancer: American College of Chest Physicians evidence-based clinical practice guidelines. Chest, 143(5), e142S-e165S.
27. **Carretero R. G., Manotas-Hidalgo M., Brugera M. R., and Mohamed L. E. B. (2016).** Pleural effusion of malignant aetiology: cell block technique to establish the diagnosis. Case Reports, 2016, bcr2016215140.
28. **Husain A. N., Colby T. V., Ordóñez N. G., Allen T. C., Attanoos R. L., Beasley M. B., . . . Dacic S. (2018).** Guidelines for pathologic diagnosis of malignant mesothelioma 2017 update of the consensus statement from the International Mesothelioma Interest Group. Archives of pathology & laboratory medicine, 142(1), 89-108.
29. **Shambayati B., and Trust A. a. S. P. s. H. F. (2011).** Cytopathology. Oxford University Press
30. **Sheaff M. T., and Singh N. (2012).** Cytopathology: an introduction: Springer Science & Business Media.
31. **Ali S. Z., and Cibas E. (2012).** Serous cavity fluid and cerebrospinal fluid cytopathology (Vol. 11): Springer Science & Business Media.

32. **Davidson B., Firat P., & Michael C. W. (2018).** Serous effusions: etiology, diagnosis, prognosis and therapy: Springer.
33. **Yu G. H., Sack M. J., Baloch Z. W., DeFrias D. V., and Gupta P. K. (1999).** Occurrence of intercellular spaces (windows) in metastatic adenocarcinoma in serous fluids: a cytomorphologic, histochemical, and ultrastructural study. Diagnosticcytopathology,20(3), 115-119.
34. **Murugan P., Siddaraju N., Habeebullah S., and Basu D. (2008).** Significance of intercellular spaces (windows) in effusion fluid cytology: a study of 46 samples. Diagnostic cytopathology, 36(9), 628-632.
35. **Jain D., Mathur S. R., Guleria R., and Iyer V. K. (2014).** Utility and pattern of positivity of p40 in the diagnosis of squamous cell carcinoma of the lung by cytology: the first study on fine needle aspiration smears. Cytopathology, 25(5), 330-335. doi: 10.1111/cyt.12105