metaplasia/dysplasia or clonal patches.[4] We speculate that the molecular changes in the airway epithelium of an affected bronchiole in the context of constrictive bronchiolitis might account for the increased risk of developing non-small-cell lung carcinoma in our patient, although this association requires further confirmation. In conclusion, constrictive bronchiolitis should be included as a differential diagnosis of DCLD and it is speculated that it may determine an increased risk of lung cancer.

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Martina Rodrigues de Oliveira,[a] Alexandre Franco Amaral,[a] Ellen Caroline Toledo do Nascimento,[b] Carlos Roberto Ribeiro Carvalho,[a] Bruno Guedes Baldi[a,*]

[a] *Divisao de Pneumologia, Instituto do Coracao, Hospital das Clinicas HCFMUSP, Faculdade de Medicina, Universidade de Sao Paulo, Sao Paulo, SP, Brazil*
[b] *Departamento de Patologia, Hospital das Clinicas HCFMUSP, Faculdade de Medicina, Universidade de Sao Paulo, Sao Paulo, SP, Brazil*

* Corresponding author.
E-mail address: bruno.guedes2@terra.com.br (B.G. Baldi).

1579-2129/

Calcified Pulmonary Nodules in an Oncological Patient

Nódulos pulmonares calcificados en un paciente oncológico

Dear Director:

A 50-year-old female patient underwent thoracic and abdominal computed tomography examinations for oncological follow-up. The images showed multiple lung nodules, some of which were calcified (Fig. 1A and B), a calcified hepatic mass, and an expansile osteolytic lesion with internal foci of calcification on the ischiopubic ramus of the right hip (Fig. 1C). The patient had undergone colonoscopy 3 years previously due to rectal bleeding, which showed an exophytic and stenosing rectal lesion. The biopsy findings were compatible with well-differentiated tubular adenocarcinoma. Surgical resection confirmed the anatomopathological

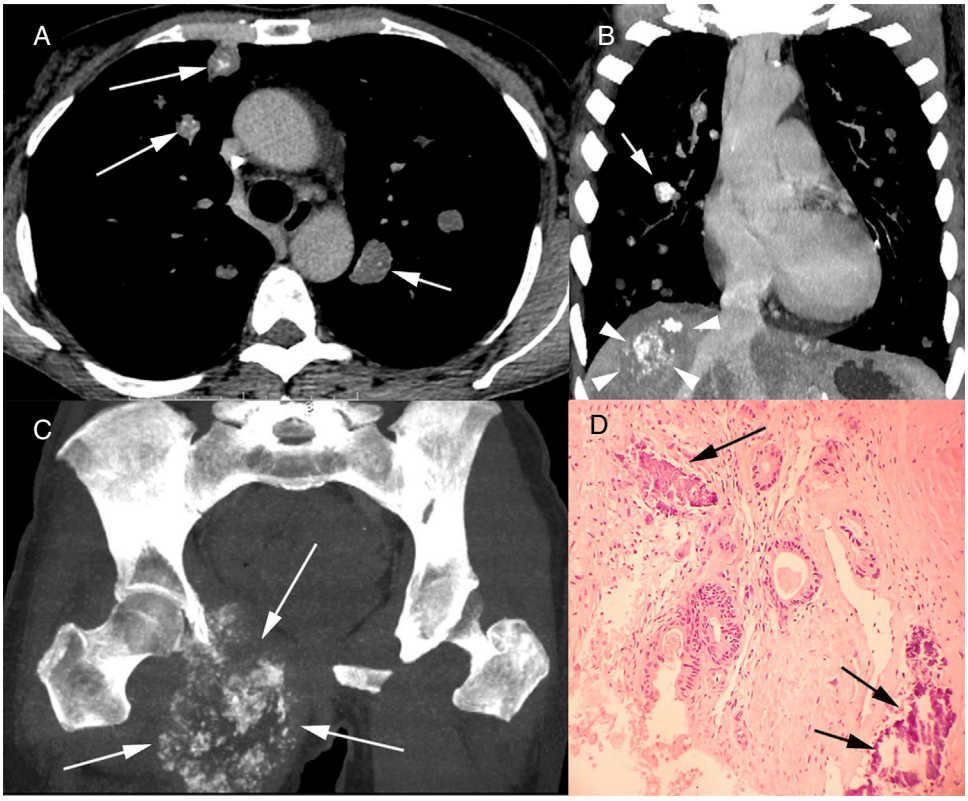

Fig. 1. Chest computed tomography with axial (A) and coronal (B) reconstruction showing multiple pulmonary nodules, some with calcification (arrows). Note also in B a calcified mass in the right lobe of the liver (arrowheads). In C, computed tomography of the pelvis with coronal acquisition MIP reconstruction, showing an osteolytic lesion with internal foci of calcification (arrows) and invasion of surrounding soft tissue. In D, histological section of the pulmonary nodule demonstrating atypical neoplastic glands infiltrating the connective tissue amid desmoplastic stroma. Note also the amorphous basophilic material, compatible with extracellular deposition of calcium adjacent to the neoplastic process (arrows; hematoxylin and eosin stain, ×100).

diagnosis and identified vascular and perineural invasion with metastasis to the peritumoral lymph nodes. The patient started chemotherapy at that time. Our main question was whether the new lesions were metastases of the rectal tumor or corresponded to a new tumor (e.g., bone sarcoma with pulmonary and hepatic metastases), which would imply the need to change the treatment strategy. Biopsies of the bone lesion and a pulmonary nodule were performed, and showed metastatic adenocarcinoma with a tubuloacinar pattern (Fig. 1D).

Calcification of a pulmonary nodule is usually suggestive of its benign nature – such nodules are most commonly granulomas and less commonly hamartomas – but calcification and ossification can also occur in malignant lesions. Multiple pulmonary nodules have numerous etiologies, but the diagnostic possibilities are considerably reduced when these lesions show calcification. The main diagnostic considerations are calcified pulmonary metastases, amyloidosis, hyalinizing granulomas, epithelioid hemangioendothelioma, necrobiotic nodules, and multiple chondromas. Amyloidosis, in its nodular form, is usually asymptomatic. The definitive diagnosis is made by histopathology, on the basis of the finding of deposition of amyloid, which stains with Congo red and shows apple-green birefringence under polarized light. Hyalinizing granulomas are rare fibrotic pulmonary lesions, usually associated with autoimmune phenomena related mainly to exposure to mycobacterial or fungal antigens. Epithelioid hemangioendothelioma is a rare multifocal pulmonary neoplasm of endothelial origin. It is considered to be a sarcoma of low aggressiveness. Necrobiotic nodules can develop in patients with pneumoconiosis associated with rheumatoid arthritis. Calcification in pulmonary chondromas is a common radiological finding. The association of these chondromas with gastrointestinal stromal tumors and extra-adrenal paragangliomas is known as the Carney triad.[1–3]

The calcification of pulmonary metastases is very uncommon. The tumors that most commonly give rise to calcified metastases are sarcomas (osteosarcoma, chondrosarcoma, synovial sarcoma, and giant cell tumor of the bone), carcinomas (particularly mucinous and papillary adenocarcinomas), and treated metastatic choriocarcinoma. Several mechanisms are responsible for the calcification of metastases: bone formation in tumor osteoid in osteosarcoma; calcification and ossification of tumor cartilage in chondrosarcoma; dystrophic calcification in papillary carcinoma of the thyroid, giant cell tumor of the bone, synovial sarcoma, and treated metastatic tumors; and mucoid calcification in mucinous adenocarcinoma of the gastrointestinal tract and breast. Calcification can develop in metastases of several other tumors after chemotherapy or radiotherapy, generally secondary to degeneration, hemorrhage, and necrosis.[1,4,5] Although tubular-type adenocarcinoma is not listed among the major causes of calcified metastases, the patient described here had undergone previous chemotherapy, which may have been the mechanism for calcification formation.

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Carla dos Santos Rossi,[b] Thiago Franchi Nunes,[a] Edson Marchiori[c,*]

[a] *Instituto de Tratamento do Câncer, Campo Grande, MS, Brazil*
[b] *Universidade Federal de Mato Grosso do Sul, Campo Grande, MS, Brazil*
[c] *Universidade Federal do Rio de Janeiro, Rio de Janeiro, Brazil*

* Corresponding author.
E-mail address: edmarchiori@gmail.com (E. Marchiori).

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Changes in the Melting Peak of Hybridization Probes Used for Genotyping in Alpha-1 Antitrypsin Deficiency Do Not Always Imply Errors☆

Las alteraciones en el pico de fusión de las sondas de hibridación usadas para el genotipado en la deficiencia de alfa-1 antitripsina no siempre implican errores

To the Editor,

Molecular analysis of the gene that encodes alpha-1 antitrypsin (AAT; *SERPINA1* gene) is the gold standard for the identification of allelic variants.[1] The different molecular methods that can be used for this purpose include hybridization probes or HybProbes,[2] which provide real-time PCR tracking. Once the amplification process is complete, these probes identify the genetic variants present in a particular region within the amplicon. This is a homogeneous genotyping test, that is to say, the entire process occurs in a single tube with no additional manipulation between the start of the test and the observation of the results. However, while it is a very reliable technique, errors may sometimes occur, especially in the interpretation of the results.[3]

We performed an analysis of the prevalence of non-S/S and non-Z/Z variants of the *SERPINA1* gene in a clinical population from La Palma (Canary Islands, Spain) by recruiting a series of 1510 patients regardless of the reason that led them to the pulmonology clinic. We identified 7 subjects in whom the peaks in the melting charts displayed by the HybProbe probes designed to identify the non-S/S variants showed a shift in respect of normal charts (Fig. 1). These 7 patients had been diagnosed with various respiratory diseases, such as diffuse interstitial lung disease, sleep apnea–hypopnea syndrome, and chronic obstructive pulmonary disease.

To rule out an error in the genotyping process due to differences in the saline concentration of the 7 DNA samples involved, these were prepared and analyzed again. In the new analysis, the real-time PCR genotyping platform software (LightCycler 480) continued to allocate these samples to a different genotype group than those defined by the standards, using the computer application's

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