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The diagnosis of cystic lung diseases: A role for bronchoalveolar lavage and transbronchial biospy?

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Summary
Pulmonary Langerhans' cell histiocytosis (PLCH) and lymphangioleiomyomatosis (LAM) are two rare cystic lung diseases of unknown aetiology and different pathogenesis. Although the diagnosis can be strongly suspected on the basis of the medical history and clinical and radiological features, at times a pathological confirmation of the diagnosis is necessary. Surgical lung biopsy is considered the gold standard in the diagnosis of both LAM and PLCH. However, bronchoalveolar lavage (BAL) and transbronchial lung biopsy (TBB) are less invasive procedures that can be useful in diagnosis. BAL has a high specificity but low sensitivity for PLCH, and in an appropriate clinical context it can be used to establish the diagnosis. However, even if a high percentage of pigment-laden macrophages are found in the BAL fluid of patients with LAM, no BAL findings are considered suggestive for the disease. TBB shows a low diagnostic yield (10–40%) in PLCH because of the small amount of tissue obtained and the patchy nature of the disease, although it may be of more use in LAM.

Pulmonary Langerhans' cell histiocytosis

Pulmonary Langerhans' cell histiocytosis (PLCH) is an uncommon interstitial lung disease characterized by the proliferation of Langerhans' cell infiltrates. It primarily affects young adults, and nearly all affected patients have a history of current or prior cigarette smoking. Lung involvement may occur either in isolation or as part of a multisystem disorder.[1]

Histologically, the pulmonary lesions begin as a proliferation of Langerhans' cells (LCs) along the small airways.[2,3] LCs are differentiated cells of monocyte–macrophage lineage, distinguished from dendritic cells by their characteristic cytoplasmic organelles (Birbeck granules) seen on electron microscopy and their strong expression of the CD1a antigen on the cell surface.[3,4] LCs also stain with S100 antibody, although this finding is not specific to these cells and can also be observed in neuroendocrine cells and some macrophages.[4]

Pathological findings vary with the stage: in the early stages numerous LCs invade the terminal and respiratory bronchioles, destroying the bronchiolar wall in an eccentric fashion and forming nodules. The nodules include several inflammatory cells, including LCs, eosinophils, lymphocytes,

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macrophages and plasma cells. These cellular nodules progress to cellular and fibrotic nodules, and finally to fibrotic nodules that can lack LCs entirely (Figs. 1 and 2). In the end stage, stellate fibrotic scars surrounding cystic spaces of variable diameter, and paracicatricial emphysema are the prominent lesions.[2]

Dyspnoea, cough and pneumothorax are the main clinical manifestations of PLCH. Other respiratory symptoms include haemoptysis and chest pain.[5] The most common radiological findings on high-resolution computed tomography (HRCT) are nodular and irregularly shaped cystic changes, which predominantly involve the middle and upper lobes, with sparing of the lung bases (Fig. 3).[6] In a young asymptomatic or low-symptomatic smoker this radiological pattern is so characteristic that the diagnosis is almost certain and may obviate the need for invasive investigations.

Bronchoalveolar lavage

In an appropriate clinical context BAL can be used to establish the diagnosis of PLCH. Differential cell counts may reveal a moderate increase in the proportion of eosinophils and neutrophils above that seen in smoking control subjects. The percentage of lymphocytes is normal or reduced and the CD4/CD8 ratio is decreased, as in cigarette smokers.[7]

LCs can be identified in BAL fluid of patients with PLCH by immunocytochemical techniques using anti-CD1a antibodies. In the early 1980s Chollet and colleagues used immunofluorescence staining with the monoclonal antibody OKT6 to detect LCs in BAL fluid. They found that cells reactive with OKT6 were consistently present in BAL fluid of patients with PLCH, although with great variability (1.8—25% of the total number of cells in BAL fluid). Subsequent analysis with immunoelectron microscopy showed that all cells reactive with OKT6 had the characteristics of LCs. However, small numbers of reactive cells (<3% of total cells recovered) were found in patients with different diseases.[8] Moreover, an expansion in the population of LCs on the epithelial surface of the lower respiratory tract, as detected by monoclonal antibody anti-T6, has been shown to be associated with cigarette smoking.[9]

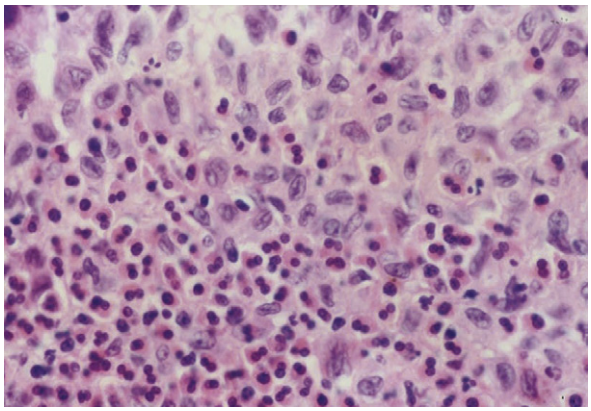

Figure 2 Detail of the cellular infiltrate in nodular lesions of pulmonary Langerhans' cell histiocytosis, showing Langerhans' cells with typical delicate and folded nuclei (haematoxylin—eosin × 400).

Comparing patients with histologically proven PLCH with patients with other lung diseases, including sarcoidosis, according to their smoking habit, Auerswald found that the percentage of CD1a-reactive cells in BAL fluid was >5% in all PLCH cases, and the dividing line of 5% CD1-positive cells was not influenced by patients' smoking habits.[10] Thus, although a low-level elevation of CD1a-reactive cells may be difficult to interpret, when the proportion of CD1a-reactive cells in the BAL fluid is >5% the diagnosis of PLCH is very likely.

More recently other authors have reported considerably lower sensitivity (<25%).[11] In our experience of 16 patients with a clinical—radiological diagnosis of PLCH, we found CD1a-reactive cells >5% in BAL fluid in four cases (unpublished data). However, in patients with an atypical clinical and/or radiological presentation BAL can be used to exclude interstitial lung diseases with more typical lavage findings (e.g. sarcoidosis) as well as pulmonary infections, such as excavated forms of *Pneumocystis jiroveci* pneumonia and mycobacterial infections.

More recently an antibody against langerin (CD207), a Langerhans' cell-specific lectin that initiates Birbeck granule formation, has been tested to detect LCs in BAL

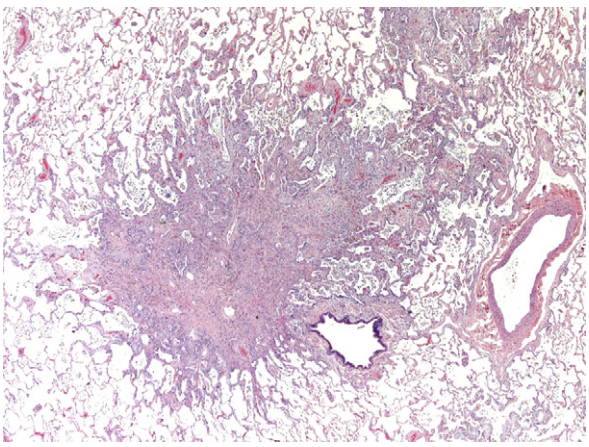

Figure 1 Lung biopsy specimen from a patient with pulmonary Langerhans' cell histiocytosis, showing a nodular lesion (haematoxylin—eosin × 100).

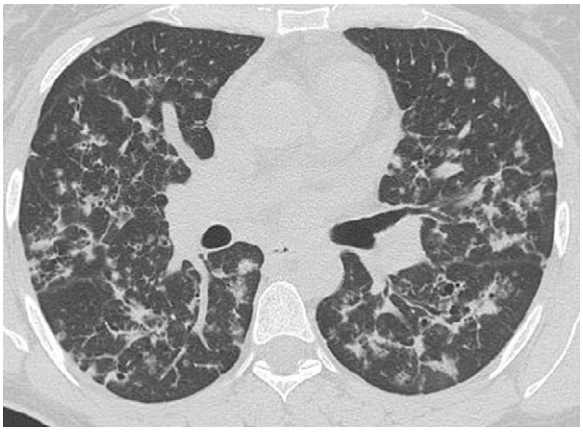

Figure 3 Computed tomography scan of the chest in a patient with pulmonary Langerhans' cell histiocytosis. The image shows a combination of nodular and cystic changes.

fluid collected from patients with PLCH, sarcoidosis and idiopathic pulmonary fibrosis.[12] The percentage of langerin-positive cells was almost identical to the percentage of CD1a-positive cells in patients with PLCH, and was significantly increased in this group in comparison with other diseases. This new LC marker may thus be useful for immunocytochemical analysis of BAL fluid in PLCH.

Transbronchial lung biopsy

TBB has shown poor sensitivity for the diagnosis of PLCH, ranging from 10 to 40%.[3,13] This low diagnostic yield is mainly accounted for by the patchy nature of the disease, with a focal distribution of the lesions, as well as the low number of active nodules in advanced disease and the small amounts of tissue obtained. Although immunohistochemical techniques using monoclonal antibodies against CD1a and S100 protein may be helpful, they do not significantly increase the diagnostic relevance of TBB because of the limitations of staining small biopsy samples (Fig. 4).[13] Furthermore, the method is frequently complicated by pneumothorax, meaning surgical lung biopsy is the safer procedure for obtaining a histological confirmation. However, the possibility of using TBB should be discussed on a case-by-case basis with an experienced pulmonologist.

Lymphangioleiomyomatosis

Lymphangioleiomyomatosis (LAM) is a rare disease that predominantly affects young women in their reproductive years. It occurs sporadically and in about 30% of women with tuberous sclerosis complex (TSC), an autosomal dominant syndrome characterized by hamartoma formation, seizures and cognitive defects.

LAM is characterized by the proliferation of LAM cells, which leads to progressive cystic lung involvement, lymphatic abnormalities and abdominal tumours (e.g. angiomyolipomas).[1,14] The lung lesions in LAM are characterized by nodules or small clusters of LAM cells near cystic lesions and along pulmonary bronchioles, blood vessels and lymphatics. LAM cells exist in two subpopulations: myofibroblast-like spindle-shaped cells, which express smooth muscle-specific proteins such as α-actin, desmin and vimentin, and epithelioid-like cells expressing glycoprotein gp100, a marker of melanoma cells and immature melanocytes that react with the antibody human melanoma black 45 (HMB45).[14–16]

Pneumothorax, progressive dyspnoea and chylous pleural effusions are the main clinical manifestations of LAM; other respiratory symptoms are cough, chyloptysis and haemoptysis.[17–21] Pneumothorax is often the first manifestation, and recurrences are common.[20] The characteristic radiological feature is cysts. On HRCT they are usually round with thin, regular walls, ranging from barely perceptible to several millimetres in diameter. They are typically distributed diffusely throughout the lungs, without sparing of the lung bases (Fig. 5).[22] A suggestive HRCT pattern in a patient with angiomyolipoma, tuberous sclerosis complex or chylous effusion (thoracic or abdominal) makes the diagnosis almost certain. In other cases a morphological confirmation of the diagnosis is necessary.

Bronchoalveolar lavage

In a comprehensive evaluation of 35 patients with LAM the results of BAL showed a higher percentage of pigment-laden macrophages than in normal subjects,[19] most likely resulting from microscopic pulmonary haemorrhages. No

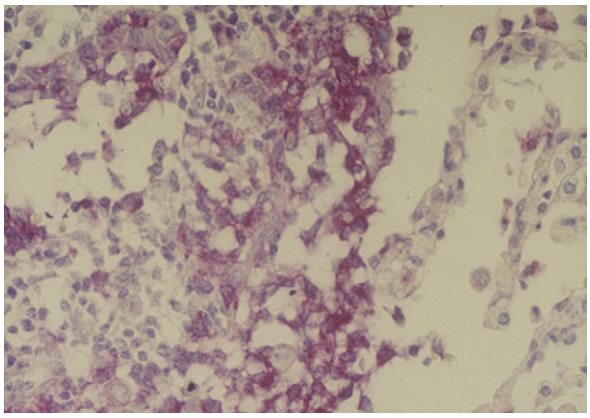

Figure 4 Detail of transbronchial lung biopsy from a patient with pulmonary Langerhans' cell histiocytosis. Langerhans' cells show intense positivity for CD1a antigen (haematoxylin–eosin × 400).

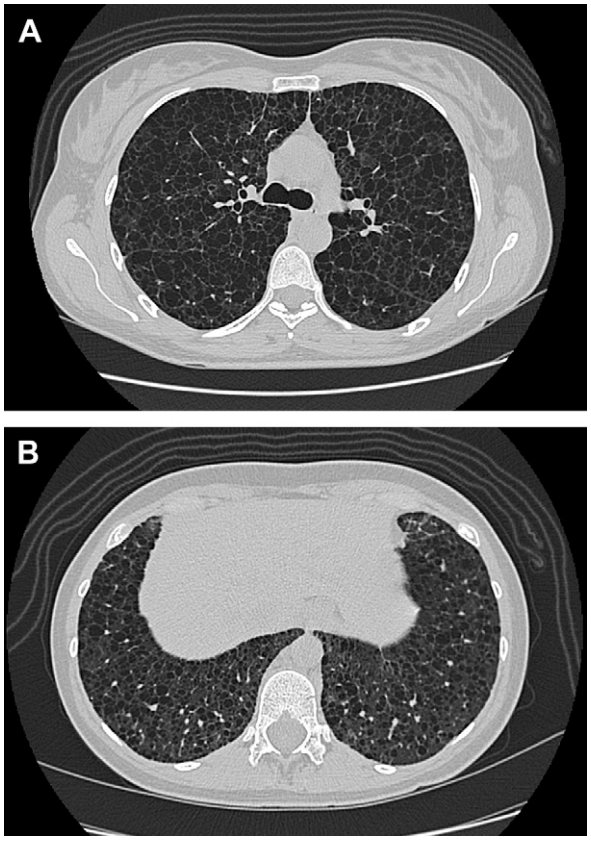

Figure 5 Computed tomography scans in lymphangioleiomyomatosis. (A) Numerous round, thin-walled cysts are distributed diffusely throughout the lungs, (B) without sparing of lung bases.

other findings in BAL fluid are characteristic of the disease and BAL does not have diagnostic relevance in LAM.

Transbronchial lung biopsy

The gold standard for the diagnosis of LAM is a tissue biopsy of lung or involved lymphatics showing nodular infiltration by LAM cells. LAM has been included on the list of interstitial lung diseases occasionally diagnosed by TBB.[23] The use of TBB findings for the diagnosis of LAM has been supported by immunohistochemical techniques. In three TBB specimens from patients with LAM (subsequently confirmed on open lung biopsies) Bonetti and colleagues found similar artefactual alveolar collapse (atelectasis). All specimens showed smooth muscle-like cells with presence of actin and epithelioid HMB45-positive cells, whereas none of 69 lesions used as controls or 20 normal lung specimens showed HMB45-positive cells.

Immunohistochemical studies of ten lung biopsy specimens from patients with LAM (seven open lung biopsies, two thoracoscopic biopsies, one TBB), using HMB45 and anti-α smooth muscle actin antibody, found LAM cells that were positive for HMB45 in all biopsy specimens, the percentage of HMB45-positive cells ranging from 17 to 67%.[24]

In our experience, among seven TBBs performed in patients with a clinical—radiological suspicion of LAM, six were diagnostic. In the remaining case the diagnosis of LAM was confirmed by surgical lung biopsy (unpublished data). No major complications were observed after any TBB procedure. TBB may be a relative safe procedure and of greater usefulness in LAM than PLCH, the more uniform distribution of histological lesions in LAM possibly accounting for this difference.

Recently, strong immunoreactivity for cathepsin K (a papain-like cysteine protease with high matrix-degrading activity) has been demonstrated restricted to LAM cells in lung biopsy specimens and angiomyolipomas.[25] This observation may provide a useful new marker for diagnosis, even in difficult cases such as TBBs providing small samples.

Conclusion

In the appropriate clinical context the presence of typical findings on an HRCT scan are often sufficient to establish the diagnosis of PLCH. A suggestive HRCT pattern in association with supportive features (i.e. angiomyolipoma, lymphangioleiomyoma, chylous collection) makes the diagnosis of LAM very likely. When pathological confirmation of the diagnosis is necessary, biopsy by video-assisted thoracoscopic surgery or an open lung approach has the best diagnostic yield in both diseases. Nevertheless, in the appropriate clinical setting, the diagnosis may be rendered based on the results of less invasive procedures.

Although BAL rarely establishes a definite diagnosis of PLCH it still represents a useful step in the diagnostic approach, to PLCH, mostly because of its safety and specificity, which in some cases can obviate the need for more invasive procedures. The routine use of TBB in PLCH is not recommended because of poor sensitivity and relatively high risk, although it is of greater use in the diagnosis of LAM.

Conflict of interest statement

None.

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