

Use of Immunohistochemistry to Establish Etiology in the Case of Primary Spontaneous Pneumotorax Patients

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Primary spontaneous pneumothorax has a complex morphopathological substrate, in which active smoking plays an essential etiopathogenic role. Inflammation of the distal airways, bronchial anomalies, perivascular eosinophilic infiltrate associated with hereditary factors and physiognomy (longilli patients) lead to obstruction of distal airways which is the essential element in the emergence of emphysematous changes. Immunohistochemistry (IHC) is a technique used to identify cellular or tissue (antigens) constituents by Ag-Ac, the Ac link site being identified either by direct labeling of the antibody or by a secondary labeling method. IHC reactions are based on tissue-antibody antigen binding, the latter being evidenced by direct conjugation to tracer molecules (direct reaction) or by another chain of other labeled free antibody linkages. We can consider the immunohistochemical method as having a potential utility, especially in selected patients, where there are sufficient clinical and epidemiological reasons to suspect a pneumothorax-causing disease but where the classical investigations did not provide diagnostic performance.

Keywords: primary spontaneous pneumothorax, Immunohistochemistry, Visceral pleura

Primary spontaneous pneumothorax has a complex morphopathic substrate, in which active smoking plays an essential etiopathogenic role [1,2].

Inflammation of the distal airways, bronchial abnormalities, perivascular eosinophilic infiltrate associated with hereditary factors and physiognomy (longiline patients) lead to obstruction of distal airways, which is the essential element in the emergence of emphysematous changes [3,4].

The association of emphysematous changes with the presence of pore in the visceral plexus and with sudden changes in the air lead to the occurrence of primary spontaneous pneumothorax [3,5].

In modern medicine, a rapid, accurate and complete anatomopathological diagnosis is mandatory for choosing a successful therapeutic strategy. Using only conventional techniques involving only optical microscopy in the histopathological examination, diagnostic concordance may sometimes only reach 75% for malignant lymphomas.

Immunohistochemistry (IHC) is a technique used to identify cellular or tissue (antigen) constituents by Ag-Ac reactions, where the Ac linking site is identified either by direct labeling of the antibody or by a secondary labeling method [6].

IHC reactions are based on tissue-antibody antigen binding, the latter being evidenced by direct conjugation to tracer molecules (direct reaction) or via another loop chain with labeled free antibodies (indirect reaction with at least two phases) [7,8].

However, in a significant number of patients considered not to have significant lung lesions, morphopathological and immunohistochemical examination of biopsy items is found to be secondary pneumothoraxes for rare pulmonary or general disorders [9,10]. This is why we consider the routine morphopathological examination of lung resection parts useful and, where possible or necessary, immunohistochemical examination.

Experimental part

Material and methods

The immunohistochemical examination was performed in 16 selected cases, all patients presenting either a chronic pneumothorax (9 cases) or more than two relapses (7 cases). Patients admitted to the Thoracic Surgery Clinic of the Pneumophtisology Hospital were between the ages of 18-36, except for a 54-year-old patient.

Markers routinely used were CD45 and CD34 for clear observation of pulmonary tissue vasculature and for evaluation of inflammatory phenomena. If the anatomopathologist considered it necessary, the marker palette was enlarged to allow a diagnosis as close as possible to certainty.

The resection pieces were processed as follows: fixation in 10% formol solution; dehydration in alcohol baths in progressive concentrations, then in xylene; paraffining; microtome processing; coloring; rehydration in successive alcohol baths in decreasing concentration; mounting using Canada balsam.

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The staining was performed according to standard haematoxylin-eosin technique. Special coloring:

a) Van - Gieson, for the detection of connective tissue. This coloring allows for the visualization of excessive fibrosis through intense red coloring of the connective tissue,

b) Gordon - Sweet, which shows the reticulin fibers or the argentic impregnation technique in which the fibers appear in black.

Reading was performed on a CETI microscope with 10x, 20x, 40x lenses.

Results and discussions

The anatomo-pathological examination performed on the resection parts provided a series of information on the morphopathologic substrate in the primary spontaneous pneumothorax. The data obtained can be seen in Figure 1, the differences between smokers and non-smokers are not significant.

It is necessary to note that suggestive results for pulmonary tuberculosis occurred after surgical treatment and that these cases were considered as primary spontaneous pneumothorax, with no symptoms or paraclinical findings suggestive of this disease (including chest radiography and BAAR sputum examination) .

The morpho-pathological descriptive elements found in the examined lung biopsies were (fig. 2-5):

1. macrophagic and haemorrhagic alveolitis, enlarged alveoli;
2. focuses of atelectasis and chronic non-specific inflammation;
3. important angiogenesis phenomena;
4. moderate peribronchial and perivascular chronic inflammation;
5. mesothelial hyperplasia;
6. perivascular eosinophilic infiltrate;

7. obliterary endarteritis of the pulmonary vessels;
8. pleurited with reactive inflammatory phenomena with eosinophils or granulomas of foreign bodies;
9. paraseptal emphysema;
10. panicular emphysema.

Anatomo-pathological examinations provided additional diagnostic data to surgical patients and biopsy pieces were obtained.

In these cases, the immunohistochemical examination of the samples was used, as this approach was financially feasible.

Regarding the characterization of inflammation, an aggregation of results was found depending on the existence of chronic pneumonia or relapse. Thus, in patients with chronic pneumothorax (9 cases) there was an inflammatory infiltration with the presence of lymphoid cells in varying degrees of intensity ranging from *rare lymphoid elements* to *lymphoid organs arranged in nodules, diffuse lymphoid infiltrate*. For relapsed patients, the results were non-homogeneous, inflammatory infiltrate not present in all cases, and lymphoid cells not mentioned. It can not, however, be assured that these differences have statistical significance, given the above-mentioned reasons.

We consider it useful to note that in two cases the immunohistochemical method had a special diagnosis, indicating the diagnosis of lymphangioleiomyomatosis, which would most probably have been unnoticed in case of not using this method.

Considering these aspects, we can consider the immunohistochemical method as having a potential utility, especially in selected patients, for which there are sufficient clinical and epidemiological reasons to suspect a pneumothorax-induced condition, but in which classical investigations did not provide diagnostic results.

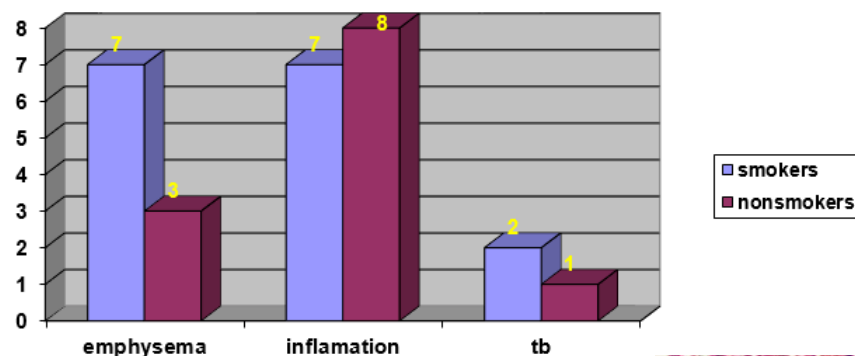


Fig.1 Distribution of anatomo-pathological lesions on the studied group

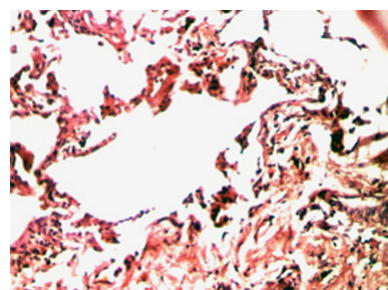


Fig.2 (Col. HE 20) Large spaces, alveolar restorations: pulmonary emphysema

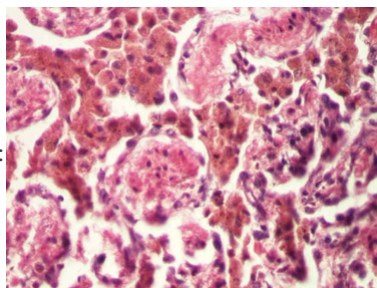


Fig.4 (Col. HE 20) Macrophagic alveolitis

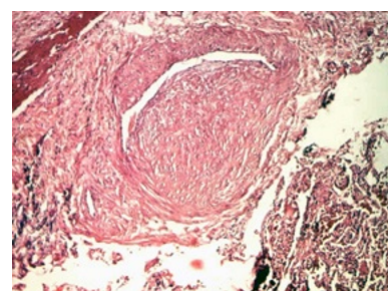


Fig.3 (Col. HE 4) Vessel with obliterative endarteritis

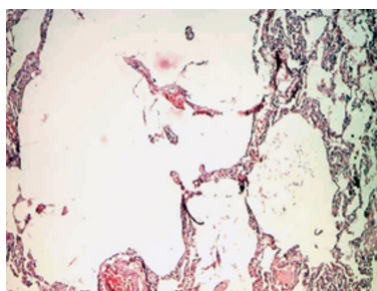


Fig.5 (Col HE 4) Enlarged alveoli, with thin walls

Conclusions

The anatomo-pathological examination performed on the resection parts brings a series of information about the morphopathological substrate in the primary spontaneous pneumothorax. It provides additional diagnostic data to surgical patients and biopsies have been obtained.

Regarding the characterization of inflammation, an aggregation of results is found depending on the existence of chronic pneumothorax or relapse.

The immunohistochemical method has a special diagnosis, indicating the diagnosis of lymphangioleiomyomatosis, which would most likely not be observed in the absence of this method.

We can consider the immunohistochemical method as having a potential utility, especially in selected patients, where there are sufficient clinical and epidemiological reasons to suspect a pneumothorax-causing condition, but in which the classical investigations did not provide diagnostic results.

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