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RESEARCH ARTICLE

Diagnostic Significance of Eosinophilic Pleural Effusion

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ABSTRACT

A pleural effusion is defined as eosinophilic when eosinophils represent ≥ 10% of the total nucleated cell count, and accounts for approximately 10% of all pleural effusions. The diagnostic significance of eosinophilic pleural effusion has yet to be determined.

Objective and Methods: A retrospective study was conducted on 65 patients with eosinophilic pleural effusion to evaluate the correlation between the percentage of eosinophils present in the pleural fluid and the benign or malignant nature of the effusion. An original aspect of current study was the evaluation of other variables in association with pleural eosinophilia, in particular pleural fluid lymphocytosis (≥ 50%), and the presence or absence of fever.

Results: Data showed the trend towards a decrease in neoplastic incidence with increasing percentages of eosinophilic counts, although this correlation was not statistically significant. The presence of fever correlated with low incidence of neoplasms (10% of neoplastic effusions in patients with fever) and was the most significant variable (p=0.001), with a Negative Predictive Value of neoplastic disease of 90%, with sensitivity 92.6% and specificity 47.4%.

When evaluated together with fever, eosinophils increased their discriminating sensitivity to the benign or malignant nature of the effusion but lost in specificity.

When evaluated as absence or presence of lymphocytosis (≥50% lymphocytes), associated with eosinophilia, lymphocytes were significantly associated with the neoplastic nature of the effusion.

Conclusions: the study showed that the finding of eosinophilic pleural effusion should not be considered an indicator of benignity of the effusion; the association of other parameters with eosinophilia, lymphocytosis of the pleural fluid and fever can provide more precise prognostic indications; a high percentage of eosinophils, the absence of lymphocytosis and the presence of fever would seem to be associated with a low probability of a neoplastic nature of the effusion.

Keywords: Eosinophilic pleural effusion, eosinophils, pleural effusion, differential cell count, malignant pleural effusion

Abbreviations: EPE (eosinophilic pleural effusion), DCC (differential cytological count), ROC (Receiver Operating Characteristic)

Introduction

Eosinophilic Pleural Effusion (EPE) is defined as effusion in which eosinophils represent ≥ 10% of the total nucleated cell count[1,2]. The prevalence of EPE is estimated to be around 10% of all pleural effusions[3-6]. Numerous studies in the last 50 years have attempted to attribute a meaning to this type of effusion, regarding the possible nature of the effusion, and consequently attributing a prognostic and diagnostic significance to eosinophilic pleural effusion.

One of the most studied aspects has therefore been the aetiology of EPE; the causes of eosinophilic effusion are numerous: neoplasms, infections, heart failure, chronic renal failure, liver cirrhosis, tuberculosis, pulmonary embolism, the presence of blood and air in the pleural space, adverse drug reactions and others[3,4,6]. The presence of air or blood in the pleural cavity as one of the most frequent causes of EPE has subsequently been challenged by more recent studies[7,8].

There are discrepancies among the studies in the literature, possibly on account of the small size of the cases studies observed and the differing epidemiology among the populations studied.

In recent years, clinical studies have been carried out on larger series, which have better characterised this condition, arriving at some common observations. The role of cytokines such as IL-5, IL-3 and GM-CSF has been highlighted: as promoters of the proliferation of this cell line, they would appear to constitute the first step in the pathogenesis of EPE[9,10].

Four mechanisms underlying EPE have been proposed[11,12]:

- increased production of eosinophils in the pleural space;
- increased proliferation of eosinophils in the bone marrow and their subsequent increase in peripheral blood and from there to the pleural cavity;
- increased eosinophilic chemotactic activity and movement of eosinophils from peripheral blood to the pleural cavity; moreover, pleural fluid eosinophilia does not correlate with peripheral blood eosinophilia and there is no correlation between the number of eosinophils in the peripheral blood and that in the pleural fluid[2]; pleural fluid eosinophilia can also occur without peripheral eosinophilia.
- increased survival of eosinophils in the pleural fluid.

An attempt was thus made to give a diagnostic/prognostic significance to the finding of eosinophilia in the pleural fluid; it was seen that, in order to express an opinion on the real prognostic value of eosinophils in the pleural fluid, it may be important to correlate the percentage of these to the final diagnosis.

From this correlation, the existence was observed of an inverse relationship between the increase in eosinophilic count and the probability of a neoplastic nature of the effusion[7]; a cut-off in the percentage of eosinophils was identified (30%) above which the probability that the effusion is neoplastic drops considerably[7,13].

However, this value has not always been confirmed, with values reported both higher[4] (40%) and lower[14] (15%); but, again, this may be due to the differing populations considered in the various clinical studies.

A recent meta-analysis[15], which aimed to establish the diagnostic significance of EPE, reconfirmed these results. Seventeen clinical studies on EPEs, involving 687 patients, were considered in the meta-analysis. The most frequent causes of EPE were found to be: neoplastic (26%), idiopathic (25%), parapneumonic (13%), pneumo/haemothorax (13%), pathologies causing transudates (7%), tuberculosis (7%) and others.

This systematic review confirmed neoplasia as the most frequent cause of EPE, specifying, however, that the eosinophil count was a factor inversely related to the probability of a neoplastic nature of the effusion when the percentage was very high; hence, it reconfirmed the observation made by previous studies regarding the existence of an inverse correlation between the percentage increase in eosinophilic counts and the prevalence of neoplasia.

Objective of the study

From the literature review, the pathogenesis, clinical significance and diagnostic role of EPE therefore remain to be ascertained. In order to compare historical experience with the scientific literature and to make our contribution, a retrospective study was conducted with the aim of correlating the percentage value of eosinophils with the nature of the pleural effusion (benign vs. malignant). Furthermore, an original and innovative aspect of the current study is that two other clinical/lab parameters, lymphocytosis of the pleural fluid and fever were also taken into consideration; in addition, it was evaluated whether these two parameters, associated with the eosinophilia of the pleural fluid, could improve the diagnostic orientation.

Materials and Methods

A retrospective was conducted on sixty-seven patients with EPE (eosinophils ≥ 10% of nucleated cells in the pleural fluid at the first thoracentesis)

treated from January 2009 to March 2018 at the Unit of Pneumology and Thoracic Endoscopy of the University Hospital of Parma. Two patients were without a clear diagnosis of the cause of the effusion as they were lost to follow-up and were thus excluded. The sample was therefore made up of 65 patients, 45 men and 20 women, with a ratio ≈2: 1, equal to that observed in many other studies[15]; the average age was 68 years ± 15.60 SD (range 17-97 years).

The diagnostic flowchart followed in patients with pleural effusion is that shown in Figure 1. All patients underwent a chemical-physical examination of the pleural fluid with a determination of proteins, LDH, glucose, amylase and pH (performed with a blood gas analyser); the distinction between exudate and transudate was made following Light's criteria[16].

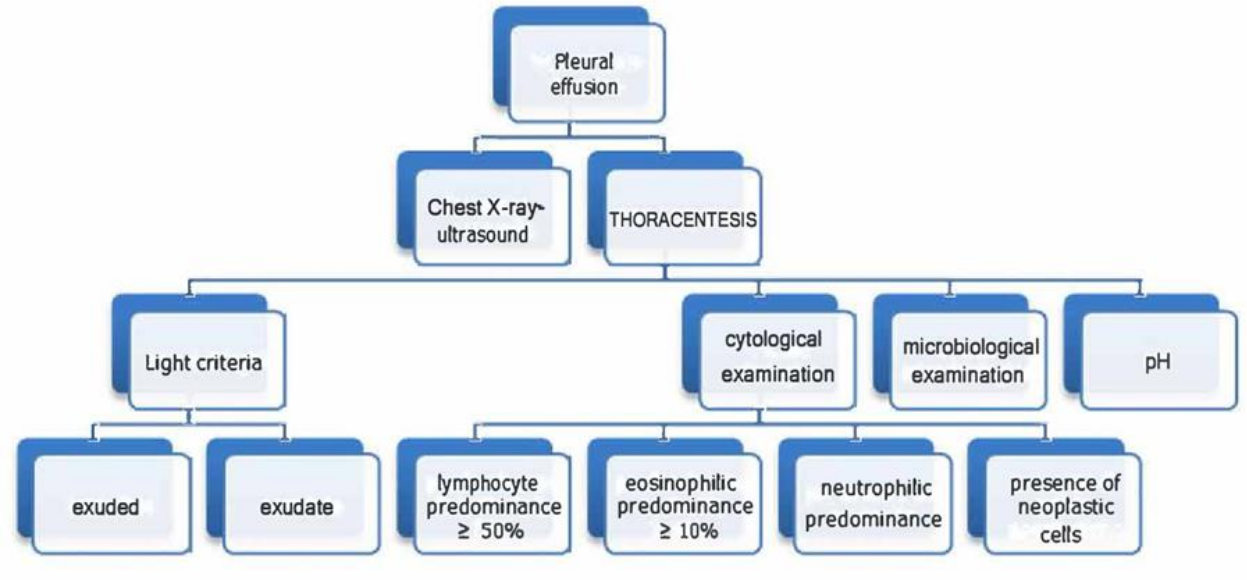

Figure 1. Diagnostic flowchart for pleural effusion

Pleural fluid samples were always sent to the Pathologist for a cytological examination for the detection of neoplastic cells and to the Microbiology lab for a complete microbiological examination (direct and cultural examination for non-specific flora, for mycobacteria and, when indicated, also for fungi).

Another aspect of this study is that a manual counting of pleural fluid cells was performed in all patients at the Laboratory of the Pneumology Clinic of the University of Parma. This investigation is performed routinely in the diagnostic path of patients with pleural effusion and the method is as follows:

- the pleural fluid sample (≈50 ml) is delivered to the laboratory immediately after the thoracentesis; the macroscopic characteristics of the fluid are examined.
- the sample is then analysed in the shortest possible time and in any case never more than two hours after the collection procedure.
- after obtaining the cell sediment by centrifugation, we carry out the analysis on slides for the differential cytological count (DCC), in which the component of macrophages, neutrophils, lymphocytes and eosinophils is reported separately, expressed in % of the total, identified (with May Grünwald-Giemsa staining) by morphological criterion[17].
- the DCC is performed using an optical microscope at 1000x on a total of at least 400-600 cells with double blind reading; finally, the average of the two readings is recorded.

In addition to compliance with the diagnostic criteria of EPE (eosinophils ≥10% of the white blood cell count), among the inclusion criteria the certainty of final diagnosis was also included.

The final certain diagnosis was obtained with Medical Thoracoscopy in 30 patients and in the remaining ones through the cytological examination of the pleural fluid, the microbiological examination of the same, the clinical evolution following the therapy carried out, other tests performed (e.g., fibrobronchoscopy) and a careful follow up until the resolution of the clinical picture.

The patients were divided into 5 groups in relation to the percentage of eosinophils: Group 1: from 10% to 19%, Group 2: from 20% to 29%, Group

3: from 30% to 39%, Group 4: from 40% to 49%, Group 5: ≥ 50%.

The other variables which were considered are listed below:

- fever, evaluated as present/absent, looking for an association between this and the benign/malignant nature of the effusion in the course of EPE;
- lymphocytosis, as an independent variable and in association with eosinophilia.

Statistical analysis

Statistical analysis was done using SPSS 25 software (IBM, Amork, NY). All data were reported as continuous variables or divided into percentile ranges. Our variables were: % eosinophils, % lymphocytes and fever. Eosinophils were reported both as a continuous variable and in percentage ranges; lymphocytes were evaluated both as a continuous variable and as the presence/absence of lymphocytosis (lymphocyte count ≥50%). Fever was evaluated as present/absent.

All three variables were, therefore, related to the nature of the effusion (benign or malignant), independently and in association with each other.

The significance of the individual quantitative variables in relation to the nature of the effusion was assessed with a *t* test for independent variables.

The association between the qualitative variables was instead evaluated with the chi-square test, or with Fisher's exact test when the frequency was too low for the chi-square test.

The Receiver Operating Characteristic (ROC) analysis was carried out with an estimate of the area under the curve to evaluate the prognostic capacity of the eosinophilic percentage count towards the neoplastic nature of the effusion. The variables were considered significant if $p<0.05$.

Results

For some patients the results of repeated thoracenteses were available but, as in other studies, only the first were considered[13,15], for a total of 65 samples. Fifty-three (81.5%) were exudate and 12 (18.5%) were transudate. The mean percentage of eosinophils was 30.6% ± 20.6 SD.

The distribution of patients according to the percentage ranges of eosinophils was as follows (Figure 2): 1: 10% -19% = 30 patients, 2: 20% -29% = 10 patients, 3: 30% -39% = 11 patients, 4: 40% -49% = 2 patients, 5: ≥50% = 12 patients.

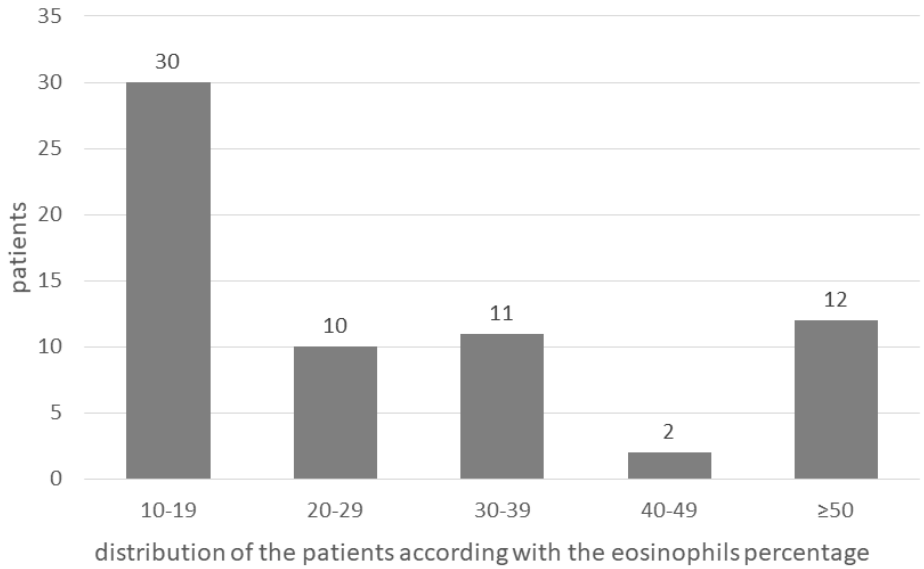

Figure 2. Distribution of patients according to the percentage ranges of eosinophils

The diagnoses obtained in 65 patients are shown in (Figure 3): neoplastic effusion 27 (41%), infectious effusion 17 (26%), cardiogenic effusion 10 (15%), idiopathic effusion 4 (6%), pulmonary embolism 2 (3%), post-traumatic effusion 2 (3%), others 3 (4.6%). All 4 patients in whom the final diagnosis was idiopathic effusion underwent medical thoracoscopy[18]: the macroscopic endoscopic picture was of the inflammatory type, and asbestos pleural plaques were not present; the microbiological tests were all negative and the histological examination of pleural biopsies revealed a picture of non-specific pleuritis.

Among the neoplasms, the diagnoses were the following: lung cancer (15), mesothelioma (4), lymphoma (2), metastasis from colon adenocarcinoma (1). In the remaining 5 patients with neoplastic diagnosis, the cytological examination was indicative for neoplasm, but Authors were unable to identify the primary tumour, because of the precarious clinical conditions of the patients and their poor life expectancy.

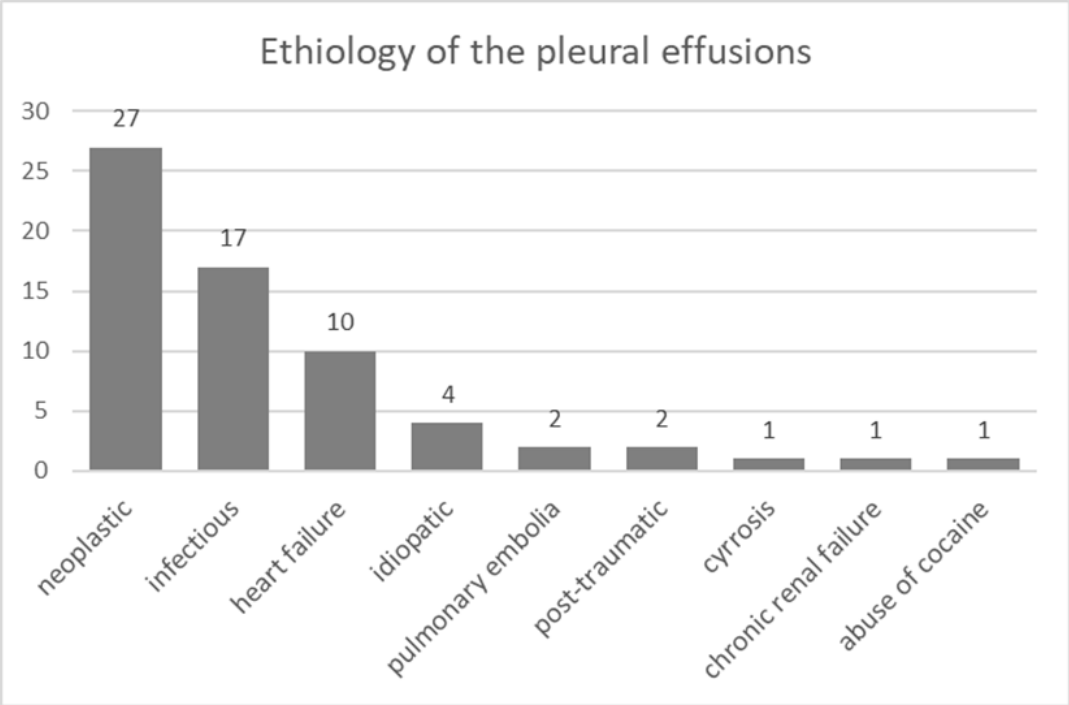

Figure 3. Aetiology in 65 patients with EPE. 27 neoplastic patients: 15 lung cancer, 4 mesothelioma, 2 lymphoma, 1 colon adenocarcinoma metastasis, 5 non-identified cancers

The current data were identified two subgroups of patients regarding the presence or absence of lymphocytosis (lymphocytes ≥50%) and fever: 35 patients had lymphocytosis, 20 had fever.

Considering the prevalence of neoplastic effusion in the various subgroups of patients by evaluating only eosinophilia, the results are those shown in Figure 4.

In the first group (10% -19%), 15 out of 30 patients had neoplastic diagnosis, in the second 6 out of 10 patients, in the third 2 out of 11 patients, in the fourth one out of 2 patients and in the fifth 3 out of 12 patients.

The prevalence of neoplastic diagnosis tends to decrease with increasing eosinophilic counts, with an average percentage of eosinophils higher in benign EPEs (33.51%) than in malignant ones (25.17%).

However, this trend, both when eosinophils were evaluated as a continuous variable and when they were evaluated divided into ranges, was not significant (p=0.087, p=0.158, respectively).

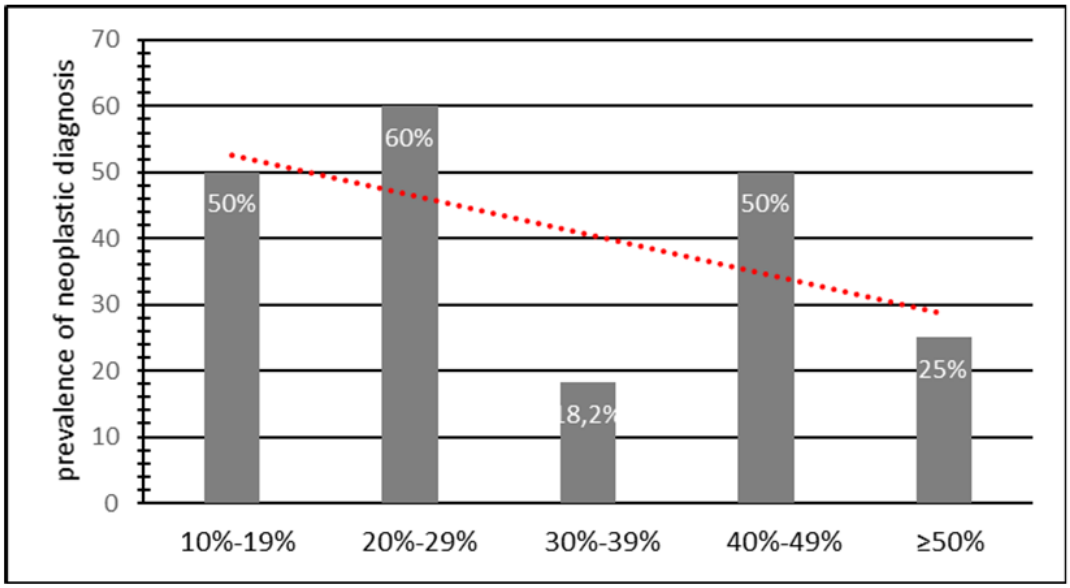

Figure 4. Prevalence of neoplastic diagnosis in the subgroups of patients.

The relationship between the percentage of eosinophils and the neoplastic probability of the effusion was also evaluated with the ROC analysis (Figure 5), which confirmed the low predictive power of the percentage of eosinophilia of the pleural fluid in relation to the neoplastic nature of the effusion, reporting an area under curve (AUC) of 0.581.

The cut-off of the percentage of eosinophils with the greatest discriminating power between benign and malignant pathology was calculated to be 28.65% with sensitivity and specificity at this value of 52.6% and 77.8%, respectively.

When independently evaluated as a continuous variable, lymphocytes did not show the same significance as eosinophilia, with minimal differences in the average lymphocyte percentage between the group with benign disease and that with malignant disease.

When the presence/absence of lymphocytosis was evaluated, 35 patients with lymphocytosis were identified, 20 (57%) of whom had a neoplastic diagnosis, and the chi-square test for the variable lymphocytosis showed significance (p=0.006) with sensitivity 74.1% and specificity 60.5%, indicating that a percentage of lymphocytes greater than 50% during EPE increases the probability of a neoplastic diagnosis.

When combined, these variables obtained values of sensitivity, specificity, PPV and NPV intermediate between the values obtained for the single variables; these are, respectively: 77.8%, 63.2%, 60% and 80%.

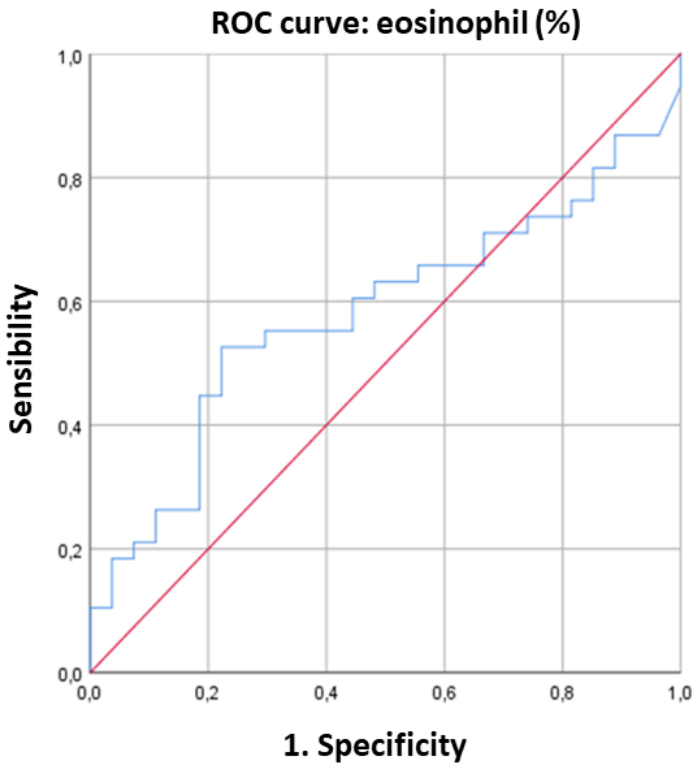

Figure 5. ROC curve. The area under the curve is small, confirming the poor predictive power of the eosinophilic count in relation to the neoplastic nature of the effusion.

As expected, when eosinophilia and lymphocytosis together were evaluated in relation to the nature of the effusion, data showed a highly significant linear and inverse correlation between the two variables (p=0.00) since as one cell line increases the others must necessarily decrease (the mean eosinophilic count in the subgroup with lymphocytosis was 18.7%).
Data analysis showed 26 patients with lymphocytosis without fever; 19 (73.5%) of these had a neoplastic diagnosis.
When correlated, both fever (p=0.003) and lymphocytosis (p=0.011) still showed significance, with values of sensitivity (70.4%), specificity (81.6%), PPV (73.1 %) and PPN (79.5%) on average higher among all those observed, confirming that the absence of fever and the presence of lymphocytosis together have the highest discriminating capacity between benign and malignant nature in an EPE.

Discussion

The diagnostic pathway of the patient with pleural effusion is complex and involves numerous steps: collection of clinical information, radiological investigations, chemical-physical examination of the pleural fluid, cytological examination for the detection of neoplastic cells and microbiological examination of the pleural fluid; in addition to this, a cytological examination with differential cell count is always performed to verify whether it is a lymphocytic, eosinophilic or neutrophilic effusion and to have a possible diagnostic orientation on the basis of this characteristic.
This differential count is particularly important in pleural effusions of infectious origin with the distinction between neutrophilic effusions (generally nonspecific infectious) and lymphocytic effusions (with a possible diagnostic orientation towards a tuberculous aetiology)[19,20].
Unfortunately, this investigation on the pleural fluid, although particularly important, is not always performed and therefore a valid diagnostic orientation is lacking.
Generally, in 75% of patients the careful evaluation of the clinical data and the results of a complete analysis of the pleural fluid, which must always include the cytological examination, the

microbiological investigations, and the pH measurement, allow us to arrive at an etiological diagnosis of the pleural effusion.

In the absence of a diagnosis deriving from clinical data and from the study of pleural fluid (this occurs in about 25% of cases), the diagnostic pathway continues, as a pleural biopsy is required. When the patient's clinical conditions allow it, the best method is to perform a medical thoracoscopy that allows us to explore the pleural cavity and to perform targeted pleural biopsies. Blind biopsy with Abrams or Cope needle can also be used which, although presenting a fairly high diagnostic yield, however are now little used.

The first important problem when faced with a patient with pleural effusion is the distinction between neoplastic effusion and effusion of another nature; a precise and above all rapid diagnostic definition allows to start a targeted therapeutic path for the patient; the presence of eosinophils in the pleural fluid has often been associated with the benign nature of an effusion[3].

Authors think that data in the current study is among the most numerous in the literature; in the meta-analysis published in 2010 (15), out of 17 clinical studies considered, only 2 cases were more numerous than ours.

Data showed that the main cause of EPE is neoplastic pathology (41%), as observed in the meta-analysis (15) and in subsequent studies (14).

The analysis of results regarding a possible correlation between eosinophils and the nature of the effusion (our main objective), in particular by dividing the effusions into classes based on the percentage of eosinophils, also led us to observe that there is a tendency to a decrease in neoplasms with increasing eosinophilic counts. This correlation was not statistically significant; however, it must be considered that the 40-49% group of eosinophils consisted of only 2 patients and that the presence of 1 neoplastic effusion therefore implies a prevalence of 50%.

We did not consider the possible correlation between the number of eosinophils in peripheral blood and pleural fluid, as a possible correlation has been denied by other authors[5].

We also analyzed the importance of the presence or absence of fever in the patient with eosinophilic effusion and observed that; the presence of fever was a significant variable when assessed independently (p=0.001), and its absence is associated with a Negative Predictive Value (NPV) of neoplastic pathology of 90% with a sensitivity of 92.6%, equal to that observed in several studies[15].

Eosinophilia and absence of fever together improve in sensitivity but lose in specificity. Data showed a highly significant inverse linear correlation between eosinophilia and lymphocytosis.

Fever and lymphocytosis are the associated variables that have the greatest discriminating power between benign and malignant disease; in particular, in an EPE with lymphocytosis and absence of fever, the diagnosis of a neoplastic nature of the effusion is highly probable.

However, we report that in our experience the most frequent benign causes of EPE are infectious (26%) and cardiogenic in nature (15%), with respectively higher and lower prevalence than those reported by the meta-analysis, although the latter reported a much higher rate of idiopathic effusions (25%) than that observed in current study (6%).

Furthermore, in the infectious effusions, contrary to what other studies have observed, we did not find any cases of tuberculosis.

From data analysis, no cases of EPE due to parasites[21] or pharmaceutical drugs[22,23] was present, but a case of EPE from the intake of cocaine was diagnosed, a substance that seems to be associated with the development of this type of effusion[24].

Conclusion

The study herein showed that the finding of EPE should not be considered an indicator of the benignity of the effusion; it showed, however, that the association of other parameters with eosinophilia, lymphocytosis of the pleural fluid and fever can provide more precise diagnostic/prognostic indications; a high percentage of eosinophils, the absence of lymphocytosis and the presence of fever would seem to be associated with a low probability of a neoplastic nature of the effusion.

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