Pathology of interstitial lung diseases

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AIMS

- General overview of interstitial lung diseases.
- Preconditions and preanalytics for ILD diagnostics.
- Histological work-up and stainings.
- Specific patterns of lung injury.

SUMMARY

Pathological assessment of interstitial lung diseases requires a multidisciplinary approach. Both conventional histomorphology and cytology can provide relevant diagnostic information.

In general, the first approach to a histological diagnosis is the categorization of morphological findings according to the current standard into ontogenetic, circulatory, inflammatory, tumor-like, and neoplastic lesions. Furthermore, it needs to be addressed if and to which extent specific changes are evident in defined anatomical structures such as the pleura, the alveolae and their structure, bronchi and bronchioli, and vessels. The distribution of morphological changes within these structures are important clues to the genesis of the disease, thus, relevant findings need to be categorized as either diffuse, focal, heterogeneous etc. Next, the extent and the composition of the cellular immune reactions need to be assessed in detail. Finally, the specimens must be thoroughly screened for specific findings like granulomas, fibroblast foci, birefringent material, microorganisms etc.

Introduction

Before starting with an in-depth pathological assessment of any specimen it is of utmost importance to know all relevant clinical and radiomorphological data in order to have a context in which the histomorphological findings need to be placed. Therefore, the following points should always be provided to the pathologist prior to the assessment of any ILD specimen:
1. Clinical data: which symptoms are present and what is the extent of the disease.
2. Patient history: Are there any known diseases? Has the patient (had) any exposition to noxious substances/drugs?
4. Lung function tests: Do/Did spirometry, body plethysmography, blood gases, or diffusion capacity show any relevant changes?
5. Imaging: Which findings are/were evident in high resolution tomography imaging analysis?

It is best practice if all findings are discussed in an interdisciplinary board (clinicians, radiologists, pathologists) to come to a final diagnosis. Occasionally, it might also be important to re-evaluate specific finding over time if new findings are not compatible with the initial diagnosis.
Cytological diagnostics

The diagnostic efficiency of cytological approaches is of limited value besides for tumor diagnostics. Usually, cytology is an additional tool to further narrow-in on specific aspects but it is not suitable to achieve a final diagnosis based on cytology alone.

Cytology allows for the assessment of single individual cells as well as the composition of cell populations within a given compartment. All cytological methods such as brushings, bronchoalveolar lavage (BAL), or other aspiration techniques (eventually guided by computer tomography or ultrasound) are suitable to provide sufficient material for diagnostic assessment. Depending on the applied method one can specifically analyze the cells within the bronchial system, within an affected lung area, lymph nodes, or the pleural space (pleural effusions). Respective cellular alterations might belong to the disease process itself, might be a bystander phenomenon or might even reflect the regular cellular state within the assessed area. Disease-specific changes can hardly be expected in cytology specimens but more or less general alterations which can be associated with a group of disease entities.

In general, cytology is an important concept in sense of step-up diagnostics. The advantage of cytology is clearly the low degree of invasiveness and thus a lower rate of critical incidents; however, it lacks data concerning the tissue composition compared to biopsy specimens which often allow a more detailed assessment and thus a more specific diagnosis. Therefore, only limited data concerning tumor invasiveness, classification of an inflammatory response, or vasculitis can be retrieved by cytology.

The differentiation of cell populations allows for the categorization and classification of inflammatory reactions as acute (predominance of granulocytes), chronic (predominance of lymphocytes and plasma cells), acute and chronic (all three cell types), or histiocytic (predominance of histiocytes).

Differential cytology is of special importance for BAL where it reflects the cellular reaction within the bronchoalveolar compartment. For subtyping of the inflammatory cells (B- and T-lymphocytes, CD4/CD8 ratio) immunocytology or FACS (fluorescence activated cell sorting) analysis are required. Sputum cytology is the easiest way to retrieve cytological material; however, since material from central airways is usually not incorporated within the sputum it is usually not helpful for the differential.

Diagnostics of infectious diseases is a domain of microbiology but cytology might also be helpful in specific cases, e.g. in patients with a suppressed/compromised immune system. Additional specimens or smears for microbiological analysis should be facilitated in these cases. Special stains to highlight specific microorganisms need to be applied depending on the potential diagnosis (e.g. Ziehl-Neelsen, Toluidin, or Grocott stain) and are helpful to diagnose for example infections by pneumocystis, mycobacteria, nocardia, and fungi. Immunocytoology as well as fluorescence in-situ hybridizations (FISH) and PCR-based approaches can also be helpful for the detection of specific microorganisms.

Histological diagnostics

Tissue specimens can be retrieved by different approaches, e.g. endobronchial biopsies, transbronchial biopsies, cryo biopsies, or transthoracic needle biopsies. Surgically resected wedges, video-assisted thoracoscopic resection specimens (VATS), segment, lobe, bilobe, pneumonectomy or pleurectomy specimens.

Transbronchial biopsies and to a lesser extent endobronchial biopsies are suitable for the differential diagnostic setting of sarcoidosis, infections, or neoplasms. The biopsy specimens should be worked-up in serial sections in order not to miss the often subtle changes like incomplete granulomas. Transbronchial biopsies are specifically helpful in immunosuppressed patients where tumors or infections may lead to pulmonary infiltrates. They can further contribute to narrow-in on drug-induced pulmonary damage; an organizing pneumonia can often be diagnosed in transbronchial biopsies. However, since changes of organizing pneumonia are also evident in various pulmonary lesions the
diagnosis of a cryptogenic organizing pneumonia requires a cautious interpretation of the findings. Endobronchial biopsies are usually only helpful if there is a specific superficial process already evident during bronchoscopy.

For idiopathic interstitial pneumonias open lung biopsies are the gold standard to assess and categorize respective diffuse alterations of the lung parenchyma. In selected cases transbronchial cryo biopsies seem to be sufficient to allow for a reliable diagnosis, however, further studies are clearly needed in this regard.

The decision for an open lung biopsy is usually a single event in the patients’ history, thus, optimal processing of the specimens is of utmost importance. The optimal position for wedge resection should be discussed together with thoracic surgeons and radiologists based on thin section computed tomography imaging. It is important not to take the most fibrotic areas or areas with signs of an advance interstitial lung disease, since areas with active remodeling of the parenchyma are much more helpful to assess the underlying process of the disease. It is recommended not to resect the tips of the lobes or the lingula since a certain degree of fibrosis accumulates there during age which has no specific pathological meaning. In order to avoid atelectasis the resected specimens should gently be injected with formalin using a small syringe; in larger resection specimens this can also be done via the bronchial system. It is important to inject or instillate the formalin with low pressure in order not to wash out intraalveolar cells (e.g. macrophages, eosinophils) which can be important clues for specific diagnoses.

Histochemical stains are helpful to highlight specific characteristics within the slide, e.g. fibrosis, amyloid, or hemosiderin-laden macrophages, and are routinely performed. The PAS (Perjod Acid Schiff) stain allows for the recognition of dyskrinia or fungi, connective tissue stains (for example Masson-Goldner (MG) or Elastic van Gieson (EvG)) improve the quantitative assessment of fibrosis and elastosis. Iron stains and Kongo red stains are helpful to recognize iron pigment or amyloid, respectively. Specific microorganisms can be highlighted by Gram or Ziehl-Neelsen stains but also by silver stains (for example Grocott stain).

Immunohistochemistry allows for the specific categorization of cell populations and structures by visualizing selected antigens. Within the context of interstitial lung diseases immunohistochemistry is of special importance for the diagnosis of langerhans cell histiocytosis (CD1a+, CD207+ (Langerin), S100+) and lymphangio(leio)myomatosis (for example ASMA, HMB45, D2-40) but can also be helpful in some cases of alveolar proteinosis. In general, the application of immunohistochemistry is necessary for the categorization of lymphoid infiltrates. In specific constellations the separation of epithelial, mesenchymal, endothelial, or mesothelial cells but also the specific classification of certain microorganisms is challenging based on conventional histomorphology alone and immunohistochemistry allows for a highly specific and sensitive classification. Amyloidosis is another disease in which subclassification by means of immunohistochemistry is often helpful.

**Electron microscopy**

Electron microscopic procedures have lost their former role where ultrastructural pathology was the cutting edge of diagnostic approaches and there are only few indications left. Transmission electron microscopy (TEM) is still used to analyze the ultrastructure of cilia, for example in primary ciliar dyskinesia (PCD). Specific microorganisms, especially viruses or intracellular species, but also lamellar bodies in alveolar proteinosis can also be visualized by TEM.

**Special procedures**

In selected rare cases special procedures are required to narrow-in on a definite diagnosis. One example is cell culture for PCD, which requires specific conditions to allow for the re-formation of cilia and is only available in few diagnostic institutes.
Reaction patterns of interstitial lung diseases

The morphologic characterization of alterations based on the anatomic localization and the pattern of injury is primarily descriptive and does not allow for a definite diagnosis. The distribution of the changes within the lungs can be heterogeneous and thus the obtained biopsy must not necessarily be representative. Thus, a correlation of the histomorphological findings with imaging parameters is inevitable.

a. Types of interstitial pneumonias
One prominent example is the specific pattern of a usual interstitial pneumonia (UIP), which is further classified in a definite, probable or possible UIP pattern according to the current guidelines. Typically the UIP pattern is characterized by a heterogeneous, predominantly subpleural and paraseptal, patchy fibrosis with (micro-)honeycombing and presence of fibroblast foci. In turn, the pattern of a non-specific interstitial pneumonia (NSIP) is characterized by a more homogeneous fibrosis with an also timely and spatially more homogeneous inflammatory reaction. In addition there might be areas with signs of an organizing pneumonia but if this pattern predominates the disease is better described as an organizing pneumonia pattern. Diffuse alveolar damage (DAD) is characterized by hyaline membranes attached to the alveolar walls. Over time these membranes are being organized and may result in an NSIP-like pattern. The pattern of a desquamative interstitial pneumonia (DIP) is characterized by dense aggregates of intraalveolar macrophages accompanied by a low to moderate fibrotic thickening of the alveolar walls. In respiratory bronchiolitis interstitial lung disease (RB-ILD) this process is accentuated around the bronchovascular bundles and does not affect the whole parenchyma. Lymphocytic interstitial pneumonia (LIP) is characterized by lymphocytic/lymphofollicular infiltrates and needs to be separated from marginal zone lymphomas (MALT).

b. Other patterns of injury
Both fresh and older bleeding and the presence of iron-positive intraalveolar macrophages (siderophages) may have different causes, which need to be specifically considered. Same is true for granulomatous inflammation where the specimens must meticulously be screened for necrosis, microorganisms, and vasculitis. Finally, bronchiolitis is another pattern with different causative factors, for example viral infections or intoxication with smoke/gas.

The described patterns of injury can be seen in different degrees, distributions, and, unfortunately, also combined with each other to different extents in various diseases, whereas they must be interpreted and categorized within the given clinical context.

REFERENCES

EVALUATION

1. **Within the context of ILDs, what is the role of cytology?** Answer B
   a. Cytology is the gold standard and inevitable for all ILD diagnoses.
   b. Cytology is suitable for the assessment of single individual cells and the composition of cell populations within a compartment.
   c. Disease-specific findings are often present in cytology specimens.
   d. Cytology provides important clues on the tissue composition in a specific compartment.

2. **Which specimens are usually suitable for specific diagnoses of ILDs?** Answer A
   a. Conventional forceps biopsies.
   b. Cryo biopsies.
   c. Transthoracic needle biopsies.
   d. Wedge resections.

3. **What is no “pattern of lung injury”?** Answer D
   a. DAD
   b. NSIP
   c. LIP
   d. MALT