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Commentary

Minimalistic immunohistochemical approach to non-small cell carcinoma of the lung in small biopsies in the context of the 2015 WHO Classification of Lung Cancer

About a decade ago, subtyping of non-small cell lung cancer (NSCLC) was not much of clinical relevance, as no differential treatment options were available then for specific histologic types[1,2]. But considerable developments in the understanding of genetics of pulmonary cancers have altered the present scenario. With the discovery of epidermal growth factor receptor (EGFR) and anaplastic lymphoma kinase-1 (ALK-1) mutations, which are effective targets for their corresponding inhibitors, the treatment options have opened up for certain major subsets of adenocarcinoma (ADC). Not only the targeted therapy but also the emergence of newer cytotoxic drugs with differential activity such as pemetrexed or drugs with limited indications such as bevacizumab have made the precise histologic typing obligatory[1,3-7].

Keeping in mind the changed scenario, essential modifications are made in the recent classifications of lung cancer including the recently adopted 2015 WHO Classification, which radically differs from its 2014 counterpart[1]. The major changes are chiefly with regard to common pulmonary malignancies such as ADC and squamous cell carcinoma (SQC) that morphologically manifest as non-small cell carcinomas (NSCCs). The changes in the current WHO Classification are greatly influenced by the 2011 Classification of Lung ADCs sponsored by the International Association for the Study of Lung Cancer (IASLC), the American Thoracic Society (ATS) and the European Respiratory Society (ERS)[1]. The highlight of the IASLC/ATS/ERS Classification is that it incorporates the diagnosis of pulmonary cancers on cytologic and small biopsy specimens[1].

As per the literature, about two-thirds of patients with lung carcinomas present in advanced stages, where the diagnosis is established usually through small biopsies or cytologic specimens. Thus, the points that are highlighted here from the currently relevant classification systems are pertaining either directly or indirectly to small biopsy/cytologic diagnosis of NSCCs. One essential aspect of the current WHO Classification is that it provides the standardized diagnostic criteria and terminologies for lung cancer on small biopsy and cytologic specimens. With the introduction of screening programmes for lung cancer, the small biopsy/cytologic materials are not only likely to assist in diagnosing malignancy at an early stage but also have the potential for providing the molecular diagnosis for targeted therapy; if pathologists can effectively manage to preserve sufficient material for molecular testing[1].

The current WHO Classification delves more into practical issues and strongly emphasizes the importance of immunohistochemistry (IHC) not only on small biopsy/cytologic material but also on resected specimens. The 2004 WHO Classification had restricted the use of IHC to tumours such as large cell neuroendocrine carcinoma (LCNEC), sarcomatoid carcinoma and carcinomas that needed to be differentiated from mesothelioma. With respect to IHC, the 2015 WHO Classification stresses the need for a high-quality immunostaining and also participation of laboratories in external quality assurance programme to ensure accurate diagnoses[1].

According to the literature, the proportion of NSCCs categorized as not otherwise specified (NSCC-NOS) on small biopsies ranges between 30 and 50 per cent; however, currently, there has been a drastic shift in it with the established therapeutic implications of the precise histologic diagnosis[1,8]. According to the current WHO Classification, biopsies or cytologic smears from NSCCs exhibiting acinar, papillary, lepidic and micropapillary patterns can be categorized as ADC while those with keratinization

and intercellular bridges can be reported as SQC even without IHC/immunocytochemistry (ICC) support. It recommends a limited ICC/IHC workup with the preservation of material for molecular testing in cases of poorly differentiated NSCCs lacking obvious squamous or glandular differentiation. NSCCs lacking both morphologic and IHC evidence of squamous or glandular differentiation should be reported as NSCC-NOS rather than non-small cell lung cancer (NSCLC). The alphabet L is excluded to keep open the possibility of metastasis. The tumours with cells expressing 'only thyroid transcription factor-1 (TTF-1)' and 'only p63 or p40' should be reported as 'NSCC favour ADC' and 'NSCC favour SQC', respectively, while a tumour with two different populations of cells separately expressing ADC and SQC markers should raise a suspicion of adenosquamous carcinoma, though its definitive diagnosis is possible only on resected specimens. SQCs are reclassified in the 2015 WHO Classification into keratinizing, non-keratinizing and basaloid subtypes, of which the non-keratinizing type always needs an IHC confirmation[1].

There have been considerable numbers of studies dealing with IHC in NSCCs on small biopsies/cell block specimens to differentiate between ADC and SQC[5,9-11]. Common markers used for ADCs are TTF-1[5], napsin A[10] and CK7[12]; while SQC markers evaluated are CK5/6, p63[7,9,10], 34ßE[5], p40[6,10,13], desmoglein, desmocollin[8], high molecular weight cytokeratins and S-100A7[2]. Of the important ADC markers, sensitivity and specificity of TTF-1 have been shown to range between 80.0-84.5 and 89.0-96.4 per cent, respectively; while Napsin A has been shown to be 58-92 per cent sensitive and 100 per cent specific[9,12]. Of the significant SQC markers, CK5/6 has been shown to be 73-100 per cent sensitive and 77.8-100 per cent specific[9,12]; while p63 has been shown to be 87-100 per cent sensitive and 78.3-92 per cent specific[7,9]. However, a sensitive marker, p63 is less specific, with a considerable proportion of ADCs and other tumours such as lymphomas (54%) also expressing it[6]. Sensitivity of the relatively newer SQC marker p40 is said to be comparable to that of p63[9] and hence considered the best among the existing SQC marker[13].

Although initial studies, as well as some of the recent studies, have dealt with the utility of multiple ADC and SQC markers[2,5,9-12], the current emphasis on the need for preservation of material for molecular testing has prompted a search for a reliable minimalistic 2-marker panel with one each of ADC and SQC markers[13]. The article by Walia *et al*[14] in this issue addresses this aspect with a fairly good sample size. The fact that their approach could bring down the number of NSCCs from 46.7 to mere 14.2 per cent and that 85.5 per cent of NSCCs could accurately be classified into ADC and SQC reflects the high effectiveness and practical utility of the 2-marker panel of TTF-1 and p40. The 100 per cent sensitivity and specificity documented for p40 in their study support and strengthened the claim that 'p40 is the best currently available SQC marker'. The only significant similar study in the Western literature is that of Pelosi *et al*[13]. As the authors have themselves admitted, the low number of resected specimens used for validation is one of the limitations of the study, which, however, has rightly been attributed by the authors to common presentation of NSCC patients at an advanced stage, not amenable to resection. Furthermore, as the study was designed before the implementation of the 2015 WHO Classification, it is understandable that the 2011 ASCLC/ATS/ERS terminologies such as 'non-small cell lung cancer' and 'NSCLC' are retained in the study. The observations that two of their cases morphologically diagnosed as ADC and NSCLC-NOS turned out to be adenosquamous and sarcomatoid carcinomas emphasize the importance of IHC on resected specimens[14]. Sensitivity and specificity of ADC marker TTF-1 documented by the authors[14] remain similar to that in the literature[9,12].

Mucin stains were not used in this study due to their low sensitivity, as well as with the intention of saving tissue sample for molecular studies[14]. In the recent years, with easy access to IHC markers, the role of mucin stains such as Alcian blue/periodic acid–Schiff (AB/PAS) is ignored. Although not all ADCs express mucin with routine histochemical stains, the stainable mucin still remains one of the defining characteristics of pulmonary ADC[15]. Loo *et al*[2] documented 23 per cent sensitivity and 100 per cent specificity for mucin with a 100 per cent positive and 76 per cent negative predictive values in pulmonary ADCs. Despite the limited published data on mucin stains in pulmonary ADCs, AB/PAS stains would retain a high probability of staining central bronchial type or poorly differentiated ADCs that are likely to be TTF-1 negative[2].

The current WHO Classification restricts the diagnosis of large cell carcinomas to resected tumours lacking obvious morphologic or IHC differentiation. Notably, it also puts the entire spectrum of pulmonary neuroendocrine tumours, including the LCNEC as

a single group[1]. LCNECs account for 2.1-3.5 per cent of all lung cancers. Although LCNECs exhibit neuroendocrine architecture on histology (which may not be consistently reflected in small biopsies), cytologically, these resemble large cell carcinomas with polygonal cells having abundant cytoplasm, variably granular pattern of chromatin and atypical or clear nucleoli. Thus, with the absence of classic neuroendocrine cytologic features, these can easily be mistaken for NSCC-NOS type or even ADC on small biopsies, the management of which are entirely different. However, a high mitotic activity of ≥11/10 hpf and more abundant necrosis (prominent features of LCNEC) are often helpful in distinguishing these from ADCs that tend to have fewer mitoses and less abundant necrosis[16]. The issue of LCNECs has not been addressed by Walia *et al*[14] as it was out of scope of their study design. Obviously, none of their resected cases represented LCNECs. However, with no mention of neuroendocrine markers being performed in their NSCC-NOS cases, probability of any of these being LCNECs remains unresolved. Zhang *et al*[10] documented neuroendocrine tumours including LCNEC to be consistently negative for SQC markers such as p63, p40 and CK5/6 while Pelosi *et al*[13] reported ignorably low scores of p63 and p40 in LCNECs. LCNEC has also been shown to be negative for napsin-A and TTF-1[10]. Thus, with the 2-marker approach, LCNEC will be interpreted as 'NSCC-NOS', wherein use of at least one highly sensitive neuroendocrine marker (after the 2-marker panel) would be of considerable help.

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