An Evaluation of Tissue Polypeptide Antigen (TPA) in the Two Bronchoalveolar Lavage Fractions of Lung Cancer Patients

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Background: It has been proven that cytokeratins (CKs) are useful tumor markers for the follow-up, treatment monitoring and prognosis evaluation of lung cancer and among these, tissue polypeptide antigen (TPA) plays an important role. Nevertheless, only a small number of studies have been reported about their diagnostic capacity. Bronchoalveolar lavage (BAL) can be divided into two fractions: bronchiolar (BF) and alveolar (AF). For the above reasons, our aims were (1) to analyze the diagnostic usefulness of TPA in the BAL of lung cancer patients and (2) to observe if, in lung cancer patients, TPA levels in the two BAL fractions are different. This should mean that the study of tumor markers in the BAL should be carried out in both fractions to increase their diagnostic capacity.

Methods: We studied 289 BALs divided into two phases. In phase I, TPA was analyzed in the BAL of six groups of subjects (healthy persons, chronic bronchitis, asthma, respiratory infections, diffuse interstitial pulmonary diseases and lung cancer). In phase II, TPA was studied in both BAL fractions of a group of patients with lung cancer.

Results: We observed that TPA levels were significantly higher in the BAL of patients with bronchogenic neoplasias. In these patients, TPA was increased in the BF of the lavage in relation to the AF. In smoker patients with pulmonary carcinomas, TPA was higher in the AF of the BAL than in the lavage of non-smokers. This did not occur in the BF. We found no relation between the TPA concentrations and cancer histology.

Conclusions: We believe that TPA is a useful tumor marker with diagnostic capacity and this capacity is increased when it is studied in the two BAL fractions. Smoking habit may play a role in the secretion of tumor markers by the tumor cells.

Key words: lung cancer – tissue polypeptide antigen – bronchoalveolar lavage – tumor marker

INTRODUCTION

Tissue polypeptide antigen (TPA) is a complex of polypeptide filaments of the cytokeratins (CKs) 8, 18 and 19 and is produced during late S and G2 phases of the cell cycle. It is secreted into the circulation during and immediately after mitosis. TPA represents the most abundant CK pattern in malignant epithelial differentiation (1). In general, CKs are proteins that form the intermediate filaments of the cellular cytoskeleton. Currently, more than 20 different CKs have been described. Their pattern is specific for each tissue and varies with cell differentiation (2,3).

Many studies (4,5) have demonstrated the usefulness of CKs in serum in staging bronchogenic carcinomas, during post-therapeutic follow-up and for prognosis evaluation. CYFRA 21-1 has been shown to have a certain usefulness in early diagnosis. In spite of this, none of the serum markers used today in pulmonary neoplasias has been shown to possess a significant value in early detection programs (6). Probably one of the...
causes that have contributed to the reduction of the early diagnostic capacity of tumor markers is the type of the sample used to study them. If these tumor markers are substances produced and secreted by neoplasias, the samples in which these biological substances are analyzed should pick up information directly from the tumor region. It has been proved that some tumor markers increase their diagnostic capacity when studied in the bronchoalveolar lavage (BAL) (7–10). Likewise, it has been demonstrated that the concentrations of neuron specific enolase (NSE) (11) and carcinoembryonic antigen (CEA) (9,12,13) in the lavage of lung cancers are superior to those found in other benign diseases and in healthy subjects, both smokers and non-smokers. In this sense, Rapellino et al. (14) observed that TPA was increased in the BAL of patients with lung neoplasias.

Rennard et al. (15) proved that BAL could be divided into two different types of samples: the bronchial fraction (BF), which picks up material from the nearest airways, and the alveolar fraction (AF), which represents the most distal airways. The origin of most lung cancers being fundamentally bronchial, we thought that the concentrations of the tumor markers in the two fractions of BAL should be distinct.

For this, our objectives were (1) to analyze the diagnostic usefulness of TPA in the BAL of patients with lung cancer and (2) to observe if, in bronchogenic carcinoma, the TPA of the two BAL fractions is different. If this were true, the tumor markers in BAL should always be analyzed in both fractions of the lavage to increase their diagnostic effectiveness. We have not found any reports in the medical literature of studies of TPA in the two BAL fractions in pulmonary neoplasias.

PATIENTS AND METHODS

This study was divided into two phases. In the first phase (phase I), TPA was analyzed in the BAL (without being separated into bronchial and alveolar fractions) of healthy smoker and non-smoker subjects and patients with different respiratory diseases, both benign and malignant, to evaluate the effectiveness of TPA in the diagnosis of lung cancer. In the second phase (phase II), the concentrations of TPA in the two fractions of BAL (BF and AF) in some of the lung cancer patients were compared.

We performed 281 bronchoalveolar lavages on as many patients who needed bronchoscopy to exclude pulmonary pathologies, of which 36 were excluded from the study for the following reasons: (1) inability to introduce the bronchoscope to carry out BAL in the lung affected with lung cancer (five cases); (2) impossibility of recovering a quantity of BAL liquid of more than 20% of that instilled (six cases); (3) presence of hemorrhagic BAL liquid, as viewed macroscopically (five cases); (4) too much time had passed between the BAL and its transfer to the laboratory for the procedure (seven cases); (5) inadequate handling of the fluid in the laboratory (five cases); (6) lack of TPA analysis (three cases); and (7) absence of data to complete the diagnosis (five cases).

In phase I, the patients were divided into six groups whose characteristics are shown in Table 1. Group 1: 41 healthy subjects, free of pulmonary disease according to their medical history, physical examination, thoracic radiograph and respiratory function tests. It was formed by 27 males and 14 females, between 18 and 73 years of age [mean (SD): 39.5 (2.9) years]; 12 were smokers [mean (SD): 22.2 (6.7) packets/year] and 29 were not. Fiber-optic bronchoscopy was performed owing to one of the following causes: possible hemoptysis that was later ruled out as pulmonary bleeding (six cases), dysphonia (five cases) and unclear radiological images (30 cases). Each case was followed up for 18 months after the bronchoscopy and none presented respiratory problems. Group 2: 12 patients with chronic bronchitis, 11 males and one female, between 14 and 80 years of age [mean (SD): 64.5 (9.1) years], eight of whom smoked [mean (SD): 52.3 (10.3) packets/year] and four did not, all being diagnosed by their clinical history, thoracic radiograph and respiratory function tests. All were receiving treatment with theophyllines, β-2 stimulants and inhaled corticoids. None had presented reactivation of their disease in the 3 months prior to the study. Group 3: 42 patients with diffuse pulmonary interstitial disease, including idiopathic pulmonary fibrosis (nine cases), sarcoidosis (nine cases), hypersensitive pneumonitis (eight cases), post-radiation pneumonitis (seven cases), Churg–Strauss syndrome (three cases), rheumatoid arthritis (two cases), histiocytosis X (two cases) and Sjögren’s syndrome (two cases), diagnosed by their clinical history, physical examination, radiograph and high-resolution computerized tomograph of the thorax, respiratory function tests, the results of cytology and cellular count of BAL and transbronchial biopsy or, in some cases, open pulmonary biopsy. The group was formed by 19 males and 23 females, between 16 and 81 years of age [mean (SD): 53.1 (16.9) years]; 11 were smokers [mean (SD): 32.3 (8.6) packets/year] and the rest were not. Group 4: 24 subjects diagnosed with different infectious

<table>
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<th>Table 1. Characteristics of the subjects grouped in phases I and II</th>
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<tr>
<td>Number</td>
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<tr>
<td>Males/females</td>
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<tr>
<td>Age (years)</td>
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<td>Smokers: yes/no</td>
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|                   | CB, chronic bronchitis; DIPD, diffuse interstitial pulmonary disease; RI, respiratory infection.
respiratory processes [pulmonary tuberculosis (10 cases), non-tuberculous pneumonia (nine cases), infected bronchiectasis (five cases)]. It was formed by 14 males and 10 females, with an age range of 18–83 years [mean (SD): 46.7 (19.9) years]; 12 smoked [mean (SD): 50.1 (9.7) packets/year] and the others did not. The diagnoses were reached from their clinical history, physical examination, thoracic radiograph and sputum culture, bronchial washing and BAL culture. In all of the cases, FBC was performed before starting antibiotherapy. Group 5: 63 patients diagnosed with asthma, 30 males and 33 females, whose ages were between 17 and 73 years [mean (SD): 33.4 (16.1) years], of whom 58 were non-smokers [mean (SD): 20.3 (7.9) packets/year] and five were smokers. These included patients with a history of bronchospasm, dyspnea and whistles and spirometry with obstructive pattern at the moment of the crisis or positive bronchial hyperactivity test. None of them presented asthma reactivation in a period of less than 3 months. Group 6: 63 patients diagnosed with lung cancer by histological methods [squamous carcinoma (38 cases), adenocarcinoma (11 cases), large-cell carcinoma (one case), oat-cell carcinoma (13 cases)]. This group was made up of 55 males and eight females with ages between 18 and 80 years [mean (SD): 64.1 (6.7) years], of whom 49 smoked [mean (SD): 60.1 (11.1) packets/year] and 14 did not.

In phase II, 52 lung cancer patients belonging to Group 6 of phase I were included, of whom 47 (90.4%) were men and five (9.6%) were women with a mean age of 64.3 (1.2) years. Out of the 52, 46 were smokers (88.5%) [mean (SD): 63.3 (9.9) packets/year] and six non-smokers (11.5%) (Table 1). The histological types were squamous carcinoma (34 cases), adenocarcinoma (six cases), large-cell carcinoma (one case) and oat-cell carcinoma (nine cases). Three patients with radiological and non-visible solitary nodules were included.

All of the participants of each part of the study gave their informed consent.

**BRONCHOALVEOLAR LAVAGE**

Fiber-optic bronchoscopy was performed on each of these patients with an Olympus BF-30 instrument (Olympus, Tokyo, Japan). The upper airways were anesthetized topically with 2% lidocaine. In the majority of the cases, the fiber-optic bronchoscope was introduced through the nose. In the healthy subjects and in those with chronic bronchopathy, diffuse pulmonary interstitial disease or asthma, BAL was carried out in the medial lobe or in the lingula. In those patients with localized pulmonary disease, such as respiratory infections or pulmonary neoplasias, the bronchoscope was introduced in the bronchi of the affected area or as close as possible.

For the BAL, 150 ml of 0.9% saline solution were instilled in three successive 50 ml fractions according to the norms set by the European BAL Task Group (16). Subsequently, the solution was retrieved through light suction. In phase I, the succioned liquid was retained without separating. In phase II, the BAL was divided into two parts: the bronchial fraction (BF), which represents the liquid recuperated after succioning the initial 50 ml of the BAL, and the alveolar fraction (AF), made up of the lavage obtained after succioning the remaining 100 ml (15,17).

The recovered liquid was centrifuged at 500 g for 15 min to separate the cellular component from the supernatant, which was frozen at −70°C until its later analysis.

**BIOCHEMICAL ASSAYS**

TPA was quantified in the BAL through radioimmunoassay (RIA), in accordance with the indications of the laboratory (Proligen TPA IRMA, Sweden), analyzing each sample in duplicate to obtain a coefficient of variation of <10%. Before this study and using the receiver operating characteristic (ROC) curves, the cut-point for the TPA in the BAL was: BF = 5680 U/g of total proteins (TP) and AF = 237 (18). The TP of the BAL was identified using the Lowry method (19). The results were expressed in units (U) of the antigen per gram of TP in the BAL (U/g TP).

**STATISTICAL ANALYSIS**

The results are expressed as mean and standard deviation (SD). To compare the concentrations of TPA among the different patient groups, the Mann–Whitney test was used and also the variance analysis as a multiple comparison of means through the Bonferroni test. An analysis of covariance was also utilized. The association of variables was valued with the χ² test. A value of p < 0.05 was considered significant (20).

**RESULTS**

**CONCENTRATION OF TPA IN THE GROUPS STUDIED**

The concentrations of TPA in the AF in the different patient groups are shown in Fig. 1. The lung cancer group reached significantly superior levels with respect to the rest (p < 0.001).

The possible effect on the TPA levels brought about by the differences in age and tobacco habit between the tumor and non-tumor groups was correct by means of a covariant...
analysis, using age and tobacco habit as covariants. In this analysis model, for the comparison of the adjusted means, the $F$-test (coefficient between the variance for the contrast of the equality of adjusted means and the residual variance of the dependent regressions) and the level of statistical significance were determined. Through this type of analysis, it was proved that the concentrations of TPA were greater in the tumor group than in the non-tumor group, after having corrected for age ($F_{\mu}=12.0, p = 0.0006$) or for tobacco habit ($F_{\mu} = 13.6, p = 0.0003$).

**CONCENTRATION OF TPA BETWEEN THE TWO FRACTIONS OF BAL**

The concentrations of TPA in the two BAL fractions (bronchial and alveolar) were compared in 52 patients with lung cancer. Significant differences were found between the two fractions, reaching higher concentrations in the BF [26 018.2 (52 240.7) vs 3447.9 (942.0) U/g TP, $p < 0.05$]. It was found that, in the subgroup of patients with bronchogenic neoplasias who were smokers, the concentrations of TPA were superior in the AF with respect to the non-smokers [5323.4 (10 566.5) vs 878.3 (936.2), $p < 0.05$], without any differences with relation to the BF [27 167.6 (5405.8) vs 20 272.6 (4476.0) U/g TP, not significant]. In addition, in those patients with pulmonary carcinomas, both smokers and non-smokers, higher concentrations of TPA in the BF than in the AF were observed (Fig. 2) [27 167.6 (5405.8) vs 878.3 (936.2) U/g TP, $p < 0.05$].

**CONCENTRATION OF TPA ACCORDING TO THE HISTOLOGICAL TUMOR GROUP**

Table 2 shows the concentrations of TPA according to the histological type of tumor in the two fractions of BAL. No association was found between the histological group and the levels of TPA in the BAL.

Three of the patients with bronchogenic carcinoma had non-visible neoplasias, of which two were squamous carcinoma and one was oat-cell carcinoma. In these subjects, the levels of TPA in the BF and in the AF were higher than average: the first patient presented concentrations of 35 482.2 and 4842.3 U/g of TP, the second patient had levels of 34 925.4 and 5640.8 U/g of TP and the third had 31 833.5 and 4592.3 U/g of TP, respectively. Other patients with macroscopically visible tumors through bronchoscopy showed concentrations of TPA that were lower than average: 4516.2 U/g of TP in the BF and 357.6 U/g of TP in the AF, and also 693.8 U/g of TP in the AF, which corresponded to a large-cell and an oat-cell carcinoma, respectively.

Neither any healthy subject nor any patient with non-malignant pulmonary pathology presented concentrations of TPA in the BAL significantly higher than the group average.

**DISCUSSION**

There have been several studies (15,21) that have demonstrated that BAL can be divided into two different fractions: that obtained from the first few milliliters suctioned, which is the bronchial fraction, and that obtained from the rest of the liquid used for the lavage. Keeping in mind that the origin of lung cancer is bronchial (except for certain types of metastatic tumors and adenocarcinomas), the concentrations of these markers were different in each lavage fraction, as we have previously proven (17,21–23).

Although great advances have been made in the histological diagnosis of malignant tumors, the field of early diagnosis has not yet been covered. In the search for new biological substances for this use, CKs seem to play an important role in helping diagnose pulmonary neoplasias (3,5–7,9), as they are secreted into the circulation during cellular lysis and tissue necrosis. For this, they can act as clear tumor markers. In 1957, Björklund and Björklund (24) isolated, for the first time, a new polypeptide antigen (TPA). TPA is a CK that is produced and secreted by cells in proliferation, for which the concentrations of this marker in the different biological samples determine the speed of cellular division and the tumor aggressiveness (3). It is clearly shown that TPA is a useful serum marker for evaluating the prognosis of the patient and the stage of the tumor and also monitoring treatment (1,5,26–29). However, in the serum,

**Table 2. Concentrations of TPA in each fraction of BAL according to the tumor histology**

<table>
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<tr>
<th>Tumor Type</th>
<th>BF (U/g TP)</th>
<th>AF (U/g TP)</th>
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<tr>
<td>Squamous carcinoma</td>
<td>27 843.7</td>
<td>5263.3</td>
</tr>
<tr>
<td>Adenocarcinoma</td>
<td>28 752.5</td>
<td>1852.2</td>
</tr>
<tr>
<td>Large-cell carcinoma</td>
<td>4516.2</td>
<td>357.6</td>
</tr>
<tr>
<td>Oat-cell carcinoma</td>
<td>24 477.0</td>
<td>2017.3</td>
</tr>
</tbody>
</table>

BAL, bronchoalveolar lavage; TP, total protein.
this usefulness diminishes when it is used in the early diagnosis of lung cancer.

Because of this, the following step was to apply the study of the tumor markers to more specific samples, such as BAL. Thus, some studies (8,10,14) analyzed this marker in the BAL liquid with an increase in its diagnostic effectiveness. We have analyzed TPA in the BAL of subjects with lung cancer and other benign respiratory diseases, and also in healthy smoker and non-smoker subjects. We found that the levels of the marker in the BAL of the tumor patients were significantly higher than in the rest of the pathologies and the healthy controls. However, the concentrations of TPA between the diverse benign pulmonary diseases and the healthy subjects were similar. Likewise, Rapellino et al. (30) also found that TPA in the BAL possesses an absolute specificity for a sensitivity of 40% in the diagnosis of lung cancer. This could be explained by the fact that the TPA would correlate better with the grade of proliferation than with the total mass of the tumor (5). In contrast, Blasco et al. (31) saw that the levels of TPA were also increased in other inflammatory processes. Most likely, as Rapellino et al. (30) indicated, the value of this report is limited because of the small number of patients studied. We believe that, with our results, it is shown that TPA clearly has a capacity to diagnose lung cancer.

Keeping in mind that with BAL two different types of samples can be obtained, we studied them in the two samples of BAL of a group of lung cancer patients. We wanted to find out if the concentrations of TPA in each fraction of BAL are different. We found that the concentrations of this marker were significantly higher in the BF of BAL. This could be explained by the fact that most pulmonary neoplasias originate in the areas closest to the bronchial tree, with the exception of bronchoalveolar carcinoma and some forms of metastasis (32). Some tumors not visible with the bronchoscope present an increase in concentration in the bronchial fractions of the BAL. This demonstrates the usefulness of the analysis of TPA in the diagnosis of the solitary pulmonary nodule. We have not found any other studies that analyzed the concentrations of TPA in both BAL samples.

When we compared the concentrations of TPA in the BAL between healthy smokers and non-smokers, we did not obtain significant differences (Fig. 1). Upon analyzing the concentrations of this marker in the two fractions of the BAL of smokers and non-smokers with bronchogenic carcinoma, we observed that, in the smoker patients with tumors, the concentrations were higher in the BF than in the alveolar. Similarly, when the levels of TPA in the BAL were compared between smokers and non-smokers with neoplasias, we saw that these levels were significantly increased in the BAL of the smoker patients. This demonstrates that the concentrations of the marker are distinct in the two fractions of the BAL. In addition, this further supports the fact that lung cancer patients who were smokers have a more increased cell proliferation than the non-smokers, as some authors have indicated (33,34). The increase in the BF would indicate that it is in the portion closest to the bronchial tree where the malignant transformation develops.

We have not found any relationship between TPA and the histological types of tumors, either when analyzed independently or when grouped as oat-cell and non-oat-cell carcinomas. In this sense, different authors (24,26) have found results similar to ours in serum, although Ferrigno and Buccheri (1) indicated that TPA had a clear prognostic value in squamous carcinoma of the lung. However, when we divided the broncho-genic carcinomas into primary and metastasis, we found that the concentrations of TPA were significantly elevated in the primary tumors. In any event, we think that more studies are needed to corroborate these latest findings owing to our limited number of secondary pulmonary neoplasias.

CONCLUSION

We consider that high levels of TPA in the BAL of patients with lung cancer could be effective in diagnosing the disease. Because of the results obtained, the tumor markers should be routinely analyzed in both fractions of the lavage. This would avoid false negatives associated with the analysis of TPA in the BAL without separating the two fractions and it would also increase its diagnostic power in lung neoplasias. We think that it is necessary to carry out a third phase of the study. This would allow us to compare the levels of TPA and other markers in the two BAL samples, not only in tumor patients, but also in healthy smoker and non-smoker subjects and in other benign pulmonary pathologies, also allowing us to verify whether this method could serve as a screening technique for lung cancer. In other words, it would allow us to recognize precancerous lesions, which would be a diagnostic benefit. Another future study that will be of great importance is to consider the patient tumor stage and to compare this stage with the TPA concentrations in both BAL fractions. This would most likely increase the value of tumor markers in the follow-up of these kinds of patients.

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References


