https://doi.org/10.48047/AFJBS.6.15.2024.3878-3894

African Journal of Biological Sciences

Journal homepage: http://www.afjbs.com

Research Paper Open Access

Use of Cytokeratin 5/6 and Epidermal Growth Factor Receptor for the differential diagnosis of non-small cell lung Carcinomas

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Article History

Volume 6, Issue 15, 2024

Received : 03 May 2024

Accepted : 28 Aug 2024

Published: 07 Sep 2024

doi:10.48047/AFJBS.6.15.2024.3878-3894

Abstract

Background: Non-small cell lung carcinomas represent the most common and the most aggressive entity of lung cancers, with a poor prognosis and decreased survival time. The clinical diagnosis is usually based on histopathology, immunohistochemistry and molecular analysis to describe the precise histological subtype and target therapies. **Patients and Methods**: Immunohistochemistry technical based on monoclonal antibodies of cytokeratin 5/6 and epidermal growth factor receptor was performed using the EnVision+ system on formalin-fixed paraffin-embedded samples for differential diagnosis of non-small cell lung carcinomas. **Results**: The imunostaining of epidermal growth factor receptor and cytokeratin 5/6 conducted on lung adenocarcinomas and squamous cell lung carcinomas sections shows a negative staining of the cytokeratins 5/6 in more than half of adenocarcinomas and an over-expression of the epidermal growth factor receptor in all cases. Only 37% of adenocarcinomas cases have provided a membrane and non-diffuse cytoplasmic immunostaining of cytokeratin 5/6. The patient having squamous carcinoma revealed an intense and diffuse positive staining of cytokeratin 5/6. **Conclusion**: Therefore, differential diagnosis using cytokeratins 5/6 must absolutely be supplemented with other specific markers.

Key words: NSCLC, CK5/6, EGFR, Squamous cell lung carcinoma, Adenocarcinoma.

Introduction

Lung cancer is considered as the most lethal of cancers worldwide. There are different histological types with high prevalence (80-85%) of non-small cell lung carcinomas (NSCLC). The most common histological type is adenocarcinoma with an incidence of 60%, followed by squamous cell carcinoma (≈30%) and large cell carcinoma (≈10%). The diagnosis is often late in 70% of cases and has usually a poor prognosis reducing survival time to 5 years in 15% of patients [1].

When the morphology of NSCLC remains unclear, several markers such as the nuclear tissue-specific transcription factor-1(TTF-1), p63 and cytokeratins (CK) 5, 6 and 7, are used in addition to the histopathological studies. Thus, lung adenocarcinomas are typically positive for TTF-1 and CK7 and negative for p63 and CK5/6. The squamous carcinomas have the opposite profile [2,3].

The cytokeratins are intermediate filaments that represent the basis of human cytoskeleton epithelial cells. This family includes twenty polypeptides classified by their multigenic diversification. The CK5/6 are basic and have an intermediate molecular weight. The CK5 is a basic cytokeratin with a high molecular weight (58kDa), expressed in the basal, intermediate and superficial layers of stratified epithelia. The CK6 is a basic cytokeratin with a high molecular weight (56 kDa), expressed in the proliferative squamous epithelia and often paired with CK16. The CK5/6 are also expressed in keratinizing squamous epithelia (epidermis), nonkeratinizing epithelia (mucosa), basal myoepithelial prostate cells, in breast and salivary glands. They are expressed in benign and malignant skin tumors, squamous mucosa and in the myoepithelial tissues [4-7]. Several immunohistochemistry studies have been performed in patients with squamous carcinomas and revealed a variable expression of epidermal growth factor receptor (EGFR), whereas CK5/6 and p63 are co-expressed in the poorly differentiated

carcinomas and suggest a squamous and metastatic epithelial origin of tumors [9-11]. Thus, many researchers describe the CK5/6 immunostaining as an identification tool of simple and stratified epithelia [4].

The EGFR is a tyrosine kinase receptor expressed in some normal epithelial, mesenchymal and neurogenic tissue. This is a transmembrane receptor with an extracellular ligand binding domain, a transmembrane domain, and intracellular tyrosine kinase and regulatory domains. It induces the cell proliferation signaling pathways and the inhibition of apoptosis. The over-expression of EGFR is implicated in the pathogenesis of many human malignancies, including NSCLC [12]. Some studies reported that metastasis, low chemotherapeutic response and reduced survival are related to the EGFR expression in NSCLC [13-15].

Our objective is to evaluate the efficiency of CK5/6 immunoreaction in the differential diagnosis of lung adenocarcinomas and squamous carcinomas, supplemented by EGFR immunostaining used usually to confirm NSCLC diagnosis.

Patients and Methods

The immunohistochemistry technique was performed on 2µm sections of 48 Algerian patients with lung adenocarcinomas and squamous carcinomas according of ethics and deontology, using the EnVision+ system (Dako) on tumor samples fixed in formalin and embedded in paraffin (FFPE). The slides were deparaffinized using xylene and rehydrated. The antigen retrieval was performed using the sodium citrate buffer (pH9.9) for 5 minutes in a domestic microwave (750W). The CK5/6 primary antibody (Clone D5/16B4; DakoCytomation, 1:50 dilution) was incubated for 1 hour at room temperature. The blocking of the endogenous peroxidase activity was realized after the application of the primary antibody by a pretreatment of the tissue sections with the hydrogen peroxide solution. The EnVision-HRP–enzyme conjugate was applied after a PBS wash during 30 minutes. The chromogen 3,3′-diaminobenzidine (DAB, DAKO) was used as substrate for the EnVision–HRP–enzymes. The

slides were washed quickly in distilled water before counterstaining with the hematoxylin-Eosin (H&E). The sections were mounted and examined with a light microscope (Leica). The immunostaining using the EGFR primary antibody (Clone H11; DakoCytomation, 1:200 dilution) was performed using citrate buffer at pH 6 following the same conditions of CK5/6 immunostaining. Positive control of CK5/6 was represented by squamous cell lung carcinoma tissue and negative control was represented by a lung adenocarcinoma that revealed a positive staining for EGFR primary antibodies.

Results

The immunohistochemistry study has been performed on primary adenocarcinomas tumors and squamous cell lung carcinoma using CK5/6 antibodies. About 37 % of lung adenocarcinomas cases revealed a positive immunostaining of the CK5/6 (Fig. 1a). This previous staining seems to be moderate in all cases, without a diffuse staining pattern. The rest of lung adenocarcinomas samples revealed a negative CK5/6 immunostaining. The CK5/6 immunoreaction is located in the membrane and cytoplasmic region (Fig. 1b) of all positive adenocarcinomas. Application of immunohistochemistry techniques using EGFR antibodies in all lung adenocarcinomas cases revealed a membrane and cytoplasmic EGFR positive immunoreaction with an expression profile characterized by a clear over-expression of EGFR in tumor tissues (Fig. 2). Most of lung adenocarcinomas revealed a negative staining of CK5/6 and a strong positive staining of EGFR in membrane and cytoplasm (Fig. 3). The CK5/6 immunoreaction performed on squamous cell lung carcinoma tissue, revealed a positive immunoreaction for all cases with an intense and diffuse expression pattern in the membrane and cytoplasm (Fig. 4).

Discussion

Target therapies need a precise histologic sebtyping of lung cancers because of the morphological misdiagnosis that seems to be more common in biopsies. This is due to the limited amount of tissue in comparison with the surgical specimens [16].

The immunohistochemical diagnosis of lung tumors is commonly made by using a panel of antibodies that reacts with adenocarcinomas but not with squamous carcinomas. Then, a lot of studies describe a positive CK5/6 staining in all squamous carcinomas studied [2], and a negative staining in all lung adenocarcinomas [11]. These results are in accordance with data

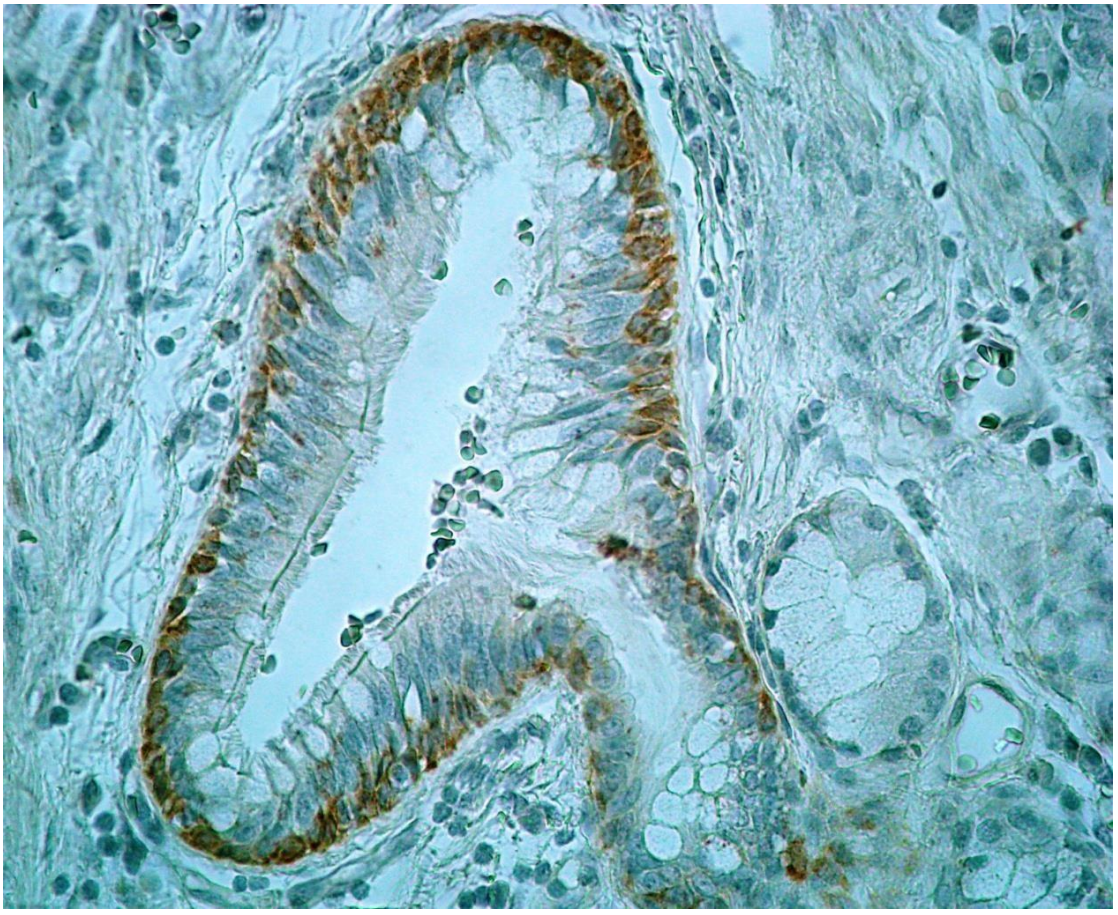

Figure 1a: **Positive CK5/6 immunostaining in lung adenocarcinomas (HE; GX10);**

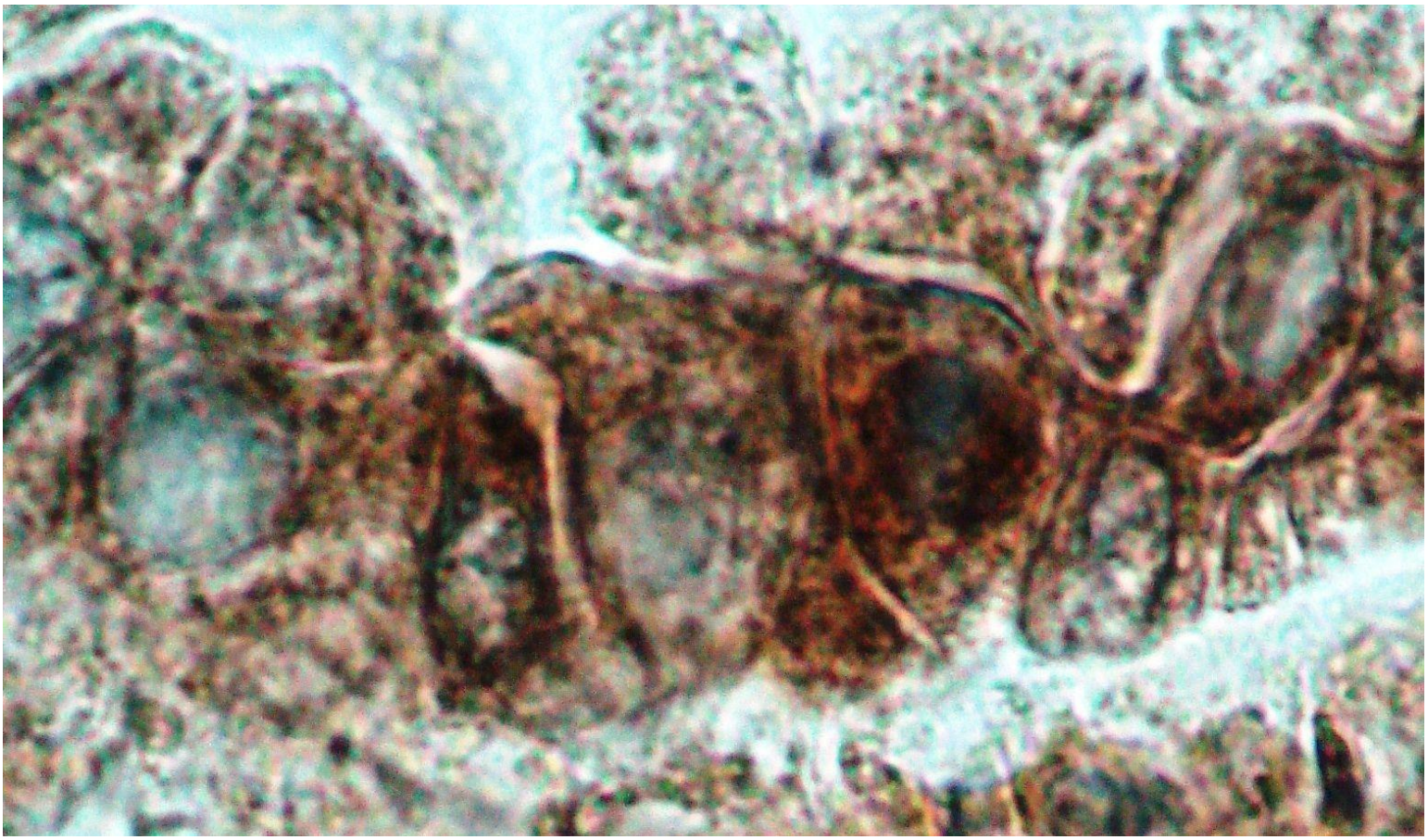

Figure 1b: **Positive CK5/6 immunostaining in lung adenocarcinomas (HE; GX100).**

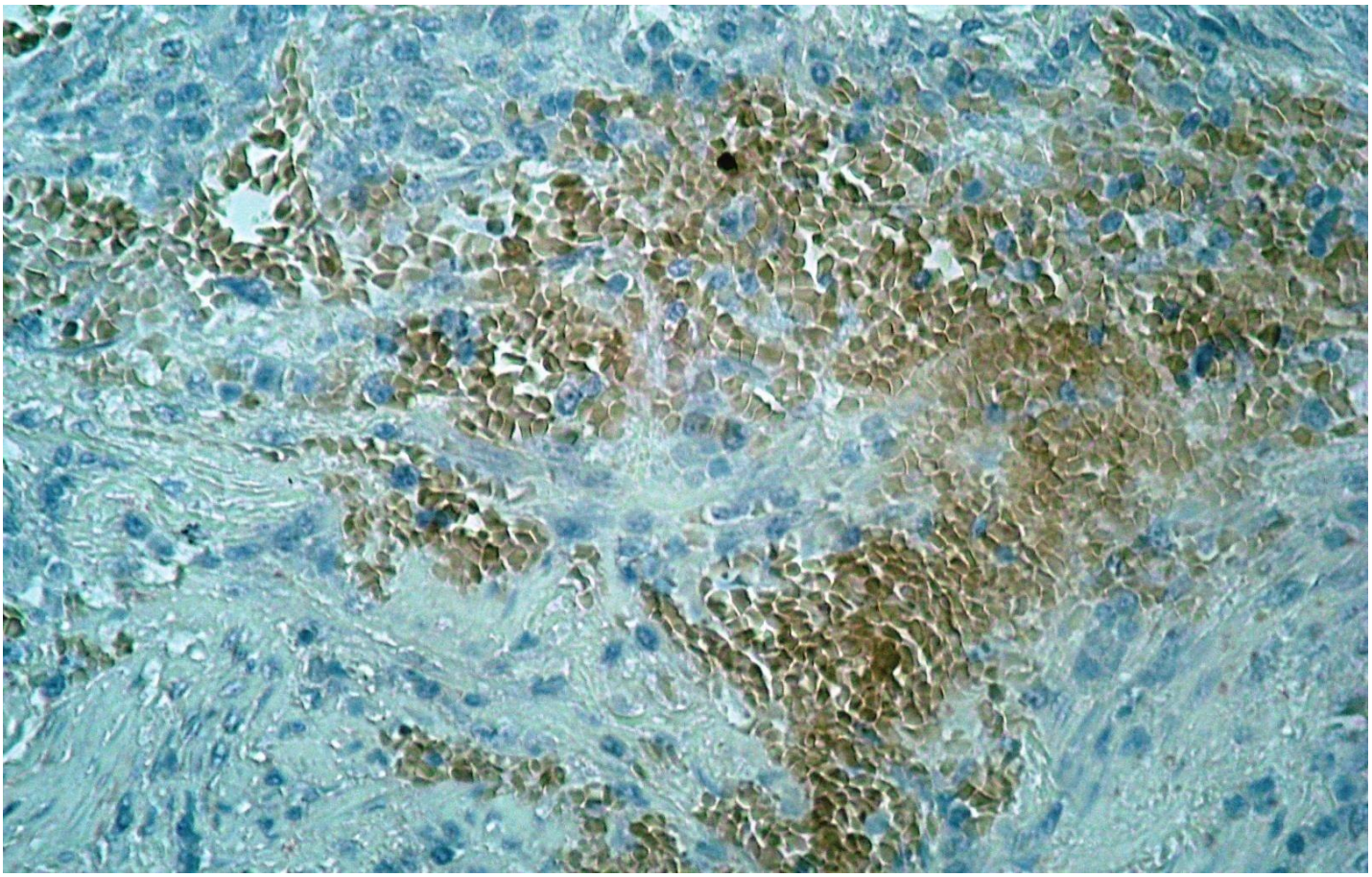

Figure 2: Over-expression of the epidermal growth factor receptor (EGFR) in lung adenocarcinomas (HE; GX40).

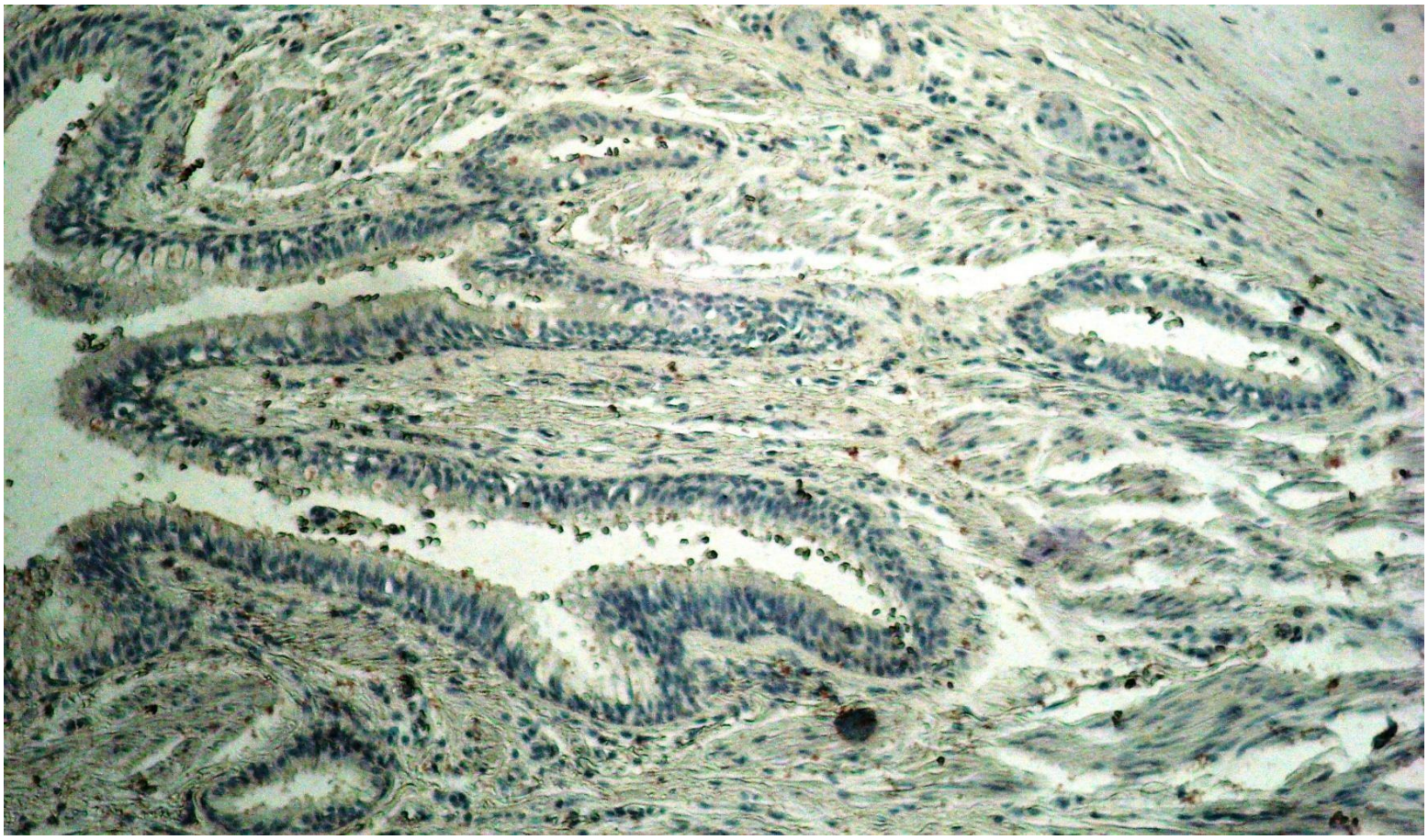

Figure 3: Negative immunostaining of CK5/6 in lung adenocarcinomas (HE; GX10).

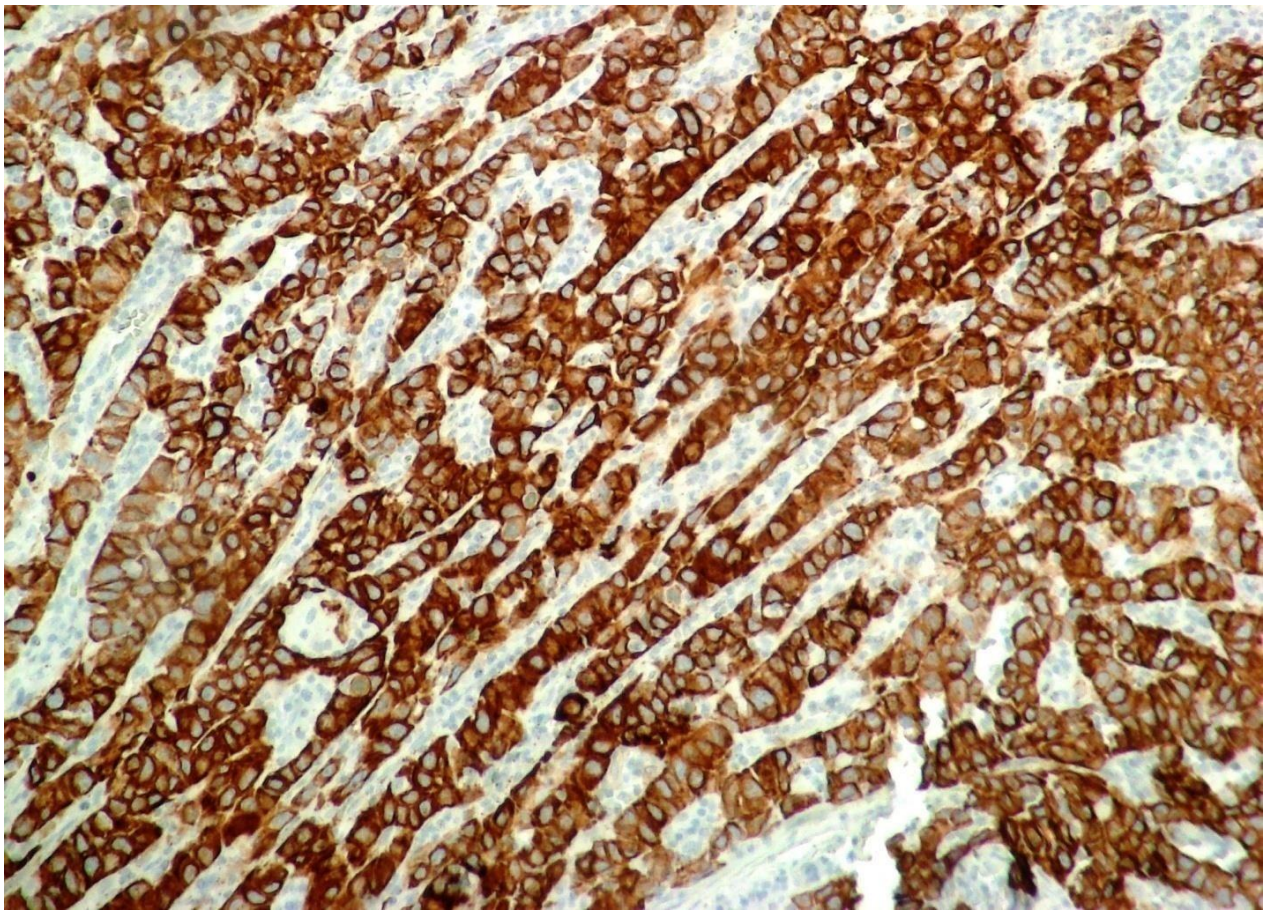

Figure 4: **Positive immunostaining of CK5/6 in patients with squamous lung carcinomas (HE; GX10).**

described by Ordonez *et al*. [21], which describe a total negativity of CK5/6 in lung adenocarcinomas and correlated with the results reported by Cury *et al*.[18]. Thus, CK 5/6 are commonly used in the differential diagnosis of adenocarcinoma and squamous carcinoma [16, 17, 19]. Some other studies reported an immunoreactivity of CK5/6 in the vast majority of malignant mesothelioma but only rarely in pulmonary adenocarcinomas [19]. Argon *et al*.[20] report that none of the 12 non-small cell lung cancers with a final diagnosis of adenocarcinoma exhibited positive staining for CK5/6. However, previous data contradict the positive CK5/6 staining reported for primary lung adenocarcinomas without metastasis in our study. Our findings align with reports suggesting a 5-14% positive CK5/6 immunoreaction in lung adenocarcinomas [17,19].

Several studies suggest the evaluation of CK5/6 and TTF-1 immunostaining in the differential diagnosis of adenocarcinoma. But additional research indicated that CK5/6 are not specific (96%) and have a low sensitivity estimated to 53%. The expression of TTF-1 is found in lung and normal thyroid tissue. It is also expressed in the derived adenocarcinomas with a sensitivity of 60% and a specificity of 98% [21-23]. Thus, TTF-1 is a reliable marker for subtyping lung cancers and seems to be more discriminating than the CK5/6 [20, 23, 24] in the differential diagnosis of lung adenocarcinoma and squamous lung carcinoma with a better specificity (100%) and sensitivity (100%) than CK5/6 and p63 [20]. Recent studies reported that TTF-1 positivity was seen in 87.7% of adenocarcinomas [25]. In our study, some adenocarcinoma samples showed co-expression of CK5/6 and TTF-1, highlighting the need for enhanced diagnosis with markers beyond CK5/6. Other studies propose a new panel composed of napsin A and p63, which have better specificity estimated to 94% and a sensitivity of 96% in differential diagnosis of adenocarcinoma and squamous carcinomas [23]. The CK5/6 staining is localized in cytoplasm and found in the squamous differentiation areas of undifferentiated carcinomas [19]. These data correlate with our results, and an

additional CK5/6 membrane staining was found and seems to be in accordance with the literature [26, 27]. Many researchers have evaluated the CK5/6 expression profile in adenocarcinomas of various origin tissue, reporting a positive CK5/6 immunoreaction in approximately 15% of cases. Other studies reported a lower percentage of 9%, probably due to the origin variation in various cases of adenocarcinomas. Interestingly, all studies revealed a positive CK5/6 staining in many adenocarcinomas derived from the uterus, ovaries, pancreas and biliary tract [17, 19].

The overexpression of EGFR has been found in all adenocarcinoma cases, with a membrane and cytoplasmic staining, due to the structure of the EGF receptor which contains a membrane and intracellular domains [12]. Lung cancer has a poor prognosis because of the EGFR overexpression and resistance to EGFR Tyrosin kinase inhibitors (TKI). The EGFR-TKI resistance involves activation of compensatory pathways due to amplification and/or overexpression of bypass survival receptors (HER2, HER3, MET, AXL and IGF1R). As a result, resistant tumors are not depending on EGFR for survival and proliferation [28, 29].

Conclusion

Our findings indicate that CK5/6 is not a highly sensitive marker for differentiating between squamous cell carcinomas and adenocarcinomas. Therefore, it should be supplemented with additional markers like TTF-1 and p63. While CK5/6 demonstrates good specificity as a marker for squamous cell carcinoma, basal cell carcinoma, transitional carcinoma, tumors of salivary glands, and thymoma, it's important to remember that tumor heterogeneity and representativeness issues can arise in some cases. Molecular analysis remains crucial for precise identification of the NSCLC subtype.

Acknowledgements

I thank the Professor **Ahmed KACIMI,** the Doctor **Nacera BELBLIDIA** and Mister **Hakim HALIMIA** from the Anatomopathology service of Mohammed Seghir Nekkache Hospital; Algiers; Algeria, for their help and human values.

Figure legends

Figure 1a, b: **Positive CK5/6 immunostaining in lung adenocarcinomas (HE; a; GX10); (HE; b; GX100).** Only 37% cases of lung adenocarcinomas revealed a positive staining for CK5/6. The staining appearance is moderately intense and non-diffuse with a membrane and cytoplasmic localization.

Figure 2: Over-expression of the epidermal growth factor receptor (EGFR) in lung adenocarcinomas (HE; GX40). The EGFR immunostaining has an intense and continuous aspect with a membrane and cytoplasmic localization**,** according to the structure of EGFR.

Figure 3: Negative immunostaining of CK5/6 in lung adenocarcinomas (HE; GX10). A negative immunoreaction of the CK5/6 has been obtained in a case of adenocarcinoma demonstrating the absence of a squamous histological structure.

Figure 4: **Positive immunostaining of CK5/6 in patients with squamous lung carcinomas (HE; GX10).** The CK5/6 staining was intense and has a diffuse aspect indicating the presence of a squamous structure.

Conflict of Interest Statement: Amal Mesli-Mostafa, Fatima Zohra El Kebir and Tewfik Sahraoui declare that they have no conflict of interest

Disclosure: All the funding sources have been supported by the Biology of Development and Differentiation laboratory of the University Oran 1 Ahmed Benbella in Algeria.

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