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High diagnostic performance of cell-block procedure in a case of typical lung carcinoid

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Abstract

In young patients, bronchial carcinoid tumors (BCT) are rare, slow growing, malignant neuroendocrine tumors, which arise from Kulchitsky cells in tracheo-bronchial tree mucosa, covered by intact epithelium. Herein, we report a case of BCT occurred in a young 20-year-old female patient presenting cough, fever and shortness of breath. Although cytological smears were suggestive for well differentiated lung tumor, only the cell block procedure allowed to define the diagnosis in the right way by the application of an immunohistochemical algorithm. The post-surgical histopathology confirmed the diagnosis of typical BCT with negative lymph nodes; no local recurrence was revealed in the 24 months follow-up after surgery.

Keywords: typical carcinoid, lung, immunohistochemistry, cytology, cell block

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Introduction

According to histopathologic criteria of the most recent World Health Organization (WHO) classification, neuroendocrine (NE) lung tumors are classified in four entities: typical carcinoid (TC), atypical carcinoid (AC), large cell and small cell neuroendocrine carcinoma (NEC)[1]. Bronchial carcinoid tumors (BCT) represent uncommon, low-grade/intermediate malignant tumors with neuroendocrine differentiation arising from the Kulchitsky cells in bronchial mucosa and the distinction is based on two morphological parameters: the presence/absence of necrosis and the mitotic index per 2 mm[2,1,2]. Although the lung carcinoids represent rare primary lung tumors, the incidence increased over the last 30 years and the typical variant represents the most frequent[3] one. It is well established that about 70% of these neoplasms are centrally located in large bronchial structures, while 30% of carcinoids may have a peripherical localization[3,4].

The mean age of patients with carcinoids is generally about 50 years, but it can occur at any age from 5 to 90 years[3,4]. Until now, in the international guidelines, classification of BTC

criteria has been established using surgical specimens, whereas bioptic and cytological approach remains a poorly explored methodology in neoplastic diagnosis with important clinical implications[5].

It is well known that in lung pathology, core biopsies yielding histological specimens can be used for immunohistochemistry (IHC) and molecular tests, which are useful for a correct diagnosis and treatment choice[5,6].

On the other hand, fine needle aspiration (FNA) has a lower rate of complications compared to core needle biopsy, but making differential diagnosis using only cytological samples can be more difficult[5,6].

For this reason in cytology laboratories, cell block (CB) methodology should be performed allowing the application of specific immunohistochemical algorithm to define lung tumors subtypes[5,6].

Herein, we present a case of a young woman with typical lung carcinoid diagnosed on cytology specimens using cell block technique.

Case presentation

A 20-year-old female presented at thoracic department with cough, fever and shortness of breath. The patient had been smoking 5 cigarettes a day for three years. Physical examination revealed decreased breath sounds in the right upper lobe. Chest X-ray revealed a consolidation in upper right hilum and right upper lobe. CT scan showed a central nodular intraluminal lesion with a wider diameter of 1,2 cm and post-obstructive pneumonia in the right upper lobe. No other endobronchial lesions or focal intrapulmonary pathology were found. There was no lymphadenopathy and no pericardial or pleural effusion. Bronchoscopy revealed a well-defined endobronchial mass in the right lower bronchus and FNA was performed.

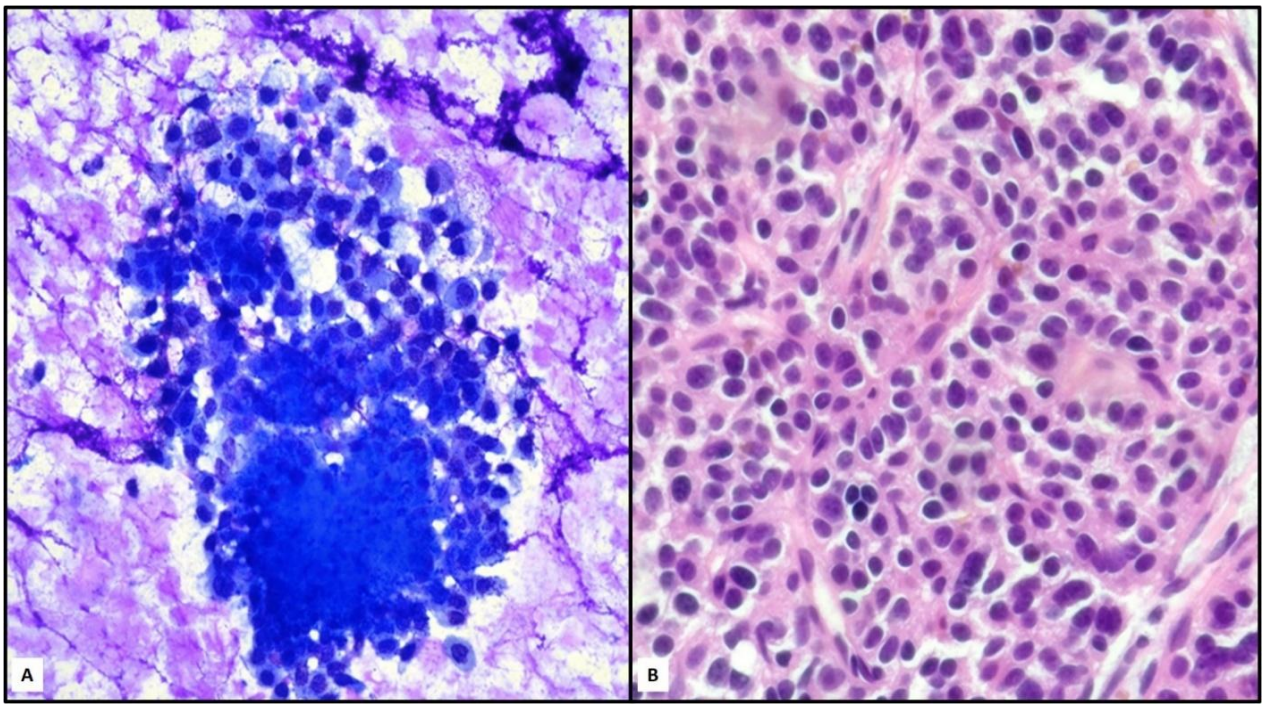
All cytological samples were prepared on Shandon cytocentrifuge. Smears were made on glass slides, which were immediately fixed in 95% alcohol and stained by Papanicolaou method. Air-dried smears were stained by May-Grünwald-Geimsa (MGG) method. Immunohistochemical analysis were performed on a Ventana Benchmark automated immunostainer on formalin-fixed paraffin-embedded CB preparations, obtained and stained utilizing the techniques previously described by Ieni et al.[7,8].

The monoclonal antibodies (Ventana diagnostics inc.) included in the diagnostic algorithm were anti-broad spectrum cytokeratin (AE1/3), thyroid transcription factor-1 (TTF-1), Ki-67 and neuroendocrine markers (chromogranin-A, synaptophysin and CD56).

Cytologically, conventional smears as well CB slides showed a moderate cellularity

characterized by uniform polygonal neoplastic elements with abundant eosinophilic granular cytoplasm arranged in sheets, trabeculae and sometimes small gland-like formations (Fig.1a,b).

Fig. 1 Conventional smear with a solid cluster of large cells with abundant granular cytoplasm and oval nuclei with scant nucleoli (a, MMG stain, original magnification X20) and CB sample with uniform polygonal neoplastic elements with abundant eosinophilic granular cytoplasm arranged in trabecular pattern (b, haematoxylin and eosin stain, original magnification X40).



Neither mitotic activity nor necrosis were observed. In CB serial sections, immunohistochemistry showed a diffuse reactivity for cytokeratin AE1/3, chromogranin-A (Fig.2a), synaptophysin(Fig.2b) and CD56 (Fig.2c).

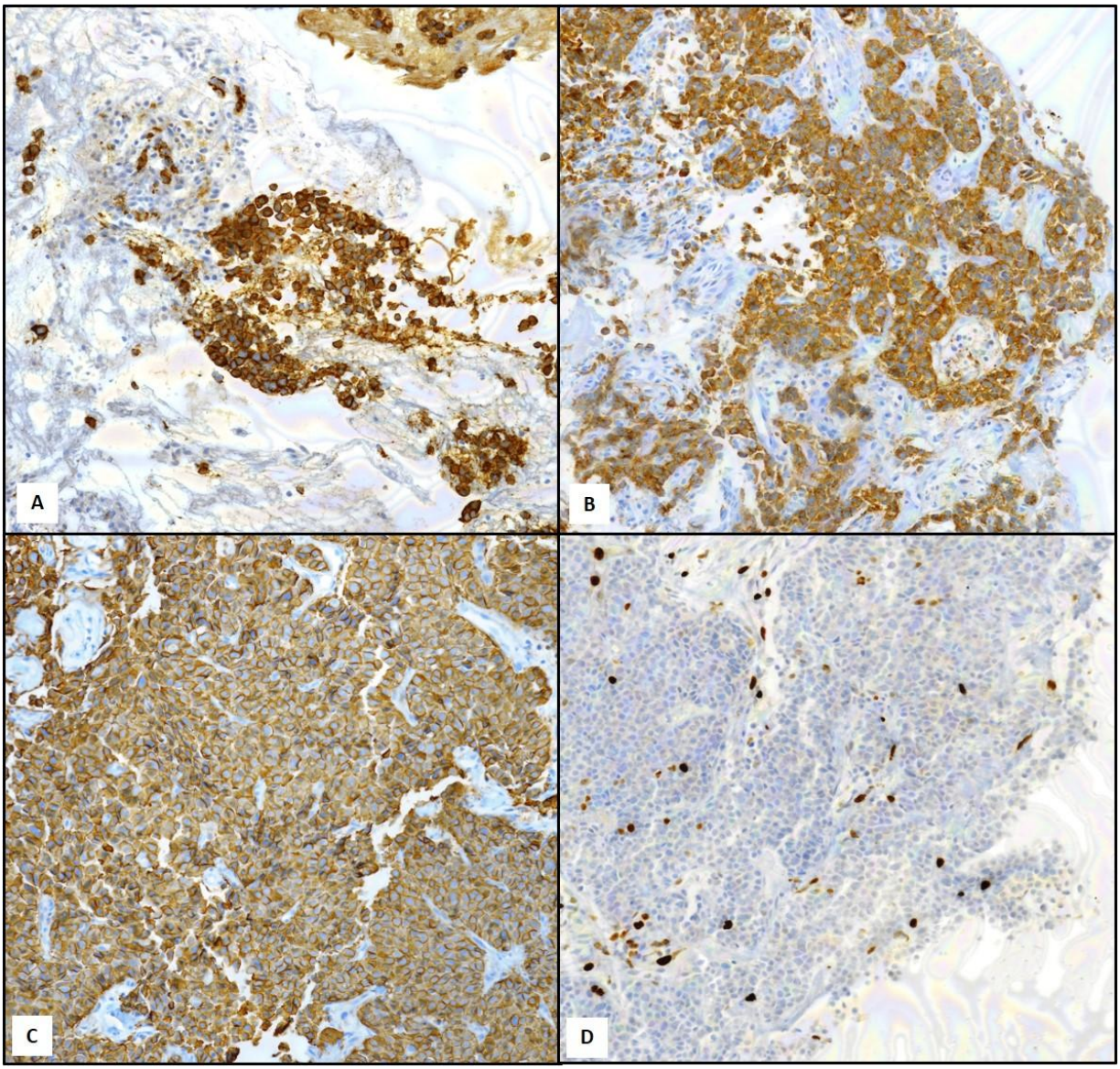
A focal immunohistochemical nuclear staining for TTF-1 was noted, while Ki-67 labelling index was < 2% (Fig.2d). The cytological diagnosis was compatible with well differentiated low grade typical carcinoid.

One week later the patient underwent a right upper lobe lobectomy and the specimen macroscopically revealed a solid, well-circumscribed lesion in the bronchial lumen measuring 1,2 cm with a color from gray to white.

Histological sectionsshowed a well circumscribed neoplastic proliferation with an organoid and nests pattern separated by thin vascular septae (Fig.3a).

The tumor cells are characterized by monomorphic round nucleus with stippled chromatin and large eosinophilic granular cytoplasm with <2/10 HPF mitotic figures. No necrosis was seen.

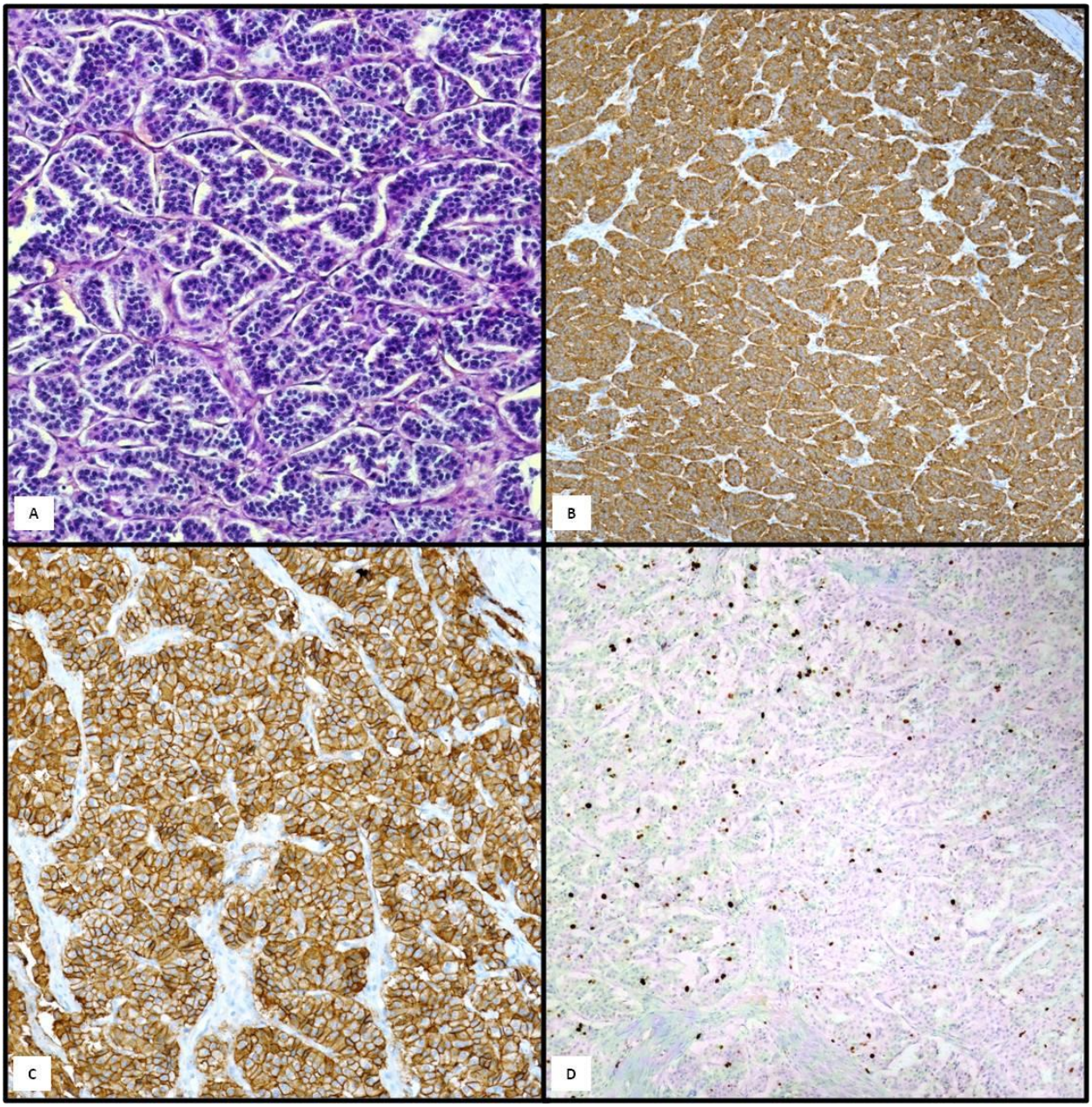
Fig. 2 Immunohistochemical results on sections cell block procedure: strong and diffuse immunopositivity in neoplastic elements for chromogranin-A (a, original magnification X20), synaptophysin(b, original magnification X20) and CD56 (c, original magnification X40). Low proliferation rate evaluated with Ki67 antibody (d, original magnification, X40)



The immunohistochemical analysis showed a strong immunohistochemical positivity for neuron-specific enolase, synaptophysin (Fig.3b), CD56 (Fig.3c), chromogranin A and Ki67 low expression < 3% (Fig.3d), confirming the previous diagnosis of typical lung carcinoid with tumor free resection margins and no lymph node involvement.

The patient has been under clinical follow-up for 24 months and she is currently in complete remission following surgery procedure.

Fig. 3 Definitive histopathological findings: neoplastic proliferation characterized by trabecular growth pattern with hyalinised and high vascular stroma (a, original magnification, X20), strong and homogeneous immunoexpression for synaptophysin (b, original magnification X20), CD56 (c, original magnification X40) and low Ki67 expression rate (d, original magnification, X20)



Discussion

Primary lung tumors in adolescent and young patients are extremely rare and approximately 75% are malignant forms[9]. In detail, this group consists of BCT (40%), bronchogenic carcinoma (17%) and pleuropulmonaryblastomas (15%)[9]. BCTs affect both sexes, although some studies reported a higher prevalence in women; interestingly, this entity shows two peaks of incidence: one in adolescence and another one close to the age of 45 years[9]. The etiopathogenesis of these tumors is largely unclear; in fact, unknown carcinogens or exposure to environmental agents

have been unequivocally implicated in carcinogenesis[3,9].

Although the association between bronchial carcinoids and smoking is unclear, a rate between one-third and two-thirds of all patients has been featured by smokers with higher prevalence of smoking and reported in patients with ACs[3].A diagnosis of BCT is often overlooked in young patients due to the absence of specific symptoms and for this reason the clinical diagnosis is controversial and very challenging[9].

Apart from symptoms like chest pain, pleural effusion, cough, wheeze, hoarse voice or atelectasis, common pulmonary manifestations are hemoptysis (18%), post obstructive pneumonitis (17%) and dyspnea (2%)[3,9]. The symptoms depend on the tumor location: central or peripheral; in the first localization the tumors are mostly located in the right lung and cause recurrent bronchial obstruction similarly to the present case.

The last classification of the World Health Organization/The International Association for the Study of Lung Cancer (WHO/IASLC) classifies BCT into: typical (76–90%) characterized by less than 2 mitosis/2 mm^2, absence of necrosis and atypical with increased mitosis (2–10 mitosis/2 mm^2) and confirmed necrosis [1-4].Cytological and bioptic sampling are the gold standard for a correct diagnosis and treatment approach, mainly in the differential diagnosis of low-grade/intermediate carcinoid and high-grade NEC[5].

FNA and core needle biopsy are two methods used to collect pulmonary tumor specimens. However, the latter method has a higher complication rate for percutaneous transthoracic CT-guided biopsies, such as hemothorax, pneumothorax and haemoptysis[10]. In contrast, FNA represents a minimally invasive procedure to diagnose lung neoplasms without dangerous complications; but this procedure has the disadvantages of providing few cellular specimens and not revealing tumor morphology, which interferes with accurate lung cancer subtyping and prognostic parameters assessment[10].

CBs are basically microbiopsies that are formalin fixed and embedded in paraffin creating the opportunity to examine the histological architecture of cytology material, perform ancillary tests (IHC and molecular tests) obviating the need for a more invasive biopsy procedure[11]. In fact, CB technique provide the opportunity to cut serial sections from the same specimens, even weeks or months after the first diagnosis or when the tumor relapses and new biopsies are not feasible[11]. This procedure allows for the retrieval of small sample fragments in cytology specimens that often cannot be processed by other cytologic techniques (direct conventional smear and liquid-based preparations). Somestudies indicated the usefulness of cell block lung in cytological specimen; in particular, the detection rate of malignancy ranges from 13% to 15%, higher in cell block than in conventional cytology[5,6]. Interestingly, the number of suspicious or positive cases

are twice higher using a combination of cytology and cell block in comparison to cytology alone, mainly applying ancillary methods[5,6].

In the present case, tumor cells encountered in cytological samples were constituted by uniform round/plasmacytoid morphology, granular/speckled (salt and pepper) chromatin with rare mitotic figures organized in trabeculae and pseudo-glandular pattern. In addition, it was possible to perform an immunoistochemical analysis on CB samples with specific NE markers allowing the correct identification of TC. Our main purpose was discriminating low-grade BCT from high grade NE carcinoma, based on mitotic count and the presence of necrosis. According to WHO classification, a mitotic count of 10 per 2 mm^2 has been defined as the absolute cut-off rate for atypical carcinoids and NE tumors exceeding this threshold, which are by default classified as high grade NEC[11].

Although Ki67 is not currently used as a WHO criteria, a Ki67 rate of < 20% is generally regarded as a cut-off rate to define lung carcinoids, similar to the result of our case[12]. However, one of the most common challenges in lung NE neoplasms diagnosis is the one ofbronchoscopic biopsies and FNA-CB samples, where significant crush artifact may cause morphological alterations in carcinoids closely mimicking high grade NEC[11,12]. In this setting, mitotic counts are difficult to assess and the critical role of Ki67 is well established[10]. It has been demonstrated that carcinoids should have low rates (1–20%, usually <10%), while most NECs have Ki67 of >50%. Consequently, High Ki-67 index above 20% has also been proposed as an indicator of high-grade NEC on small biopsies sans CB samples[13]. Although the issue of crushed carcinoids over diagnosis as NEC is well-documented in pathology literature[13] this remains one of the most common pitfalls in practice. Furthermore, the histologic diagnostic criteria to define TCT and ACT were established only on resection specimens and sometimes this parameters should not be applied in the metastatic setting where mitotic rates may be higher than seen in the primary tumor[13]. For this reason, the new 2021 WHO classification has introduced in this issue the term "carcinoid tumor, not otherwise specified-(NOS)" to define carcinoids encountered in metastic/advanced disease and in small biopsies/poorly sampled specimens. In these cases, the diagnosis of carcinoid tumor-NOS should be reported utilizing the following parameters: mitotic count per 2 mm^2, presence of necrosis, Ki-67 labeling index[13].

However, a new designation of "carcinoid tumors with elevated mitotic counts and/or Ki-67 proliferation rates" has been identified as an emerging concept in the last WHO classification when mitotic counts exceeding the upper threshold for AC (>10 mitoses per 2 mm^2) and an increased Ki-67 labeling index up to 30% in lung carcinoid[13]. Finally, a cutoff value of Ki-67 able to distinguish TCT from ACT is still not standardized and therefore, it is in discussion so

far; in fact, many proposed different cutoffs have been variously reported[2,3,5].

Conclusions

The present case highlights the diagnostic importance of CB technology in NE lung tumors diagnosis, even if the exact identification remains very difficult, mainly in cases with sampling artifacts. This event could lead to an incorrect distinction between low-grade/intermediate tumor and high grade NEC. The combined application of CB and IHC on FNA samples can provide a precise and accurate diagnosis, also taking into consideration the growth fraction of BCT. Thus, we recommend CB application to evaluate cytological samples in lung pathology allowing the chance for genetic testing, prognostic assessment and determining an appropriate therapeutical management.

Conflicts of interest: The authors declare no conflict of interest.

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