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Lung cancer cytology: potential pitfalls- a review

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ABSTRACT

Lung Cancer, a cancer that forms in tissues of the lung, usually in the cells lining air passages, has traditionally been classified into two major types: non-small cell lung cancer (NSCLC) and small cell lung cancer (SCLC). About 85 percent of all lung cancers are identified as non-small cell, and approximately 75 percent of these are metastatic or advanced at diagnosis. Recent findings have changed our understanding of the disease, and today distinct molecular subsets of lung cancer have been identified that can be classified by a biomarker profile of a patient's tumor. In spite of advances in early diagnosis and standard treatment, non-small cell lung cancer is regularly analysed at advanced stages and has a poor prognosis. The treatment and prevention of lung cancer are major needs that can most likely be enhanced by a better understanding of the molecular process in cancer and development of cancer. However, significant progress is underway in both the prevention and treatment of lung cancer. Lung cancer therapy has now emerged as a "role model" for precision cancer medicine. Cytology is increasingly being used in the evaluation of lung lesions. There are several potential pitfalls encountered in the evaluation of respiratory cytology specimens, making interpretation of respiratory cytology challenging.

Keywords: Lung Cancer, non-small cell lung cancer, small cell lung cancer, Cytology, potential pitfalls.

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INTRODUCTION

The lungs are a pair of sponge-like cone-shaped organs in the chest, which are a part of our respiratory system. The left lung is smaller because the heart occupies space on the left side. Furthermore, the lungs are slightly different on each side; the right lung has three lobes, whereas the left lung has two lobes. The lungs are covered by a thin membranous covering called "pleura," which protects and helps the lungs to move back and forth as they expand and contract during breathing. A thin, dome-shaped muscle below the lungs, called "diaphragm," separates the chest from the abdomen. The diaphragm moves up and down during breathing forcing air in and out of the lungs [1].

Main function of the lungs is to exchange gases between the air we breathe and the blood. When we breathe in (inhale), oxygen enters into our body through the lungs and when we breathe out (exhale) carbon dioxide is sent out of our body. Air enters the lungs through the nose or mouth via windpipe (trachea), which divides into the right and left lungs. These airways are called "bronchi" (singular, bronchus). Inside each lung, the bronchus divides into smaller tubes, the "secondary bronchi," which further sub-divide into smaller branches called bronchioles. At the end of the bronchioles are tiny air sacs known as "alveoli," which receives many tiny blood vessels. These tiny alveoli perform the function of exchange of gases [1].

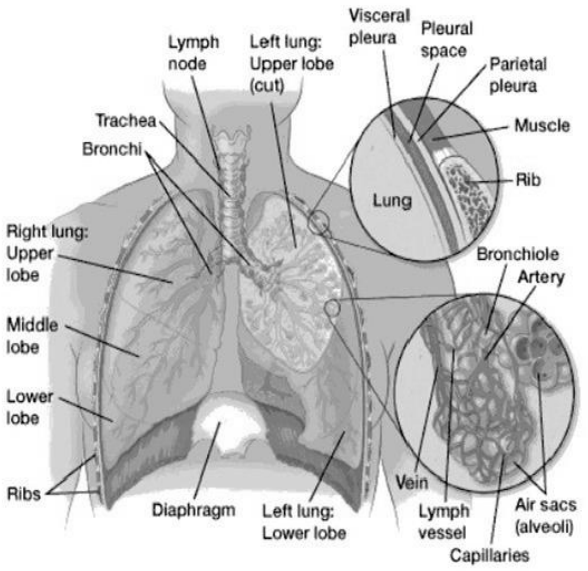

Figure 1: Anatomy of lungs

Lung cancer appears to arise in the bronchi in response to repetitive carcinogenic stimuli, inflammation, and irritation. Disruption of cell development occurs in the mucosal lining and progresses to elevate or erode the basal

membrane. The tumor then spreads throughout the lung and will eventually metastasize to the lymph nodes and other parts of the body. There are four main histological classifications of lung cancer: squamous cell carcinomas, adenocarcinomas, small cell carcinomas, and large cell carcinoma. Because the behavior and management of squamous cell carcinoma, adenocarcinoma, and large cell carcinomas are very similar, they are often grouped together as non-small cell lung cancer (NSCLC) in contrast to small cell lung cancer (SCLC), which has a distinct natural history and management. Squamous cell carcinoma is most commonly found in men and shows the strongest relationship with smoking. It arises in the larger and more central bronchi and tends to spread locally, later metastasizing to other types but grows rapidly at its site of origin. Adenocarcinoma was previously known as the most common type of lung cancer in women and non-smokers; however, the incidence of adenocarcinoma has increased in the last two decades, and it is now the most common histological subtype in both males and females. The reason for the increasing incidence of adenocarcinoma is not well understood, but may be related to changing patterns of smoking. Cell carcinomas are likely to be undifferentiated squamous cell and adenocarcinoma. They usually consist of large polygonal cells with vesicular nucle [2].

Lung cancer is the most common cause of cancer mortality worldwide, accounting for 25% of all cancer deaths with an incidence rate of 1.2 million people per year [3].The vast majority (85%) of cases of lung cancer are due to long-term exposure to tobacco smoke. About 10–15% of the cases occur in people who have never smoked. These cases are often caused by a combination of genetic factors and exposure to radon gas, asbestos, or other forms of air pollutants, including second-hand smoke [4].

PATHOLOGY OF LUNG CANCER

The most common primary malignant tumors occurring in the respiratory tract arise from the endobronchial epithelium. They are subdivided into small cell lung cancers (+/- 25%) and non-small lung cancers (+/- 75%).These are further subdivided into squamous cell carcinoma, adenocarcinoma, and undifferentiated large cell carcinoma. The cancer growth frequently leads to endobronchial obstruction, which represents the classical indication for palliative endobronchial brachytherapy. In selected early cases cancer growth is very limited and superficial and is confined to the dimensions of the bronchial wall. These cases may be considered for definitive treatment with curative intent with brachytherapy playing a major role, often in combination with photodynamic therapy. Lung metastases from other primary sites such as e.g. renal cell carcinoma, breast cancer, soft tissue sarcoma, osteosarcoma, or malignant melanoma only represent an indication for intraluminal brachytherapy if there is endobronchial obstruction caused by intraluminal tumour growth, which is rather rare [5].

It is generally accepted that the pathogenesis of human cancer involves the accumulation of multiple molecular abnormalities over time. Those alterations lead to acquired cellular capabilities that can be classified in the following six functional sets: a) self-sufficiency in growth signals due to mutations in proto- oncogenes, b) insensitivity to antiproliferative signals as a result of mutations affecting the tumour suppressor genes, c) evading of apoptosis by up-regulation of antiapoptotic or down-regulation of proapoptotic molecules, d) limitless replicative potential due to the activation of telomerase, e) sustained angiogenesis and f) capability for tissue invasion and capability for dissemination into distant sites (metastasis) [6]. Those molecular alterations can occur at the level of gene up-regulation or down-regulation, DNA sequence changes (point mutations), loss of heterozygosity (i.e., deletion of one copy of allelic DNA sequences), DNA segment amplification or whole chromosome gains or losses with the simultaneous genomic instability and alterations in microsatellite DNA [7, 8].

The carcinogens from the tobacco or other environmental pollutants lead to the loss of the 3p21.3 allele in thousands of cells on different sites of the respiratory epithelium. Later, the tumor suppressor genes located in the 3p21.3 chromosome arm become haplo-insufficient. The next hit occurs in genes that are critical for the cell proliferation, such as *RB, p53, p16* or other genes either by the mutational inactivation or by the promoter hypermethylation. That permits a clonal outgrowth of the initially transformed cells. Some authors suggest that the molecular pathogenesis differs significantly between SCLC and NSCLC main tumor types[9].It is proposed that during the pathogenesis of the SCLC neoplastic cells arise directly either from normal or hyperplastic epithelial cells without passing through characteristic preneoplastic intermediate pathological stages (parallel theory of lung cancer pathogenesis). On the contrary, the NSCLC pathogenesis is accompanied with sequential morphological changes [10].

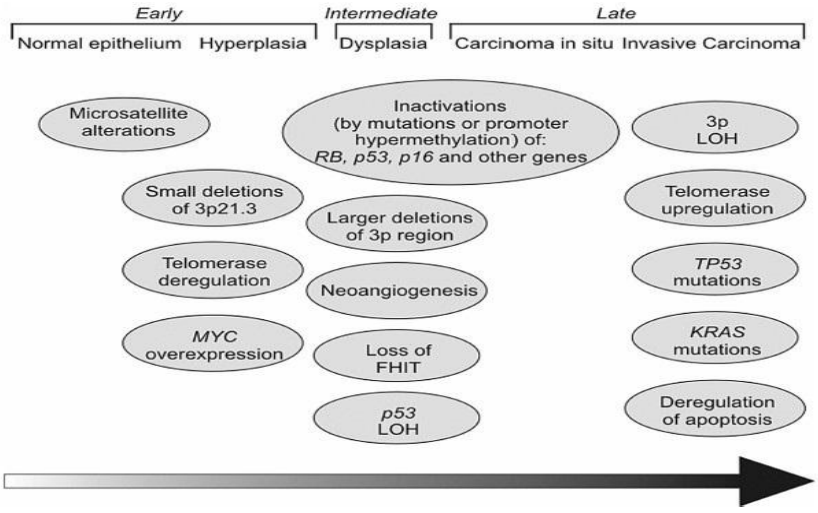

Figure 2: Main molecular abnormalities occurring during lung cancer pathogenesis

RISK FACTORS FOR LUNG CANCER [11, 12]

Tobacco smoking – Smoking causes almost 9 out of 10 lung cancers. Compared with nonsmokers, smokers are 25 times more likely to develop lung cancer. Second-hand smoking Breathing in other people's tobacco smoke (passive or second-hand smoke) can cause lung cancer. People who have never smoked but who have been frequently exposed to second-hand smoke are 20–30% more likely to develop lung cancer than nonsmokers who have not been exposed. People who have never smoked and have not been around second-hand smoke have about a 0.5% risk of developing lung cancer.

Exposure to asbestos – People who are exposed to asbestos have a greater risk of developing cancer.

Radon- Radon is a radioactive gas that is produced during the breakdown of naturally-occurring uranium in soil and rocks, particularly granite. It can pass through from the ground into homes and buildings. Exposure to excessive levels of radon is thought to be a significant causative factor in patients with lung cancer who have never smoked.

Genetic susceptibility-It is thought that some people may be more likely to develop lung cancer based on their genetic makeup. Having a family history of lung cancer, or other types of cancer, increases the risk of developing lung cancer to some degree. In people who are genetically predisposed to lung cancer, smoking further increases the risk.

Exposure to other elements – Contact with the processing of steel, nickel, chrome and coal gas may be a risk factor. Exposure to radiation and other air pollution, such as diesel particulate matter, also increases the risk of lung cancer.

Personal history – The risk of developing lung cancer is increased if you have been previously diagnosed with another lung disease such as lung fibrosis, chronic bronchitis, emphysema or pulmonary tuberculosis.

Family history – Having a family member diagnosed with lung cancer increases the risk.

Older age – Lung cancer is most commonly diagnosed in people aged 60 years and older, though it can occur in younger people.

DIAGNOSIS OF LUNG CANCER [13,14,15,16]

Chest x-ray: A chest x-ray is painless and can show tumours 1 cm wide or larger. Small tumours may not show up on an x-ray or may be hidden by other organs within the chest cavity. After a chest x-ray, you may need more detailed tests.

CT scan: A CT (computerised tomography) scan uses x-ray beams to take many pictures of the inside of your body and uses a computer to compile them into one detailed, cross-sectional picture. It can detect smaller tumours than those found by chest x-rays, and provides detailed information about the tumour, the lymph nodes in the chest and other organs. CT scans are usually done at a hospital or a radiology clinic. You may be asked to fast (not eat or drink) for several hours before the scan to make the scan pictures clearer and easier to read. Before the scan, you will be given an injection of dye into a vein in your arm. This dye is known as the contrast and it makes the pictures clearer. The dye may make you feel hot all over, and leave a bitter taste in your mouth, and you may feel a sudden urge to pass urine. The CT scanner is a large, doughnut-shaped machine. You will lie flat on a table that moves in and out of the scanner. The scan itself takes 10–20 minutes, but you will also need to prepare and then wait for the scan. While a CT scan can be noisy, it is painless.

PET scan: A PET (positron emission tomography) scan is a specialised imaging test, which is available at most major hospitals. It is used to stage lung cancer, usually after the diagnosis is confirmed. Before the scan, a small amount of radioactive glucose solution will be injected into a vein, usually in your arm. This makes cancer cells show up brighter on the scan because they take up more of the glucose solution than normal cells do. You will be asked to sit quietly for 30–90 minutes while the glucose solution moves around your body, then you will lie on a table that moves through the scanning machine. The scan will show 'hot spots' that have taken up the high levels of radioactive glucose.

Lung function test (spirometry): this test checks how well the lungs are working. It measures how much air the lungs can hold and how quickly the lungs can be filled with air and then emptied. You will be asked to take a full breath in and blow out into a machine called a spirometer.

Sputum cytology: A sputum cytology test examines a sample of mucus (sputum) from your lungs. Sputum is different to saliva as it contains cells that line the respiratory passages. To collect a sample, you will be asked to cough deeply and forcefully into a container. This can be done in the morning at home. The sample can be refrigerated until you take it to your doctor, who will send it to a laboratory to check under a microscope for abnormal cells.

Biopsy: If a tumour is suspected after an x-ray or CT scan, a sample of tissue will be taken to confirm whether you have lung cancer. The sample can be collected in different ways.

CT-guided core biopsy: This is used to obtain cells when the tumour is in the outer parts of the lungs. A CT scan will be used to guide the needle through your chest wall and into position. A small piece of tumour can usually be removed with the needle. A core biopsy is done in a hospital or radiology clinic. You will be observed for a few hours afterwards, as there is a small risk this procedure can damage the lung.

Bronchoscopy: A bronchoscopy allows the doctor to look inside the large airways (bronchi). A bronchoscope is passed down your nose or mouth, down your windpipe (trachea) and into the bronchi. The bronchoscope is a flexible tube with a light and lens for viewing. It may feel uncomfortable, but it shouldn't be painful. You will be given sedation to help you relax or a general anaesthetic, and the back of your throat will be sprayed with a local anaesthetic to numb it. If the tumour is near your main respiratory tract, the cells can be collected using the washing or brushing technique. During 'washing', a small amount of fluid is injected into the lung and withdrawn for further examination. 'Brushing' involves the use of a brush-like instrument to remove some cells from the bronchi.

Endobronchial ultrasound: An endobronchial ultrasound (EBUS) is a type of bronchoscopy that allows the doctor to see cancers deeper in the lung. Samples may also be taken from a tumour or a lymph node in the middle of the chest or next to the airways. In other cases, samples can be taken from the outer parts of the lung.

Mediastinoscopy: This is not used as often as other biopsy methods, but is sometimes used if a sample is needed from the area between the lungs (mediastinum). A small cut is made in the front of the neck and a rigid tube is passed down the outside of the trachea. Some tissue is removed from the mediastinal lymph nodes. A mediastinoscopy is usually a day procedure but you may need to stay overnight in hospital for observation.

Thoracoscopy: A thoracoscopy is an operation used to take a tissue sample (biopsy). It is usually done if other tests are unable to provide a diagnosis. For a thoracoscopy you will have a general anaesthetic. The surgeon will make one or two small cuts in your chest and insert a surgical instrument called a thoracoscope, which has a camera attached. You will wake up with a drain coming from your side and stay in hospital for a few days.

TREATMENT OF LUNG CANCER

There are several types of treatment for lung cancer. You and your health care team will determine which choice is best for you after reviewing the type of lung cancer, the stage of your disease, your symptoms, and other health problems you may have. Lung cancer treatments are continuing to improve as new discoveries are made so it is important to thoroughly discuss all your options with your clinicians.

Surgery: Surgery provides the best chance of a cure for patients with stage I or stage II NSCLC. It involves removal of the tumour through resecting one or more lobes of the lung (lobectomy or bi-lobectomy) or through removal of the whole lung (pneumonectomy). The surgery is challenging and should only be undertaken in a unit with an appropriate level of expertise. The postoperative mortality rate is about 5% and patients can experience significant complications after surgery, including haemorrhage, respiratory failure, infection and arrhythmias. It is of vital importance, therefore, that patients are carefully selected and are fit for surgery[17].

Radiotherapy: Radiotherapy uses x-rays to kill or damage cancer cells. It can be used to treat all types of lung cancer. It may be offered on its own or in combination with surgery or chemotherapy. Radiotherapy with curative intent is the choice for patients with stage I to III NSCLC who are not suitable for surgery. Traditionally the total radiotherapy dose is calculated and the dose is then divided into smaller portions known as fractions. These fractions are given once daily over three to four weeks. Recent research has focused on delivering the fractions more quickly in a schedule known as CHART (continuous, hyperfractionated, accelerated radiotherapy), CHART has a small but significant survival advantage over traditional radiotherapy[18].

Chemotherapy: Chemotherapy is the treatment of cancer with anti-cancer (cytotoxic) drugs. The aim is to destroy cancer cells while causing the least possible damage to normal, healthy cells. Chemotherapy is usually delivered through an intravenous drip. Each chemotherapy treatment is called a cycle and is followed by a rest period to give your body time to recover. The number of treatments you have will depend on the type of lung cancer you have and how well your body is coping with the side effects.combination of a single third generation medicine (eg, vinorelbine, gemcitabine or docetaxel) plus a platinum-based drug (carboplatin or cisplatin).Platinum-based chemotherapy combinations are the standard first-line treatment for advanced NSCLC[19].

Lobectomy: A lobectomy, the surgical removal of a single lobe, is the optimal procedure for the management of early stage disease. For patients who are medically fit for surgery, a lobectomy is preferred over a sublobar resection[20].

Sublobar resection: A sublobar resection is recommended over non-surgical interventions for patients with stage I NSCLC who cannot tolerate a lobectomy or anatomic pulmonary resection due to co-morbid disease or decreased pulmonary function. A sublobar resection can either be a segmentectomy, which is removal of one or more anatomical segments, or a non-anatomical wedge resection. While lobectomy is currently the standard of care for all medically fit stage I patients, recent evidence suggests that sublobar resection may be appropriate for some patients with a small (1 cm or less) peripheral nodule, or for patients with bronchioloalveolar carcinoma histology [21, 22].

Targeted therapy: New types of drugs known as targeted therapy or personalised medicine target specific mutations within cancer cells and often work by blocking cell growth. Targeted therapy drugs are generally used for advanced NSCLC (stage IV) or if the cancer has come back (recurred).

Epidermal Growth Factor Receptor (EGFR) Inhibitors: Epidermal growth factor receptor, a member of the HER/Erb-B family of receptor tyrosine kinases, mediates cell proliferation, differentiation, survival, angiogenesis, and migration.This molecule consists of an extracellular domain that binds EGF, transforming growth factor alpha (TGF-a), and other growth factors; a short transmembrane region; and an intracellular tyrosine kinase domain. Ligand binding leads to homodimerisation of EGFR or heterodimerisation of EGFR with another receptor of the Erb-B family and phosphorylation of specific EGFR tyrosine residues. Tyrosine-phosphorylated receptors then recruit intracellular signaling proteins, converting extra-cellular signals to intra-cellular signal transduction events. Epidermal growth factor receptor is expressed more abundantly in malignant than in normal tissues, and has been shown in 40%-80% of NSCLCs [23, 24].There are two main types of EGFR inhibitors that are available currently: *(i)* monoclonal antibodies (MAbs) directed at the extra-cellular domain of the receptor, and *(ii)* small molecule intra-cellular EGFR tyrosine kinase inhibitors. The MAbs include drugs, like cetuximab, panitumumab (ABX-EGF) and matuzumab (EMD 72000). The later two are currently in early phases of investigation[25]. The kinase inhibitors gefitinib and erlotinib have been studied extensively throughout the world in different population groups.Patient characteristics from these trials that have been associated with responsiveness to EGFR inhibitors include adenocarcinoma histology, female sex, absence of history of smoking, and Asian ethnicity[26].

Anti-EGFR MAbs: Antibodies generally have the advantages of less frequent administration, induction of receptor down regulation, the potential to engage the host immune response in direct tumour cell cytotoxicity, and a favourable toxicity profile (notably the absence of gastrointestinal adverse effects). Antibodies specific for EGFR are among the first targeted therapies to demonstrate effectiveness in treating cancer, including NSCLC[27].

Cetuximab: The monoclonal antibody, cetuximab, is an IgG1 MAb that binds specifically and with high affinity to the extra-cellular portion of the EGFR and acts as a competitive antagonist, preventing endogenous ligand

binding. This EGFR blockade affects all cellular functions implicated in tumour biology, such as cell proliferation, cell survival, DNA repair, tumour angiogenesis, cell motility, and cell invasion. Internalisation of EGFR may lead to down regulation of cell surface receptors and reduced receptor signaling [28].

EGFR TKIs: Small-molecule TKIs are another class of EGFR-targeted agents. The TKIs can be orally administrated, have a rapid onset of action, and potentially have better tumour penetration than mAbs.Among drugs of this class, the two most extensively evaluated in NSCLC are gefitinib and erlotinib. Both have demonstrated single agent activity in NSCLC[29].

Gefitinib: Gefitinib, an anilinoquinazoline, was the first TKI selective for EGFR evaluated in NSCLC. It is orally active and given once daily. Gefitinib Monotherapy, Two randomised, double-blind trials of gefitinib monotherapy in daily doses of 250 mg or 500 mg given to patients with advanced NSCLC who had previously received chemotherapy regimens [30].

Erlotinib Like gefitinib, erlotinib is an orally-active, EGFR-specific quinazoline TKI that demonstrated anti-tumour activity in xenograft models.14,15,18,62 In addition to the first-line treatment for advanced pancreatic cancer, erlotinib is currently approved for second-line treatment of locally advanced or metastatic NSCLC [31]**.**

EGFR and Tumour Angiogenesis (Combination treatment): New blood vessel formation is required for the growth and progression of most tumours. The EGFR is also involved in angiogenesis: the EGFR ligands, EGF and TGF-a, induce angiogenesis, and TGF-a promotes the expression of VEGF, which induces vascular growth and vascular cell permeability,providing a strong rationale for combined anti-VEGF/anti-EGFR therapy. The VEGF expression is upregulated in many tumours, resulting in an imbalance between pro- and antiangiogenic factors in the tumour microenvironment, promoting vascularisation and growth[32].

Rexinoid Agonists: Lung cancers are known to be defective in retinoic acid signalling with low levels of RARba and RXRa. Patients with low levels of RXRa have a shorter survival than those with normal levels. Bexarotene is a rexinoid agonist that produced long disease stabilization (> 3 months in 36%) in Phase I single agent trials and produced long time to progression in a randomized maintenance study [33]**.**

Herbal Remedy for Lung Cancer: Medicinal plants play an important role in the management of cancer especially in developing countries where resources are meager. Herbal remedies are focused in the pharmaceutical industry to evolve a safe route for liver disorders. Therefore, hepatoprotective natural products are, *Withania somnifera, Pomegranate, Curcuma longa, Green tea, Lithosperum radix, Chinies herbs, Juzen-Taiho-To(Japanes medicine), Tinospora cardifolia, Ziziphus nummularia* etc.[34].

CONCLUSION

Lung cancer appears to arise in the bronchi in response to repetitive carcinogenic stimuli, inflammation, and irritation. Disruption of cell development occurs in the mucosal lining and progresses to elevate or erode the basal membrane. Tremendous progress in understanding the pathogenesis of lung cancer has occurred over the past century. There are several types of treatment for lung cancer. You and your health care team will determine which choice is best for you after reviewing the type of lung cancer, the stage of your disease, your symptoms, and other health problems you may have.

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