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Original contribution

Accuracy of classifying poorly differentiated non–small cell lung carcinoma biopsies with commonly used lung carcinoma markers☆

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Summary Immunohistochemical (IHC) staining is an important adjunct to the classification of non–small cell lung carcinoma (NSCLC). Several studies have used tissue microarrays derived from resection specimens to evaluate the accuracy of IHC staining for classifying NSCLC, but few have used actual biopsies of poorly differentiated carcinomas, and the question of how often biopsy IHC in such tumors leads to the correct classification has received little attention. We identified 40 cases of NSCLC that, on biopsy, could not be subclassified by morphology and that had subsequent resection specimens. TTF-1, napsin, p63/p40, and CK5 or a subset thereof were used for IHC classification. Of the 40 cases classified by IHC on biopsy, 33 (82%) had no change in diagnosis after resection. Of the remaining 7 cases, 3 were classified as NSCLC–not otherwise specified on biopsy and subclassified as either adenocarcinoma or squamous cell carcinoma (SCC) on the surgical specimen. One adenocarcinoma biopsy was reclassified as pleomorphic carcinoma. Two SCCs were changed to adenosquamous carcinoma, and 1 SCC was changed to large cell lung carcinoma. Only 1 antibody pair (2%) was discordant between biopsy, and almost all reclassifications were done based on morphologic features rather than change in IHC pattern. We conclude that IHC staining allows accurate subclassification of poorly differentiated NSCLCs on small lung biopsies in most cases, but there is still a substantial "miss" rate (here, 18%). Surgical resection specimens allow further subclassification, mainly due to architectural features not present in the biopsies.

1. Introduction

In the past, differentiating between adenocarcinoma (AC) and squamous cell carcinoma (SCC) of the lung may have provided an element of prognostic information but realistically did not alter management [1]. The recent development of targeted therapies has created a need for accurate subtyping of non–small cell lung carcinomas (NSCLCs). In particular, newer therapies that target specific molecular alterations as well as differences in the types of antitumor chemotherapeutic agents used between AC and SCC make differentiation between these 2 tumors of great importance, as medicine moves toward individualized therapy [2-4].

Traditionally, subtyping of NSCLCs only uses morphologic features evident on hematoxylin and eosin (H&E)–stained slides. The diagnosis of SCC requires the presence of

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intercellular bridges or evidence of keratinization, whereas *ACs* are defined as epithelial tumors with glandular differentiation or with mucin production [1,5]. A poorly differentiated NSCLC without these defining features is labeled as large cell lung carcinoma (LCLC) on surgical resection or NSCLC–not otherwise specified (NSCLC-NOS) on biopsy.

Morphology is often difficult to elucidate on small biopsy samples, where architecture may not be apparent. Interpretation is further hampered by crush artifact that frequently accompanies these biopsies. Moreover, biopsies are fractional samples of the tumor and may not be representative of the whole lesion, making accurate subtyping particularly challenging [5].

Immunohistochemistry (IHC) allows for greater diagnostic accuracy in subtyping NSCLCs [6]. There are a number of studies that correlate lung cancer diagnoses of AC and SCC with particular staining patterns, and, often, the use of IHC in LCLCs has allowed identification of histologic subtypes despite the lack of specific morphologic findings on H&E-stained slides [7,8]. This is reflected in the 2011 International Association for the Study of Lung Cancer, American Thoracic Society, and the European Respiratory Society multidisciplinary update on lung AC and the plan to incorporate IHC in the 2015 World Health Organization criteria [9]. There have been several recent series validating the use of IHC in lung biopsies, with reports that IHC reduces the frequency of NSCLC-NOS from 40% to less than 10% of all biopsies [5,6,10-21]. Most of these studies have used morphologically evident tumors as the basis of IHC staining, and some have created tissue microarrays from resection specimens to simulate small biopsies [17-20], but few have used actual biopsy specimens and correlated them with subsequent resections (Table 1) [12-16].This approach is important because biopsies present problems with limited sampling and crush artifacts that hinder interpretation and could potentially create aberrant IHC staining patterns.

In this study, we selected a series of biopsy cases that, on H&E, could only be classified as NSCLC-NOS and that had subsequent surgical resections, to examine how accurate IHC staining of the biopsies might be when compared with the resections and whether the resection specimens provided additional information for tumor classification.

2. Materials and methods

Surgical pathology files were searched for cases of NSCLC diagnosed on lung biopsies between the years 2010 and 2013, yielding 946 results (Fig. 1). We then narrowed the search to the 112 cases in which subsequent surgical resections were performed at our hospital, a tertiary care center. Cases with descriptors within the biopsy pathology report of "lepidic," "bronchioalveolar carcinoma," "AC in situ," "acinar," and "keratinization" were removed, as they implied easily identifiable morphologic findings allowing subclassification without IHC. This step further reduced the potential number of cases to 78, which were selected for review. Cases finally were included in the study if (1) both the biopsy and surgical resection specimen were available for review, (2) the biopsy showed no differentiating features on H&E, and (3) IHC had been performed on the biopsy (or corresponding bronchial washing/brush cytology block if insufficient tissue on biopsy) with a resulting diagnosis of AC, SCC, or NSCLC-NOS. Fifty-nine paired biopsies with surgical resections and corresponding IHC were reviewed. The resected tumors were considered the criterion standard. Tumors were classified based on the 2004 World Health Organization and the updated 2011 International Association for the Study of Lung Cancer/American Thoracic Society/European Respiratory Society criteria [1,9].

IHC stains on the biopsies and resection specimens usually included TTF-1 (8G7G3/1, dilution, 1:100; DAKO) and p63 (EPR5701, 1:2000; Epitomics) or p40 (rabbit polyclonal antibody, 1:200; Biocare) in later years. Additional stains in some cases included CK5 (XM26, 1:200; Novocastra/Leica) and napsin A (rabbit polyclonal antibody, 1:400; CellMarque). Our interest in this study was to see how a "real-world" approach to classification would work, and, for that reason, we only added stains beyond those done by the original sign-out pathologist if we believed them necessary to reach a histologic diagnosis. If the diagnosis was morphologically obvious on the resection specimen, no additional stains were done for this study. Tumors showing TTF-1 and/or napsin A staining were classified as ACs, whereas tumors showing diffuse p63 or p40 staining or diffuse CK5 staining were classified as SCCs [5-23]. Focal p63 or CK5 staining in the presence of TTF-1 staining was interpreted as an AC [16].

The study received approval from the University of British Columbia and Vancouver General Hospital Office of Research Ethics.

3. Results

Of the 78 potential cases, 19 were unavailable for review, and another 19 were found to have obvious differentiating features on review of the H&E biopsy and excluded from the study. Forty cases had biopsies of NSCLC that could not be subclassified on H&E-stained slides alone based on morphologic criteria and were used in our study. Twenty-six of the biopsies were endobronchial or transbronchial, and 14 were computed tomography–guided needle core biopsies. Using IHC staining, the 40 cases were classified as 15 SCCs, 19 ACs, and 6 NSCLC-NOS (Table 2). IHC was generally performed on the biopsies, with the exception of 2 cases, where there was insufficient tissue on biopsy, and the staining was performed on the corresponding bronchial washing cytology block.

The resection specimens changed the diagnoses to 13 SCCs, 20 ACs, 4 LCLCs, 2 adenosquamous carcinomas, and