Application of International association for the study of lung cancer/American Thoracic Society/European Respiratory Society criteria for the diagnosis of lung carcinomas on small biopsies: A tertiary care center experience

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Abstract

Background: The new IASLC/ATS/ERS classification provides standardized terminology for lung cancer diagnosis in small biopsies and cytology specimens. **Objectives:** The aim was to study the feasibility of the guidelines using one marker for adenocarcinoma (ADC) and one for squamous cell carcinoma (SQCC) in non-small cell lung carcinomas (NSCLCs). **Subject and Methods:** In this study, we reviewed all the formalin-fixed paraffin-embedded tissue blocks diagnosed as lung carcinoma between July 2016 and December 2017. Cases were labeled as SCLC, ADC, SQCC, NSCLC favor ADC, NSCLC favor SQCC, NSCLC-not otherwise specified (NOS), and NSCLC-NOS possible adeno-SQCC (ADSQCC) as per IASLC/ATS/ERS 2011 guidelines. A three-step approach incorporating morphology, immunohistochemistry (IHC), and molecular analysis was used. **Results:** One hundred and nine cases were included. Six of the 109 cases were SCLC and 1 case was of large-cell neuroendocrine type. Of the remaining 102, 51 were diagnosed based on their classical histomorphology into SQCC (8) and ADC (43). Remaining 51 cases required IHC/special stains for categorization. The panel comprised anti-CK7, anti-thyroid transcription factor-1 (TTF-1), and anti-p63. Twenty-nine were positive for anti-TTF-1 and thus labeled as NSCLC favor ADC. Fifteen were labeled as NSCLC favor SQCC as they were highlighted by anti-p63. Four cases showed reaction to both the antibodies in different sets of tumor cells and thus were classified as NSCLC-NOS, possible ADSQCC. Remaining 3 cases did not show reaction to any of the antibodies and hence were labeled NSCLC-NOS. **Conclusion:** The need for every laboratory to use minimal tissue for ancillary tests to diagnose lung carcinoma on small biopsies is reemphasized. Tissue from small biopsies needs to be preserved not only for the diagnosis but also for molecular testing and evaluation of markers of resistance to therapy, in this era of personalized medicine.

Key words: IASLC/ATS/ERS guidelines, lung cancer, nomenclature, small biopsies, three-step approach

Introduction

Lung cancer is one of the leading causes of cancer-related deaths all over the world and accounts for 13% of all newly diagnosed cancer cases and 19% of cancer-related deaths worldwide.[1]

Conventionally, lung tumors have been subclassified purely on histomorphology into SCLC and NSCLCs. NSCLC is further categorized into squamous cell carcinoma (SQCC), large-cell carcinoma, and adenocarcinoma (ADC).[2] The World Health Organization (WHO) 2004 classification on lung tumors primarily addressed the resection specimens of lung and did not offer uniform guidelines/nomenclature for reporting of small biopsies and cytology specimens.

The New International Classification (IASLC/ATS/ERS)[3,4] and 2015 WHO classification[5] have addressed this issue and proposed standardized terminologies to be used for reporting, especially in ADCs. The IASLC/ATS/ERS recommendations on the approach of a positive sample can be compared to a three-step ladder where the first step is akin to histomorphological assessment. The diagnosis should be made as far as possible on histomorphology alone, and the pathologist needs to resort to immunohistochemistry (IHC) or special stains only if it is imperative. The whole essence of this is to curtail the tissue consumption in diagnosing to exploit the tissue available for testing the molecular strikes.[3]

The last decade has seen a paradigm shift in molecular understanding of lung carcinomas, and molecular testing of lung carcinoma has proved integral to the success of new targeted therapies and their use is now standard of care. KRAS was the first molecular marker discovered in 1987, but the list of markers is vast now, including EGFR,[6] ALK rearrangement, BRAF, and MET-1 and is ever expanding.[7] The clinical utility of the molecular subtyping lies in the fact that the different subtypes respond differently to therapies. The presence of EGFR mutation makes patients more sensitive to tyrosine kinase inhibitors (TKIs),[6] ALK rearrangement leads to sensitivity to crizotinib while KRAS mutation implies resistance to TKIs.

We, in this study, tried to incorporate the IASLC/ATS/ERS guidelines for diagnosing lung carcinomas on small biopsies using the minimal IHC panel. We further attempted to correlate clinicopathological features with EGFR mutations/ALK rearrangement analysis, wherever feasible.

Subject and Methods

Study sample

This single-center study was conducted at the Department of Pathology of a tertiary care center, after the approval from the institutional ethical committee. All the cases of primary lung carcinoma were selected from July 2016, onwards.

A total of 109 cases were included in this descriptive study. Relevant clinical details – age, sex, tobacco use status, clinical diagnosis, and location of the tumor were obtained from the hospital records, departmental database, and patients wherever required. All the hematoxylin and eosin-stained slides were reviewed and classified according to the IASLC/ATS/ERS guidelines. Those showing classical features of squamous differentiation in the form of keratin pearls, individual cell keratinization, and/or intercellular bridges were defined as

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classic morphology: SQCC [Figure 1]. The malignancies which showed the architectural layout of lepidic, papillary, acinar, and/or micropapillary type pattern were grouped under classic morphology: ADC [Figures 1 and 2]. If there was no conclusive evidence of the type of carcinoma on histopathological examination (HPE), then the sections were subjected to IHC.

Immunohistochemistry

An ancillary panel of one marker each for SQCC and ADC was put in for cases where HPE was indecisive. p63 was chosen as marker for SQCC and thyroid transcription factor-1 (TTF-1) for ADC. Some neuroendocrine immunostain markers such as synaptophysin and chromogranin were chosen where the cases were suspected to be of small-cell carcinoma or large-cell neuroendocrine carcinoma (LCNEC) type. Evaluations of the expression of these markers were carried out on 3–4 μm sections which were subjected to immunostaining as per manufacturer's protocol. The antibodies employed were p63 (anti-p63; clone PM163AA; Eurobio Life Science), TTF-1 (anti-TTF-1; clone 8G7G3/1; Sigma Aldrich), synaptophysin (Anti-synaptophysin; clone PR102; PathnSitu Biotechnologies), chromogranin (Anti-Chromogranin; clone LK2H10; Sigma Aldrich), CD56 (Anti-CD56; clone 6016816), and cytokeratin 7 (CK7) (Anti-CK7; clone OV-TL12/30; Thermo Fisher). All the markers were graded as positive or negative. Appropriate positive and negative controls were used. Nuclear staining of >10% of tumor cells was considered positive for TTF-1 and p63.

Molecular testing

EGFR mutational testing was carried out using amplification-refractory mutation system-polymerase chain reaction for detecting mutations in the exon 18, 19, 20, and 21. The target exons were amplified with mutation-specific primers, and mutant amplicons were detected using a fluorescent probe. ALK rearrangement testing was performed on fully automated IHC staining on the BenchMark XT Autostainer, Roche Diagnostics using the Ventana anti-rabbit monoclonal antibody (D5F3). The presence of strong granular cytoplasmic staining in any percentage of tumor cells was considered positive.

Results

Patient profile

A total of 109 cases formed the study sample, which included 72 male and 37 female patients (M:F – 2:1). Age of patients ranged from 29 to 85 years with a mean age of 58.5 years. The mean age was 59.3 years (age range: 29–85) in males and 57 years (age range: 37–77 years) in females. The mean age was 57.5 years (age range: 29–85 years) for ADC cases while it was higher in SQCC with an average age of 61.7 years (age range: 39–75 years).

About 44.4% of ADC cases were females in our study (32/72 cases) while males formed the bulk of SQCC category amounting to 78.3% (18/23 cases). Nicotine consumption history in the form of smoking cigarettes and bidis or chewing gutka was obtained in all cases. However, the smoking history was not quantified and categorized further in the study. Seventy-two (66.1%) patients consumed tobacco in some or the other form whereas the remaining 37 (33.9%) did not consume. Nearly 44.4% (32/72 cases) of the ADC patients denied any form of nicotine consumption, while only a minority 17.4% of SQCC cases (4/23) were nonsmokers. All the SCLC and LCNEC cases were males and smokers.

The left lung involvement was marginally higher, accounting for 56 (51.4%) cases while the remainder being in the right lung (53 cases; 48.6%).

Histological subtypes

Of the 109 cases, 6 cases (5.5%) were of small-cell type (SCLC) and 1 case (0.9%) was of LCNEC type with the remaining 102 (93.6%) being of non-small-cell type (NSCLC). Of the NSCLCs, ADC cases surpassed other subtypes and made up for the 72 of 102 cases (70.6%). SQCC accounted for 23 cases (22.5%) and adeno-SQCC (ADSQCC) accounted for 4 cases (3.9%) while 3 cases (3%) belonged to NSCLC-not otherwise specified (NOS) subtypes.

Of the 109 cases, 51 cases (46.8%) could be diagnosed directly on histomorphology alone. Of these 51 cases, 43 were classic morphology-ADC and remaining 8 were classic morphology – SQCC. On the 43 ADC cases which could be opined directly on histomorphology itself, 31 cases (72.1%) showed an acinar pattern, 6 cases (13.9%) revealed a lepidic morphology, a solitary case (2.4%) had a papillary histomorphology while the remaining 5 cases (11.6%) showed a mixed pattern of acinar, papillary, and lepidic histomorphology.

Remaining 58 cases (53.2%) required the help of ancillary techniques in the form of IHC. Six cases were suspicious of small-cell carcinoma and one was suspicious of LCNEC type on histomorphology, so they were subjected to neuroendocrine panel of IHCs [Figure 3]. Of the remaining 51 cases, 29 cases were highlighted solely by the ADC marker (anti-TTF1) [Figure 4] and 15 cases were positive for SQCC marker (anti-p63) alone [Figure 5]. Four cases were

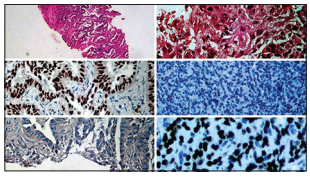

Figure 1: On the left (top to bottom): Classical morphology adenocarcinoma (H and E, thyroid transcription factor-1 and p63). On the right (top to bottom): Classical morphology squamous cell carcinoma (H and E, thyroid transcription factor-1 and p63)

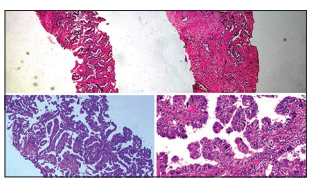

Figure 2: Various adenocarcinoma patterns encountered (from top clockwise): Acinar pattern, papillary, and lepidic

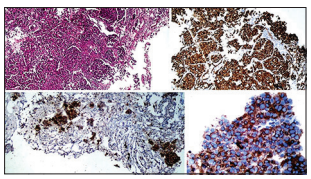

Figure 3: Large-cell neuroendocrine carcinoma: Tumor cells are positive for CK7 (top right), CD56 (bottom left), and chromogranin (bottom right)

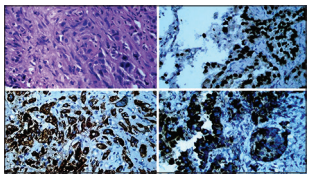

Figure 4: Non-small cell lung carcinomas favor adenocarcinoma: Morphology not discernible but tumor cells are strongly and diffusely positive for thyroid transcription factor-1 (top and bottom right) and negative for p63. Tumor cells are positive for CK7 (bottom left)

highlighted by both anti-TTF1 and anti-p63 in different sets of tumor cells while the remaining 3 cases were nonreactive to either of the immunostains while being positive for anti-CK7 [Figure 6].

Molecular analysis

The cases diagnosed as ADC, NSCLC favor ADC, NSCLC-NOS, and NSCLC-NOS probable ADSQCC were advised for molecular analysis (EGFR and ALK testing). A subset of patients got themselves tested for EGFR mutation and ALK rearrangement. Of the 79 cases who were advised molecular analysis, 14 got themselves tested for the same. Four of the 14 patients (28.5%) were positive for EGFR mutation. Three of the 14 (21.4%) patients were positive for ALK rearrangement by IHC. Remaining 11 were negative. The molecular hits found in our study were correlated with the clinicopathological features [Tables 1 and 2].

Discussion and Conclusion

Many changes have been introduced in the latest IASLC/ATS/ERS classification compared to the previous WHO classification in the form of implementation of special stains and immunostains to subtype histomorphologically unclassifiable cases into ADC or SQCC, using small biopsies and cytological samples to diagnose malignancies and the necessity to manage the tissues for further molecular studies.

The amendments as far as nomenclature are concerned include discontinuation of the term bronchoalveolar carcinoma and the introduction of newer terminologies such as lepdic pattern for the erstwhile bronchoalveolar carcinoma, invasive mucinous ADC instead of the previously used mucinous bronchoalveolar carcinoma, and the introduction of the term micropapillary variant of ADC.[7]

The previous editions of the WHO classification of lung tumors released in 1967, 1981, and 1991 primarily classified lung carcinomas on the resection specimens and not on cytology or small biopsies.[8-10] Furthermore previously, the WHO recommendations on subtyping lung carcinomas were purely based on histomorphology only.[8,9] Mucin stain was the only ancillary test recommended by the WHO up till 1999 where the third edition suggested the use of IHC for the diagnosis of LCNEC, sarcomatoid carcinoma, and differentiation of malignant mesothelioma from carcinomas.[10]

The new IASLC/ATS/ERS classification has, for the first time, provided a realistic insight into the terminologies used in lung carcinomas, especially ADCs on small biopsies and cytology specimens. This classification recommends that on small biopsies and cytology, NSCLC be further divided into a more specific and accurate histological type, wherever possible.[3]

It is further suggested that if clear squamous or glandular differentiation is evident on light microscopy on hematoxylin and eosin slides only, then a lesion can directly be diagnosed on body fluids, aspiration cytologies as well as small biopsies as SQCC or ADC.[11]

A tumor can be termed SQCC if it reveals features such as keratin pearls, intercellular bridges, and/or single-cell keratinization. If features of glandular differentiation such as acinar, papillary, lepidic, and/or micropapillary are visible on histomorphology, the tumor can be directly categorized as ADC. This is the first step of a three-tier approach.

If a definite conclusion cannot be arrived at after histomorphological evaluation, we next move further ahead in the flow of events. The recommendations are for using a single ancillary marker for SQCC and ADC each to achieve a judicious use to exploit the tissue for further molecular testing. We used p63 as marker for squamous cells differentiation and TTF-1 for glandular differentiation.

If the tumor is highlighted by ADC marker and/or mucin with it being simultaneously negative for SQCC marker, it is apt to name it NSCLC favor ADC. Similarly, if a tumor expresses positivity for squamous markers and negativity for ADC markers, then it will be termed as NSCLC favor SQCC.

If sections from the specimen is positive for both TTF-1 and p63 although in different set of tumor cells, then this may raise a possibility of the carcinoma being of adenosquamous nature although this can be confirmed only on a resection specimen accurately. If the tumor is negative for both markers, then it is mandatory to confirm the lesion to be carcinoma by adding a cytokeratin marker. If it is negative for cytokeratin, then it is prudent to add other markers such as vimentin, HMB45, and other requisite markers to rule out a sarcoma or malignant melanoma.[12]

The cases which are counseled for molecular analysis are advised to get themselves checked for EGFR mutation and ALK rearrangement which together account for the most common mutation amenable to targeted therapies. As, is evident from the literature, the first randomized clinical trial (the Iressa Pan-Asia study) revealed that in cases of advanced

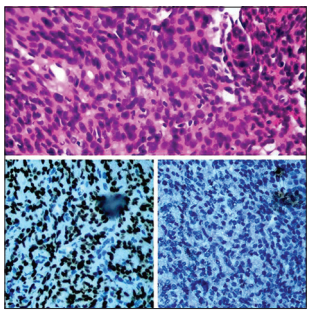

Figure 5: Non-small cell lung carcinomas favor squamous cell carcinoma: Morphology not only discernible but also tumor cells are strongly and diffusely positive for p63 and negative for thyroid transcription factor-1

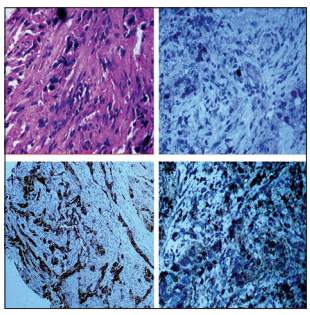

Figure 6: Non-small cell lung carcinomas not otherwise specified: Morphology not discernible and the tumor cells are not highlighted by either p63 or thyroid transcription factor-1 but are positive for CK7 (bottom left)

Table 1: Correlation of epidermal growth factor receptor mutation with patient characteristics and histological pattern

EGFR mutation	Age	Sex	Histological pattern	Smoking status
Exon 20-p.S768I and exon 21-p.L858R	58	Female	Lepidic pattern (ADC)	No
Exon 21-p.L858R	58	Male	Lepidic pattern (ADC)	No
Exon 19 deletion	33	Male	Acinar pattern (ADC)	No
Exon 19 deletion	34	Male	NSCLC favour ADC	Yes

EGFR=Epidermal growth factor receptor, NSCLC=Non-small cell lung carcinomas, ADC=Adenocarcinoma

Table 2: Correlation of anaplastic lymphoma kinase rearrangement with patient characteristics and histological pattern

ALK rearrangement	Age	Sex	Histological pattern	Smoking status
Positive	48	Male	NSCLC favor ADC	No
Positive	60	Male	Papillary pattern (ADC)	No
Positive	72	Male	NSCLC favor ADC	Yes

ALK=Anaplastic lymphoma kinase, NSCLC=Non-small cell lung carcinomas, ADC=Adenocarcinoma

NSCLCs which harbor EGFR-activating mutations, the TKIs are far more superior than conventional platinum-based chemotherapy.[13] The testing guidelines currently placed on record mention that EGFR and ALK testing be carried out on all advanced stage ADC regardless of age, sex, race, smoking history, or other clinical risk factors.

The most common mutations in the EGFR involve the exons 18, 19, 20, and 21. These are commonly seen in East Asian ethnicity, women more than men, and in those who have never smoked or are light smokers.[13-19] Apart from EGFR mutations, ALK rearrangement can be seen in a subset of ADCs which is due to inversion on chromosome arm 2p resulting in the creation of EML4-ALK fusion gene, the prevalence which is seen in a subset of ADC cases who are never or light smokers.[20,21]

At present, the recommendation for EGFR mutation testing and candidacy for pemetrexed or bevacizumab therapy is for the diagnosis of ADC, NSCLC favor ADC, and NSCLC-NOS cases.[3] Hence, the reporting pathologist should be able to definitely distinguish between SQCC and ADC accurately and rightly so because of the novel targeted therapies in use these days.

To conclude, advances in pulmonary pathology are largely due to progress made in the field of thoracic oncology. As evident from the literature, majority of the patients present to the tertiary care setup in a fairly terminal stage. The implications of this are that pathologists can foresee only small biopsies or cytological specimens in the form of aspiration or effusion fluids and yet are expected to give out accurate histopathological subtype of the malignancy observed under the scope. Hence, it becomes even more imperative that we are well versed with the latest guidelines and classification so that the right lesion goes into the right basket.

The tissue received for evaluation needs to be preserved not only for precise diagnosis but also for molecular testing as well as evaluation of markers of resistance to therapy since targeted therapeutics have vastly improved the prognosis of patients with advance NSCLC in terms of progression-free survival.

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Conflicts of interest

There are no conflicts of interest.

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