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BRIEF REPORT

ALK-rearranged primary mixed mucinous and non-mucinous lung adenocarcinoma: A case report

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KEYWORDS
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Abstract Lung cancer exhibits a diverse array of morphological manifestations and molecular changes, significantly influencing patient diagnosis, prognosis, and treatment strategies. We present the case of a 47-year-old man with a history of smoking, who presented to the emergency room with a 12-month history of haemoptysis. A chest computed tomography (CT) scan revealed a mass in the right upper lobe of the lung and bilateral lung nodules. He underwent a diagnostic wedge resection, which confirmed mixed mucinous and non-mucinous lung adenocarcinoma exhibiting acinar, papillary and micropapillary growth patterns. Molecular studies identified rearrangements in the *ALK* gene, and staging images revealed central nervous system and bone metastases. This case presents an unusual morphology of mixed mucinous and non-mucinous lung adenocarcinoma and highlights the importance of using immunohistochemical and molecular markers to determine tumour biology.

PALABRAS CLAVE
Cáncer de pulmón;
Mucinoso mixto;
ALK

Adenocarcinoma de pulmón mixto mucinoso y no mucinoso primario reordenado con ALK: presentación de un caso

Resumen El cáncer de pulmón presenta una amplia variedad de manifestaciones morfológicas y cambios moleculares, que influyen de manera significativa en el diagnóstico, pronóstico y estrategias de tratamiento del paciente. Presentamos el caso de un hombre de 47 años con

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antecedentes de tabaquismo, que acudió a urgencias con un historial de 12 meses de hemoptisis. Una tomografía computarizada (TC) de tórax reveló una masa en el lóbulo superior derecho del pulmón y nódulos pulmonares bilaterales. Se le realizó una resección en cuña diagnóstica, que confirmó un adenocarcinoma pulmonar mixto mucinoso y no mucinoso, con patrones de crecimiento acinar, papilar y micropapilar. Los estudios moleculares identificaron reordenamientos en el gen ALK y las imágenes de estadificación revelaron metástasis en el sistema nervioso central y en los huesos. Este caso presenta una morfología inusual de adenocarcinoma pulmonar mixto mucinoso y no mucinoso y subraya la importancia de utilizar marcadores inmunohistoquímicos y moleculares para determinar la biología del tumor.

Introduction

In 2022, lung cancer had the highest mortality rate among men and the second highest among women worldwide.[1] It is classified into two main types: small cell lung cancer and non-small cell lung cancer. The latter is further subdivided into adenocarcinoma, squamous cell carcinoma, and large cell carcinoma. Within the lung adenocarcinoma group, there are both mucinous and non-mucinous tumours. Among the non-mucinous types, the lepidic, acinar, papillary, solid, and micropapillary growth patterns are identified, each with a reported 5-year survival rate of 90%, 84%, 83%, 70%, and 67%, respectively.[2] Acinar adenocarcinomas may present with a cribriform pattern, which is associated with a worse prognosis and is characterized by a cluster of tumour glands fused without stroma, forming poorly structured glandular lumens.[3] The broad morphological spectrum of lung adenocarcinoma can complicate its precise classification and differentiation from other types of cancer.

Conversely, invasive mucinous adenocarcinoma (IMA), characterized by mucus-producing tumour cells, accounts for approximately 10% of all lung adenocarcinomas.[4] When it coexists with a non-mucinous pattern comprising more than 10% of the tumour, it is classified as mixed invasive mucinous and non-mucinous adenocarcinoma, representing half of all IMAs.[5] In this article, we present a case study of multifocal pulmonary adenocarcinoma with a mixed mucinous and non-mucinous pattern. Our discussion encompasses both the morphological features and the molecular alterations observed in this case.

Clinical case description

A 47-year-old man, with a smoking history of 2.3 pack-years, presented with a 12-month history of haemoptysis. Chest computed tomography (CT) revealed a spiculated subpleural pulmonary mass measuring 37 mm × 24 mm in the anterior segment of the right upper lobe, accompanied by numerous bilateral lung nodules, some of them cavitated (Fig. 1A). Furthermore, an abdominal CT scan showed blastic-type masses with poorly defined margins in the sacral and iliac bones, suggesting secondary involvement (Fig. 1B). Subsequently, he underwent a diagnostic wedge resection of the lung, during which surgeons found grey nodular lesions and pleural retraction in the pulmonary parenchyma.

The pathology study revealed a multifocal pulmonary adenocarcinoma with a mixed mucinous and non-mucinous pattern, including acinar, papillary, and micropapillary subtypes, along with areas of cribriform morphology grade 2 (moderately differentiated) (2021 WHO) (Fig. 2). Furthermore, the tumour exhibits spread through air spaces (STAS), involvement of the section margin, and no evidence of malignancy in the pleura. Immunohistochemistry (IHC) studies showed tumour positivity for TTF-1, Napsin A, CK7, CK19, CEA, and CA19.9, while PAX8, CK20, CDX2, MUC2, CD117, S100, P53, P40, and SATB2 were negative (Fig. 3). The molecular tests showed genetic rearrangements involving the anaplastic lymphoma kinase (*ALK*) gene, as confirmed by both immunohistochemistry using the D5F3 antibody, and fluorescence in situ hybridization (FISH) using the *ALK* probe (2p23) (Fig. 3). No rearrangements in *ROS1* were identified. PD-L1 expression (22C3) was negative, and no mutations were identified in the *EGFR* or *KRAS* genes. A post-operative brain magnetic resonance imaging (MRI) revealed supratentorial and infratentorial metastases (Fig. 1C). A stage IVb (8th TNM edition) mixed mucinous and non-mucinous pattern adenocarcinoma was diagnosed. Based on the tumour's molecular profile, the patient was considered eligible for treatment with a tyrosine kinase inhibitor. Brigatinib was initiated at a dose of 90 mg orally once daily for seven days, followed by a maintenance dose of 180 mg per day. After one year, the patient remains under follow-up with the same treatment plan, demonstrating good tolerance to the treatment and moderate clinical improvement.

Discussion

Invasive mucinous adenocarcinoma, a rare form of cancer, accounts for approximately 10% of all lung adenocarcinomas.[6] Approximately half of these cases are classified as mixed mucinous and non-mucinous adenocarcinoma, with only 12% of such cases exhibiting ALK rearrangements.[5] Very few similar cases have been reported in the literature. One report from Japan described a mixed

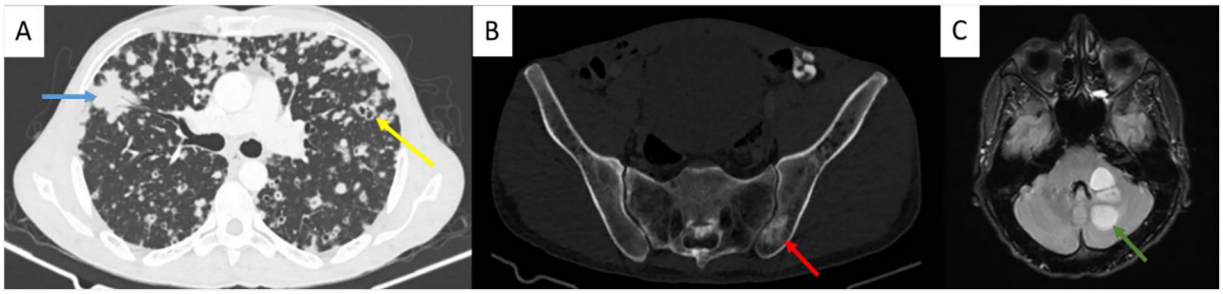

Figure 1 (A) Chest CT Scan revealing a solid mass with spiculated margins (blue arrow) in contact with the anterior segmental bronchus of the right upper lobe, measuring 37 mm × 33 mm, associated with numerous bilateral lung nodules, some of them cavitated (yellow arrow) (B). Abdominal CT scan showing left iliac bone metastasis (red arrow). (C) Post-operative brain MRI showing left cerebellar hemisphere metastases (green arrow).

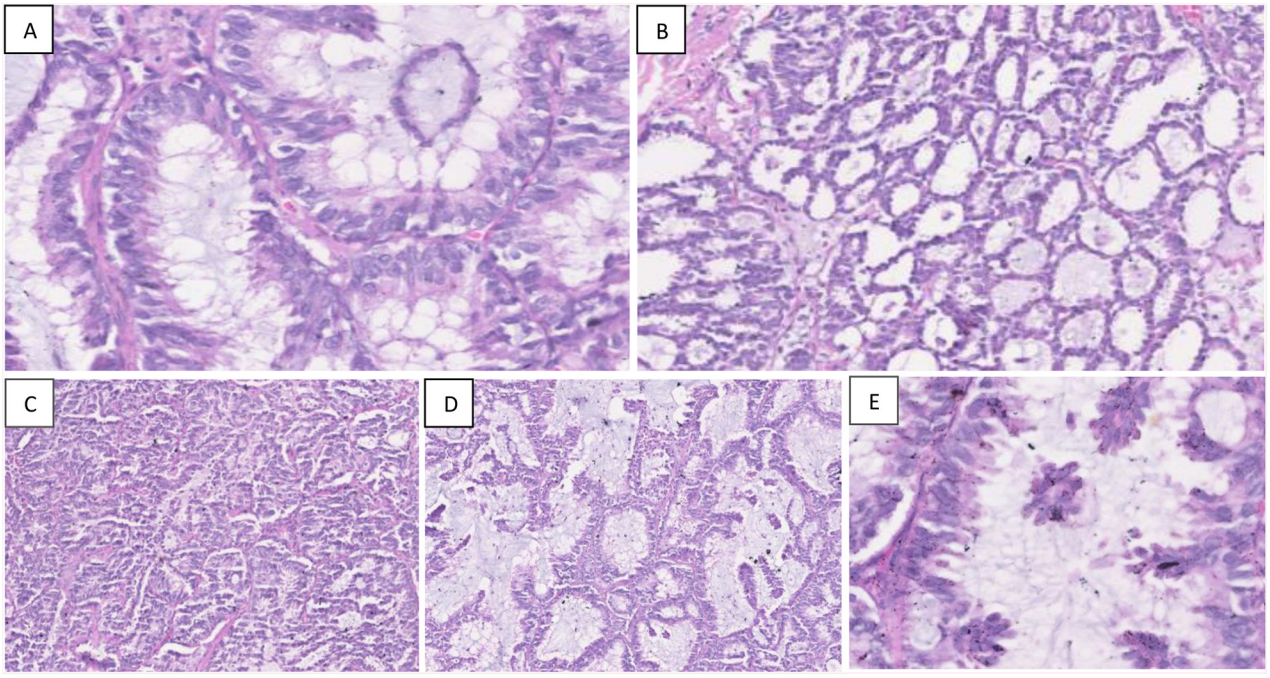

Figure 2 Haematoxylin and eosin (H&E) staining shows the morphology of the individual's lung adenocarcinoma. Mucinous (A) and non-mucinous cribriform (B) regions can be identified, as well as acinar (C), papillary (D), and micropapillary (E) growth patterns.

mucinous and non-mucinous tumour positive for CA19.9.[7] A study of 330 individuals found that, compared to mucinous and non-mucinous invasive adenocarcinomas, mixed mucinous and non-mucinous adenocarcinomas are significantly associated with the presence of micropapillary pattern, STAS, and lymph node metastasis. These findings are consistent with the tumour characteristics observed in this case.[8]

Boland et al.[5] compared the clinicopathological characteristics and survival of 750 individuals with lung adenocarcinoma, observing a 14.6% prevalence of mucinous tumours and a 7.1% prevalence of mixed mucinous and non-mucinous tumours. They also observed a higher percentage of this cancer type in male smokers, who often presented with tumours at more advanced stages compared to those with non-mucinous tumours. When prognosis was evaluated, mixed mucinous and non-mucinous adenocarcinoma had a worse overall survival rate and worse progression-free survival rate than purely mucinous adenocarcinoma or non-mucinous adenocarcinoma. Similarly, Luo et al.[9] studied 145 cases of lung adenocarcinoma with a mucinous component and found that disease-free survival was worse in mixed adenocarcinomas compared to the pure mucinous group.

Rearrangements in the *ALK* gene can activate multiple cellular signalling pathways, such as MAPK, PI3K, and AKT, leading to increased cell proliferation and survival. The prevalence of ALK alterations in lung cancer among the Latino population is approximately 5%.[10] Notably, Boland et al.[5] observed that, among 25 cases of mixed mucinous and non-mucinous adenocarcinomas, 12% exhibited *ALK* rearrangements, and most of these tumours had regions with micropapillary and cribriform growth. Similarly, Yoshida et al.[11] found that *ALK* rearrangements are frequently found in mucinous lung adenocarcinomas displaying micropapillary and cribriform regions. Another study found that the presence of *ALK* rearrangements is associated with mucinous adenocarcinomas presenting high-grade non-mucinous subtypes.[12] Overall, these findings suggest a potential association between *ALK* alterations and mixed

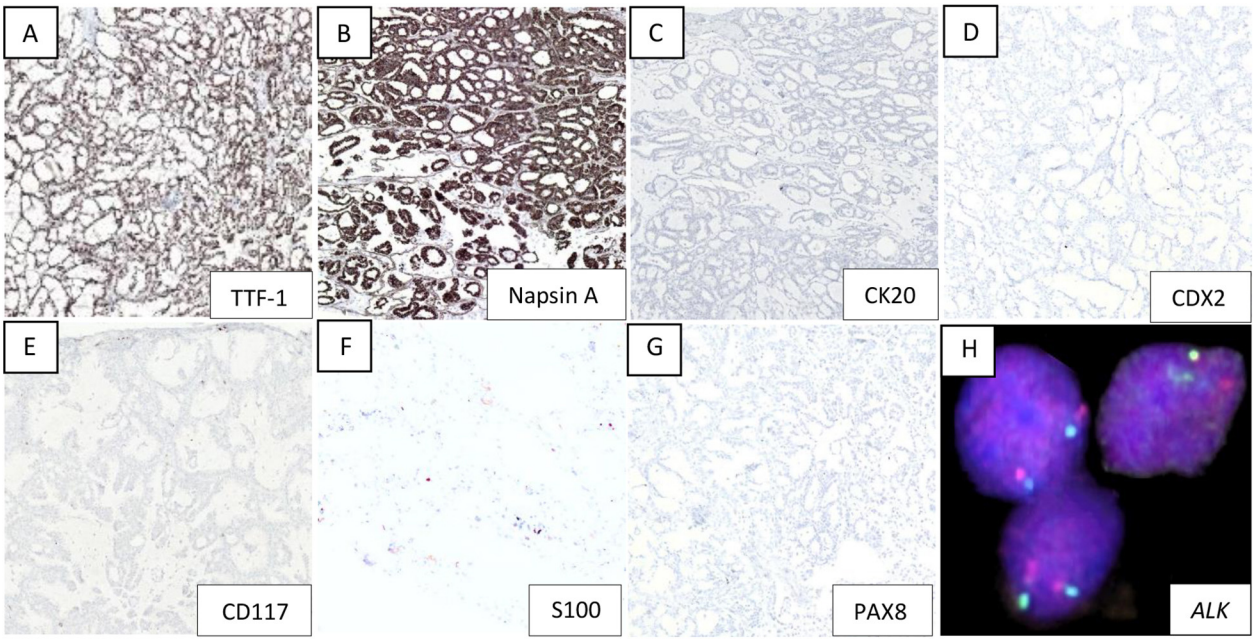

Figure 3 Immunohistochemical analysis showing a positive staining for TTF-1 (A) and Napsin A (B), and a negative staining for CK20 (C), CDX2 (D), CD117 (E), S100 (F) and PAX8 (G). (H) Tumour cells exhibiting *ALK* rearrangements detected via fluorescence in situ hybridization (FISH).

mucinous/non-mucinous lung adenocarcinomas featuring micropapillary and cribriform patterns. Moreover, *ALK* rearrangements have been significantly associated with the presence of STAS, as observed in this individual's case.[13] The presence of *ALK* expression has also been observed in mucinous adenocarcinomas of the lung with signet-ring cell.[14]

No *KRAS* mutations were identified in the tumour, despite the prevalence of *KRAS* mutations among Latinos being close to 14%.[10] Boland et al.[5] observed a lower occurrence of *KRAS* mutations in mixed mucinous and non-mucinous adenocarcinomas compared to pure mucinous adenocarcinomas, with prevalence rates of 68% and 76%, respectively. Furthermore, Kadota et al.[15] found a significant difference within invasive mucinous adenocarcinomas in a cohort of 864 individuals. Pure mucinous tumours were substantially more likely to carry *KRAS* mutations compared to mixed mucinous/non-mucinous tumours, at rates of 85% and 31%, respectively ($P = 0.002$). Meshima et al.[16] also reported that, among 47 individuals, *KRAS* mutations were less prevalent in mixed mucinous and non-mucinous adenocarcinomas compared with pure mucinous tumours and non-mucinous tumours, with frequencies of 73%, 10% and 0%, respectively. These findings imply that *KRAS* mutations are involved in the pathogenesis of mucinous adenocarcinomas, whereas their influence appears to be less pronounced in mixed mucinous and non-mucinous adenocarcinomas.

These types of tumours can pose a diagnostic challenge due to their morphology. Mucinous gastrointestinal carcinomas were ruled out based on the negativity of CDX2 and CK20 markers. Similarly, lung colloid carcinoma was considered, but ruled out because this case did not exhibit the characteristic morphology and tested negative for CK20 and CDX2, while showing positivity for TTF-1 and Napsin A. On the other hand, the possibility of a primary or metastatic tumour of salivary gland origin was evaluated, which usually tests positive for CD117 and S100, both of which were negative in this case. Furthermore, the negativity of PAX8 ruled out a thyroid origin.

Currently, the molecular profile in non-small cell lung cancer is crucial for diagnosis and treatment. Various oncogenic driver alterations, such as *EGFR*, *KRAS*, *BRAF*, *ALK* and *ROS1*, as well as PD-L1 expression, must be assessed in patients at both early and late stages.[17,18] These driver alterations can be detected using various techniques, such as immunohistochemistry, FISH, or next-generation sequencing (NGS). The implementation of NGS has the potential, compared to conventional methods, to identify a broader range of actionable and non-actionable molecular alterations and co-mutations with clinical prognostic significance in lung tumours.[19]

In conclusion, this case presents an unusual morphology of mixed mucinous and non-mucinous lung adenocarcinoma with different concurrent histological patterns and rearrangements in the *ALK* gene. Immunohistochemical markers were helpful in ruling out other differential aetiologies. This capability is crucial for optimizing treatment strategies for cancer patients with complex morphological presentations. Further studies involving Hispanic populations should explore the potential association between mixed mucinous/non-mucinous adenocarcinomas with micropapillary and cribriform growth patterns and the presence of *ALK* rearrangements.

Ethical considerations

For this case report, informed consent was obtained from the affected individual. The article contains no patient data, ensuring that the patient's privacy and confidentiality are

not compromised, and no information is provided that could lead to the identification of the patient.

Statement

Samples stored in the Anatomical Pathology departments were collected for diagnostic purposes to promote and safeguard patient health and not for experimentation on patients.

Compliance with ethical standards

Informed consent was obtained from all patients for their inclusion in the study. This study was performed in accordance with the ethical standards laid down in the 1964 Declaration of Helsinki and its subsequent amendments or comparable ethical standards. The samples stored in the Anatomical Pathology departments were collected for diagnostic purposes to promote and ensure patient health, not for experimental procedures on patients.

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Conflict of interests

The authors declare that they have no conflict of interest.

Data availability

All data generated or analyzed during this study are included in the published article.

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