Metaplastic human lung ossification: MicroCT virtual histology

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Learning objectives

The important role of MicroCT for the evaluation of bone structure is already well established, whereas in literature there are a few studies on animal or human lungs [1,2]. The main limit of pulmonary investigation is the low contrast of lung parenchyma.

Stock et al mapped the microstructure of calcium deposits in soft tissues specimens of patients with juvenile dermatomyositis but, to the best of our knowledge, lung metaplastic ossifications had never been evaluated by MicroCT [3]. The aim of our study was to utilize the intrinsic high contrast of calcifications to quantify by MicroCT the amount of pulmonary ossifications and to provide information about their structure.

Background

Diffuse pulmonary ossification is a chronic metaplastic process characterized by the histologic presence of bone in the interstitial or alveolar space. This rare entity, described for the first time in 1856 by Lushka, is usually associated with diffuse and chronic cardiac or pulmonary diseases [4]; it might be due also to other systemic disorders (e.g. amyloidosis). Diffuse pulmonary ossification is a marker of the chronicity and/or severity of the pathology. Two patterns are described in literature: the nodular and the less common dendriform type [5].

The nodular type has a rounded appearance and it is histologically made of calcified or ossified nodular masses within expanded alveoli [6]; it affects preferentially the lower lobes, and it’s encountered in the clinical setting of passive congestion, such as in mitral valve stenosis [7, 8]. This type of ossification is due to intra-alveolar exudates which consolidate after chronic congestion, ancient organized pneumonias or old intra-alveolar hemosiderin accumulation [9].

The dendriform type might be due to chronic pulmonary disease or it could be idiopathic. Dendriform is the preferred term because of the delicate, dendritic, coral-like, branching appearance of the metaplastic mature bone. This kind of pulmonary ossification preferentially affects the alveolar interstitium of an increasingly fibrotic lung, expanding the alveolar septa rather than the alveolar spaces with the bone piercing the interstitium and projecting into the alveolar spaces at focal points [5].

It is evident that the small size and the diameter of the bony spiculae preclude a confident diagnosis of bony metaplasia, especially on routine chest x-rays. High resolution computed tomography (HRCT) is more accurate in the depiction of calcium in the affected lungs and might provide useful information about the associated disease especially when the calcifications are extensive, but histopathological confirmation is
anyway required [10-13]. Although it is a difficult radiographic diagnosis, awareness of the entity and inclusion in the list of differential diagnoses might improve its detection.

Even at postmortem examination, which is currently the most common method of identification, its recognition depends on awareness of the disease. Palpation of lungs finding bony or calcified lung tissue should alert the pathologist to this possibility [5].

**Findings and procedure details**

The autopsy of a patient dead after 17 years of permanent vegetative state showed diffuse pulmonary subpleural and lobar metaplastic ossification areas which appeared as calcified granular concretions, involving the alveolar walls and septa, surrounded by fibrosis and chronic inflammation [14]. Because of the prolonged and severe clinical conditions of the patient the pathogenesis of the diffuse pulmonary ossification was supposed to be multifactorial even if frequent infections and ventilatory imbalances could have had a main role.

Postmortem chest radiograph showed a diffuse reticular pattern.

Microscopic analysis of the lungs revealed spiculae of lamellar bone; osteocytes, osteoblasts and osteoclasts were detected. At Goldner trichrome, Azan-Mallory and Alcian-PAS stainings, lung ossifications did not demonstrate homogeneous mineralization but osteoid features were found (Fig 1, 2).

Six specimens (1.2 x 1.2 cm) representative of all lobes were excised from the lungs of the patient, placed in a cylindrical polyethylene container (1.3-cm-diameter) with formalin and scanned by a Skyscan 1172 MicroCT.

MicroCT settings were as follows: 66 Kv of voltage, 100 µA of current; 1.280 x 1.024 pixel of Field of View (FOV); 13 µm of isotropic voxel size. All the samples underwent a 360° rotation with a rotation step of 0.4. The acquired raw data were reconstructed with N-Recon Software (Skyscan, Aartselaar, Belgium) which uses the back-projection algorithm to reconstruct axial subsequent images saved as bitmap format. Bitmap images were analysed by CT-An Software (Skyscan, Aartselaar, Belgium). All the samples were binarized applying the same parameters (Grayscale Threshold 85-255). 3D images were reconstructed by CTVox Software (Skyscan, Aartselaar, Belgium).

The examined volume of interest (VOI) for each lung sample was 980 mm$^3$.

The volume of the ossifications and the percentage of the VOI occupied by ossifications were considered respectively as the volume of binarized solid objects and as the proportion of the entire examined VOI occupied by binarized solid objects.
The analysis revealed that each sample contained a total volume of ossifications ranging from 0.27 to 17.6 mm$^3$ (average 7.79 mm$^3$). The average volume occupied by ossifications in all the examined samples was 0.79% (Table 1).

MicroCT 3D reconstructions demonstrated two kinds of lung calcifications: nodular and dendritic (Fig 3, 4), in some samples both were coexisting in the same examined area (Fig 5). These evidences supported the hypothesis that in our patient the pathogenesis of the disease was multifactorial.

### Images for this section:

<table>
<thead>
<tr>
<th>Lung Samples</th>
<th>Total analyzed VOI* (mm$^3$)</th>
<th>Total lung ossifications Volume (mm$^3$)</th>
<th>Percentage of lung ossifications Volume (%)</th>
</tr>
</thead>
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<tr>
<td>1</td>
<td>980.22</td>
<td>0.27</td>
<td>0.03</td>
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<td>980.22</td>
<td>17.06</td>
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<tr>
<td><strong>Mean Values</strong></td>
<td><strong>980.22</strong></td>
<td><strong>7.79</strong></td>
<td><strong>0.79</strong></td>
</tr>
</tbody>
</table>

*VOI: Volume of Interest

**Table 1:** MicroCT analysis of lung samples
Fig. 1: Lung section showing pulmonary ossifications (Haematoxylin-Eosin)
**Fig. 2:** Lung section stained with Azan-Mallory demonstrating osteoid features with only partial mineralization in a dendriform ossification.
Fig. 3: 3D reconstruction demonstrating nodular areas of metaplastic ossification
**Fig. 4:** 3D reconstruction demonstrating areas of dendriform metaplastic ossification

**Fig. 5:** 3D reconstruction demonstrating coexisting areas of nodular and dendriform metaplastic ossification
**Conclusion**

Diffuse pulmonary ossification is a relatively rare entity, but its knowledge is important for pathologists, radiologists and clinicians so that it is not confused or misdiagnosed as other forms of calcium deposition or granulomatous process. In literature, it is well demonstrated that the diagnosis of this disease is still made most of the time at postmortem examination and imaging techniques, especially chest x-rays, have a marginal role.

This study suggests that MicroCT could supplement conventional histology in the investigation of this pulmonary disorder. Indeed the application of MicroCT virtual histology provided unique data as 3D morphologic and structural information about the type and the amount of ossifications.

The main advantage of this technique is that samples do not require a peculiar preparation because of the intrinsic high contrast of calcifications. MicroCT analyses might be applied for the evaluation of other diseases that lead to calcifications in the lungs (e.g. granulomatous disease, alveolar microlithiasis) as much as for other ossifying pathologies in different organs or systems (e.g. kidney, heart, vessels).

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**References**
