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Original Article

Intravascular malignant cell dissemination pattern in KRAS mutant advanced lung adenocarcinoma: autopsy findings

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Abstract: Lung cancer is the most common cause of cancer-related death in the United States. Adenocarcinoma is the most common primary malignancy of the lung. Approximately 50-80% of pulmonary adenocarcinomas have a known oncogenic driver mutation, *KRAS* mutation being the most common. In the current study, we report the autopsy findings of two patients with advanced stage lung adenocarcinoma presenting with massive intravascular dissemination. Both cases showed a predominantly solid pattern as well as a *KRAS* mutation. The clinicopathologic implications are discussed.

Keywords: Non-small cell lung cancer, adenocarcinoma, *KRAS* mutation, disseminated intravascular tumor, pulmonary tumor microembolism, brain metastasis

Introduction

Lung cancer is currently the most common cause of cancer-related death in both men and women in the United States, with an estimated 221,200 new cases of lung cancer and 158,040 deaths in 2015 [1]. The primary risk factor for the development of lung cancer is smoking, which has been implicated in 90% of lung cancers. The risk increases with both the number of cigarettes smoked and the number of years spent smoking. Other risk factors for the development of lung cancer include prior radiation therapy, asbestos exposure, and genetic factors [2].

Based on the morphology, lung cancer can be divided into several histologic subtypes. Adenocarcinoma (ADC) is the most common type of pulmonary cancer, accounting for approximately 50% of all cases [3]. Pulmonary ADC can be further subtyped into 5 growth patterns (acinar, solid, papillary, micropapillary and lepidic) and 4 histologic variants (mucinous, colloid, enteric and fetal) [3]. The solid and micropapillary patterns have a worse prognosis.

Approximately 50-80% of pulmonary ADC have a known oncogenic driver mutation [4, 5]. The main molecular alterations include *KRAS* (Kirsten rat sarcoma viarl oncogene homolog) mutations, present in approximately 20-35% of the cases. Epidermal growth factor mutations (*EGFR*) are seen in 10-20% of the cases. Other important alterations include *ALK* (anaplastic lymphoma kinase) rearrangements present in approximately 3-5% of the patients [6]. Targeted inhibitors have been used against epidermal growth factor receptor (EGFR) and anaplastic lymphoma kinase (ALK), in tumors with specific *EGFR* mutations and *ALK* rearrangements, respectively, and more recently for tumors with translocated *ROS1* and RET [7].

KRAS mutation is the most common oncogenic driver mutation seen in pulmonary ADC, and it is more frequent in Caucasian patients with smoking history [4, 7, 8]. Of clinical relevance, it is associated with poor prognosis, high recurrence rates as well as resistance to chemotherapy. Approximately 95% of these mutations are present as single amino acid substitutions in condons 12 and 13, and less frequently at codon 61 [8]. These are activating mutations that result in downstream signaling cascades and cell proliferation [8].

Studies aimed at identifying microscopic evidence of malignant cell invasion of blood vessels in bronchogenic carcinoma, found it in 88%

of studied specimens. Of these 40% showed malignant cells in contact with blood returning to the heart, and 80% of these patients died with widespread vascular deposits. This invasion was reported in 80% of squamous cell carcinoma and in 100% of large cell carcinoma, small cell carcinoma, and adenocarcinoma [9, 10].

In the current study, we report the autopsy findings of two patients with advanced stage lung adenocarcinoma with *KRAS* mutations presenting with massive intravascular dissemination, and discuss clinicopathologic implications.

Materials and methods

Clinical histories were abstracted from retrospective chart review. Pathologic and autopsy records and all available H&E stained slides and immunostains were reviewed.

Results

Case histories

Patient 1: A 38-year-old white male with a 0.5-pack-year smoking history, presented to the emergency room with a history of four weeks of intermittent bilateral buttock pain, urine incontinence and strain. He had a history of hypertension, pulmonary embolism, and *KRAS* mutation-positive stage IV non-small cell lung cancer (NSCLC) diagnosed from an excisional biopsy of a cervical lymph node in a previous year. He was on daily lovenox and was initially treated with paclitaxel, carboplatin, bevacizumab, and metformin for 6 cycles; followed by bevacizumab plus metformin maintenance. Clinical follow-up showed progression of disease; thus therapy was changed to programmed cell death 1 (PD-1)/PD-1 ligand 1 (PD-L1) under a clinical trial. Computed tomography (CT) of the chest was remarkable for some areas of ground glass opacities, a right pleural effusion, as well as progression of his disease. A repeat CT chest showed dense ground glass consolidations suspicious for multifocal pneumonia, new metastasis or pulmonary infarct. Eventually he became increasingly hypoxic requiring oxygen by non-rebreather mask. He developed increased troponins and an echocardiogram showed an akinetic inferior and lateral left ventricular wall, and he subsequently died.

Patient 2: A 69-year-old white female arrived in the emergency department with acute shortness of breath. Her past medical history was significant for a T3N2 *KRAS* mutant lung adenocarcinoma in the left lower lobe status post lobectomy (07/2011), hypertension, right hip surgery, and chronic obstructive pulmonary disease (COPD). She had a 100-pack-year smoking history. After her diagnosis of lung adenocarcinoma and surgery, she was subsequently found to have an enlarging, spiculated right upper lobe lung mass which was resected with a wedge resection followed by completion lobectomy. A few months later, she developed recurrent pleural effusions on the right side), and underwent right wedge biopsy and catheter placement. The biopsy results were positive for recurrent adenocarcinoma. On a subsequent admission, a CT scan of the chest showed right loculated pleural effusions and nodular densities in the right pleural space along with a developing pneumonia in the right lower lobe. There was also extensive lymphadenopathy, and peribronchovascular consolidation in the right chest, radiographically suspicious for lymphangitic carcinomatosis. The patient experienced worsening shortness of breath and a pulmonary embolus was identified in the right lower lobe and treated with heparin. She was transferred to the palliative care service. The family changed the goals of care to comfort care only, and she died shortly after extubation.

Pathology

Patient 1

Previous pathology: Pathological examination of cervical lymph nodes performed at an outside institution was positive for metastatic adenocarcinoma consistent with pulmonary origin. Immunohistochemistry showed neoplastic cells to be positive for napsin-A, TTF-1, and CK7, with a high proliferation index (Ki-67), while being negative for EGFR and CD5/6.

A mutation screening panel was also done by next generation sequencing (NGS), indicating that the tumor tissue was also positive for a *KRAS* p.G12D mutation. Investigation of the following genes showed no mutations: *AKT, BRAF, ERBB2, NRAS, PIK3CA*, and *EGFR.* Of note, *EGFR* presented a polymorphic variant (rs-17289589; c.293G>A; pR98Q) within exon 3.

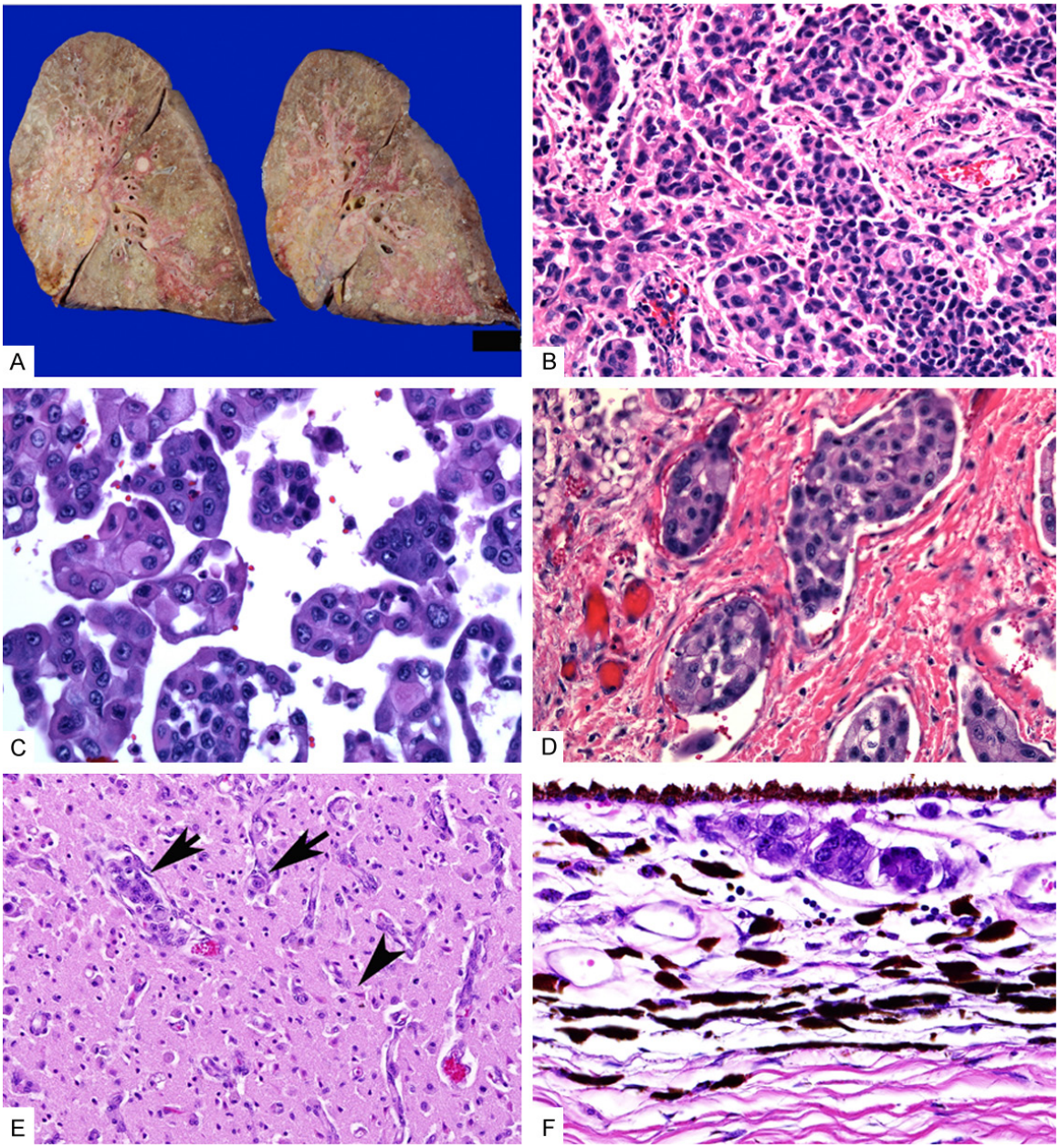

Figure 1. Autopsy findings in patient 1. The lung parenchyma grossly demonstrates a military pattern, consistent with lymphovascular dissemination (A). Histologic patterns included solid (B) and micropapillary (C). Promnent intravascular spread was evident in the lung (D), the brain (arrows) with associated with acutely ischemic neurons (arrowhead) (E), and choroidal vessels in the eye (F).

Autopsy: The right lung weighted 1100 g (reference range, 360-570 g) and the left lung 970 g (reference range, 325-480 g). The pleura showed multiple areas of nodularity and pleural puckering bilaterally (**Figure 1A**). The right lung demonstrated complete replacement of the right middle lobe by a firm, white-tan, poorly circumscribed neoplasm with areas of focal hemorrhage extending to the inferior portion of the right upper lobe. The remaining right lung parenchyma was remarkable for innumerable stellate white-tan nodules located both centrally and peripherally ranging from 0.3 to 1.0 cm in greatest dimension.

The left lung showed diffuse, innumerable, stellate white-tan nodules throughout the lung parenchyma centrally and peripherally ranging

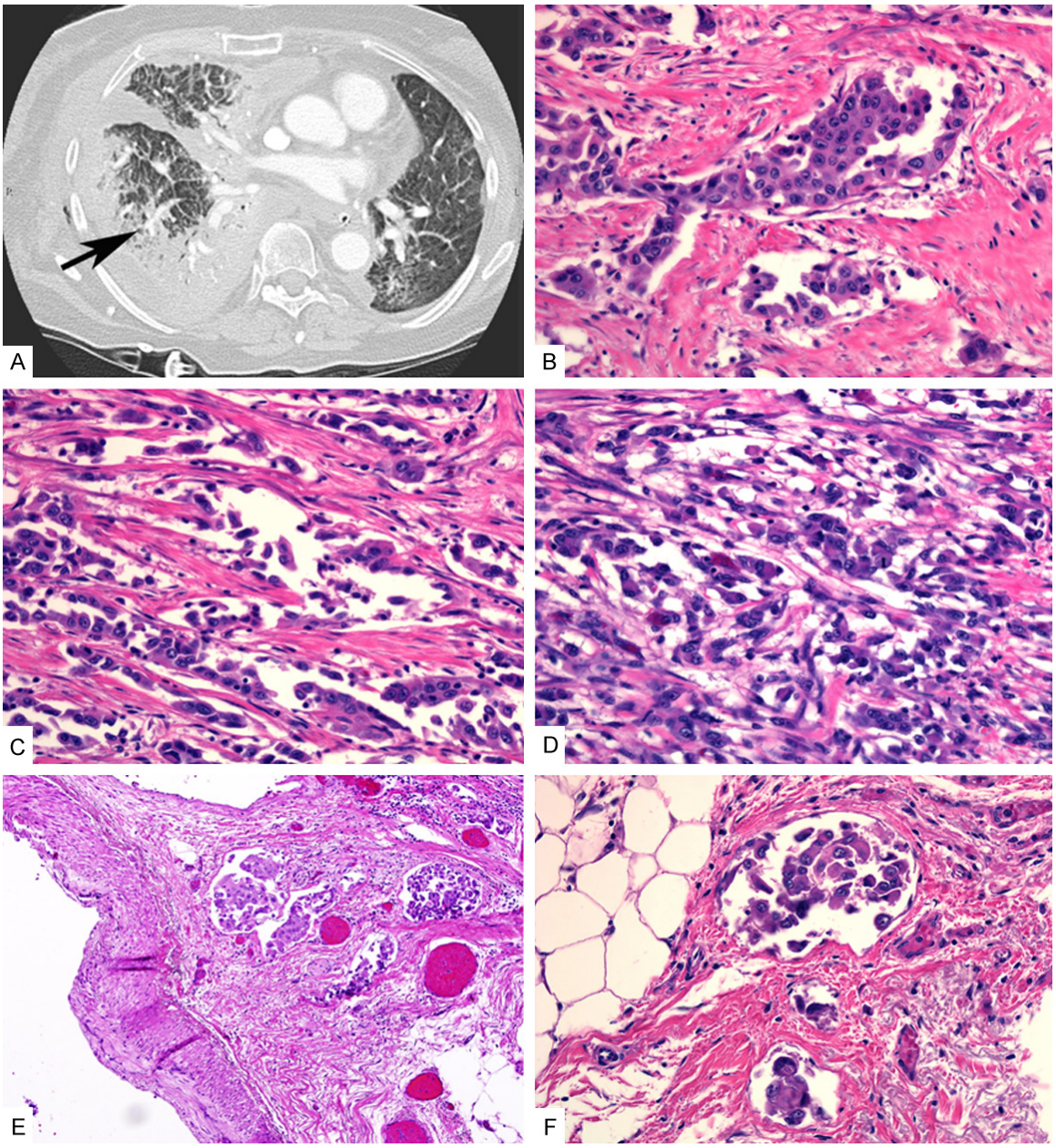

Figure 2. Radiographic and autopsy findings in patient 2. Chest CT prior to death demonstrate lung consolidation and possible septal/interstitial lymphangitic spread (arrow) (A). Histologic patterns included solid (B), acinar (C) and rhabdoid (D). Lymphovascular dissemination was present in mediastinal soft tissues (E) and lung (F).

0.3 to 1.0 cm in greatest dimension. There was hilar and peribronchiolar lymphadenopathy. Histologically examination demonstrated a poorly differentiated adenocarcinoma, of predominantly solid pattern, with extensive lymphovascular invasion and lymphangitic spread involving all five lobes and sampled peribronchial and mediastinal lymph nodes. Acute diffuse alveolar damage involved parenchyma of both upper lobes. The remainder of the non-neoplastic parenchyma showed non-specific changes, i.e. focal chronic inflammation and focal intraalveolar accumulation of macrophages consistent with mass effect.

Additional examination demonstrated widely metastatic adenocarcinoma with a predominantly solid pattern. A minor component of micropapillary pattern was also seen, as well as cribiform formations involving bilateral lungs,

liver, left and right adrenal, left kidney, pancreas, vertebral bone marrow, thyroid, anterior chest wall, right diaphragm, and esophagus with marked lymphangitic spread (**Figure 1B, 1C**). There was also widespread dissemination involving the periesophageal, gastro-epiploic, peripancreatic, periaortic, bilateral pulmonary hilar, peribronchiolar, mediastinum, bilateral cervical lymph nodes, central nervous system and eyes, with intravascular involvement of choroid, iris and ciliary body (**Figure 1D-F**).

Patient 2

Previous pathology: A wedge resection of left lower lobe nodule showed moderate differentiated adenocarcinoma (1 cm in greatest dimension). The tumor was present at the inked surgical resection margin. Completion left lower lobe lobectomy showed a tumor size of 2.5 cm in greatest dimension. Metastatic carcinoma was found in 5 of 10 lymph nodes. One hilar lymph node, 2 L-10 lymph nodes, and 2 station 7 lymph nodes were positive for metastatic carcinoma. Tumor extended to the visceral pleura. Tumor was classified using the 7th edition, AJCC as pT3, pN2, margins involved by invasive carcinoma in parenchyma. Lymphatic (small vessel) invasion was present, but no invasion to venous/arterial (large vessel) was identified. In addition, emphysematous changes were found.

Immunohistochemistry showed neoplastic cells to be positive for TTF-1 and negative for P63. In addition, stains in the lymph nodes for AE1/AE3 highlighted few carcinoma cells. *KRAS* mutational analysis, was done by RTPC method, showed that the tumor tissue was positive for a *KRAS* p.G12F mutation. *EGFR* mutational analysis was not performed due to the *KRAS* result. Subsequent histological studies performed on the right upper lung lobe nodule reported a pleomorphic carcinoma with mixed giant cell, rhabdoid, spindle cell and adenocarcinoma components. The tumor size was 1.9 cm in greatest dimension; histologic grade was G3 (poorly differentiated). All 13 lymph nodes were negative for tumor. Tumor classification (7th edition AJCC) was pT1a, pN0. Margin uninvolved by invasive carcinoma. Venous/arterial (large vessel) invasion was present. Lymphatic (small vessel) invasion was absent. Additionally, findings consistent with pneumonia were present. Analysis of a complete right upper lobectomy showed atypical adenomatous hyperplasia arising in an area of subpleural scarring. Two lymph nodes and associated fibroadipose tissue were negative for tumor. Ten lymph nodes (R10 dissection), one lymph node (station 7 dissection) and associated fibroadipose tissue were negative for tumor.

Autopsy: Permission was given for a limited autopsy restricted to the chest cavity. Autopsy findings were significant for extensive tumor caking the entire right pleural space and right chest wall with involvement of the soft tissues adjacent to the pericardium. A separate focus of tumor was present within the muscle of the left ventricle. On histologic examination, the tumor was a poorly differentiated adenocarcinoma, morphologically similar to that from the prior right upper lobe resection. The tumor was present within small vessels and lymphatics within the interlobar septae, and it grew out and along the septa in a lymphangitic pattern (**Figure 2**). There was an organizing thromboembolus without tumor cells in the right lower lobe. The left lung was congested and patchy, with acute bronchopneumonia in the left upper lobe. There was also extensive hilar, peribronchial, paraesophageal, and mediastinal lymphadenopathy with representative sections showing metastatic adenocarcinoma. The heart was not enlarged, but there was a focus of metastatic tumor within the wall of the anterior left ventricle, distant from the site of pericardial involvement. The tricuspid valve showed nonbacterial thrombotic endocarditis, with fibrin deposition and nodular fibrosis of the valve leaflets. The mitral valve leaflets had evidence of myxoid degeneration and fibrosis. Sections of the tumor were compared to sections from the tumor in the right upper lobectomy performed at Johns Hopkins Hospital on 02/11/2014. The tumor in both cases had a similar morphology. The autopsy diagnosis was extensive metastatic poorly differentiated adenocarcinoma predominantly solid and acinar components involving right pleural cavity, right interlobar septae, right pericardial sac/heart, and right chest wall, and intravascular adenocarcinoma, involving septal vessels and lymphatics.

Discussion

The cases we are describing here represent advanced stage lung adenocarcinoma with extensive intravascular spread. Both cases presented with malignant effusion, incurable, stage IV disease and were managed with palliative

therapy, as described above. Both patients had tumors with a predominant solid pattern, massive intravascular dissemination and *KRAS* mutations.

Significant risk factors for death in these patients was likely the hypercoagulable state induced by metastatic adenocarcinoma. Although the exact mechanism of thrombosis is not clear, it appears to be that neoplastic cells are able to secrete factors, such as inflammatory cytokines, that promote the coagulation cascade, induce endothelial damage, and may be responsible for areas of decreased blood flow. Some studies report that up to 50% of cancer patients show evidence of hypercoagulability at autopsy; however, rarely is thrombosis the initial presentation of malignancy [11]. It has been reported that different signaling pathways could be involved in the mechanism of the increased coagulation pattern found in cancer. For example, activation of MET, loss of PTEN, activation of KRAS, and/or loss of p53 in several experimental models of human cancers have been associated with activation of clotting pathways as an integral feature of neoplastic transformation [12, 13]. Thromboembolic findings have been reported to have an increased incidence in lung cancer as confirmed by autopsy studies [14]. Five different pathogenetic processes have been described as the causes of pulmonary vessel embolism by tumor cells: 1) large tumor emboli occluding either the main pulmonary arteries or the large segmental branches, 2) generalized lymphatic involvement, 3) pure microscopic tumor emboli involving the small arteries or arterioles, 4) combinations of 1, 2, and 3 above, and 5) widespread involvement of the alveolar septal capillaries [15, 16]. In patient 1 at autopsy, both leaflets of the mitral valve showed adherent blood clot. The occlusion of the left circumflex artery by embolus abruptly stopped blood flow to a portion of the left ventricle resulting in infarction. A similar process was observed in the brain manifesting as multiple diffuse infarcts of the grey and white matter.

Because patient 1 participated in a clinical trial for PD-L1 antibody, a known potential adverse effect of pneumonitis was clinically suspected. However, the histopathological features specific to the pneumonitis induced by PD-1/PD-L1 antibody are not fully described yet [17, 18]. Although evidence of pneumonitis was not observed, the possibility remains that the steroid therapy and discontinuation of the therapy had effectively treated a pneumonitis. He also presented diffuse alveolar damage, with underlying etiologies including sepsis, diffuse pulmonary infection, gastric aspiration, trauma, inhaled irritants, chemical injury, multiple transfusions, radiation, chemotherapy, and hypersensitivity to organic solvents and drugs, among others. In many cases, a combination of predisposing conditions is present.

Of note, both of these patients had tumors with *KRAS* mutations albeit involving different codons. Approximately 35 different *KRAS* missense mutations have been reported in patients with lung adenocarcinoma. Including our cases, G12D represents approximately 17% and G12F 0.53% of all missense *KRAS* mutations reported so far. *KRAS*-mutated lung adenocarcinoma's patients have been characterized by having a smoking history, adverse prognosis, and more recently with histopathological patterns [19-22]. Reckhtman et al, 2013 [23] found that lung adenocarcinomas harboring a *KRAS* mutation are more frequent to have a solid growth, mucinous patterns, or tumor-infiltrating leukocytes. Additionally, *KRAS* mutations have been linked to several mechanisms of complications in cancer, as increased on coagulation [12, 13] which both of the cases had.

Interestingly, the presence of vascular invasion in the most common NSCLC groups, adenocarcinoma and squamous cell carcinoma, does not affect patients' prognosis. On the other hand, lymphatic permeation has been associated with a poor prognosis [24]. The first patient also had massive intraalveolar tumor spread as defined by Warth et al [25].

Patient 1 reported here had a massive intravascular dissemination of the cancer cells, invading not just the pulmonary vessels but also the brain and eyes microvasculature, presenting multiple neocortical grey and white matter, basal ganglia, and cerebellum microinfarcts, associated with fibrin and tumor thrombi, diffuse ischemia and infarcts of the liver and spleen. This patient harbored the third most common 35G>A (located on exon 2), transition, G12D KRAS mutation, which results in an amino acid substitution at position 12 in KRAS, from a glycine (G) to an aspartic acid (D). Located on the P-loop region of the G domain [26]. On the other hand, patient 2 who present-

ed less severe pulmonary intravascular tumor cell dissemination, has the less common 34_35GG>TT, transversion, G12F KRAS mutation. Glycine (G) is an aliphatic nonessential AA, Aspartic acid (D) is and acidic nonessential AA, while, Phenylalanine (F) is an aromatic, essential AA. It is possible that KRAS mutations played a role in the massive cancer cells dissemination into the vascular system in the patients reported here, a finding that deserves further investigation.

The presence of a polymorphic variant R98Q within exon 3 of the *EGFR* gene found in patient 1 does not have a known clinical significance. The minor allele frequency is 0.2% according to genome.ucsc.edu. In this context, the presence of silent polymorphisms in the *EGFR* exons, as well as their co-occurrence with other mutations without clear clinical implications have been reported [27, 28, 22]. However, Choi et al, 2007 [29] suggested that the 181946C>T (Q787Q) *EGFR* polymorphism, could be used as a marker for the genetic susceptibility to lung cancer in a Korean population. They found that the 181946C allele was associated with a statistically significantly increased risk of lung cancer compared to the 181946T allele. It has been shown that *EGFR* and *KRAS* mutations are mutually exclusive in lung adenocarcinomas [22, 30-33]. Thus, once a *KRAS* mutation in patient 2, not further mutational screening was performed.

In conclusion, the patients reported here presented advanced lung adenocarcinoma with prominent intravascular dissemination, and both had a *KRAS* mutation in codon 12. The *KRAS* mutation could contribute to more aggressive features of lung adenocarcinoma, including intravascular dissemination. Further studies in the future with larger number of cases of this curious phenotype should be helpful in this regard.

Disclosure of conflict of interest

None.

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