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New and old biomarkers in the differential diagnosis of lung cancer: Pro-gastrin-releasing peptide in comparison with neuron-specific enolase, carcinoembryonic antigen, and CYFRA 21-1

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Abstract

Background: Testing for circulating biomarkers in lung cancer is hampered by the insufficient specificity. We aimed to assess the relative diagnostic accuracy of pro-gastrin-releasing peptide (ProGRP) for the differential diagnosis of small cell lung cancer and compare it with more conventional biomarkers.

Methods: We enrolled a cohort of 390 patients with a clinical suspicion of lung cancer and for whom a histologic assessment was available. Serum or plasma samples were assessed for ProGRP, carcinoembryonic antigen, CYFRA 21-2, and neuron-specific enolase. The performance of each biomarker in discriminating the small cell lung cancer and squamous cell carcinoma/adenocarcinoma from non-malignant lung disease, and small cell lung cancer from squamous cell carcinoma/adenocarcinoma, was assayed by receiver operating characteristic curve analysis.

Results: At the cut-off levels suggested by the manufacturers, ProGRP and neuron-specific enolase showed an almost identical sensitivity of 55.2% and 55.6%, respectively, in discriminating small cell lung cancer with respect to non-malignant lung disease. In order to quantify the added value of ProGRP to other conventional markers, we ran a multivariable logistic regression analysis, but the results showed that no markers improve the performance of ProGRP.

Conclusions: ProGRP and neuron-specific enolase individually appear more accurate than other conventional biomarkers for small cell lung cancer, but the union of two markers does not increase the accuracy. The very small target group of patients with small cell lung cancer is a limitation of this study, which can explain why ProGRP alone does not show a sensitivity higher than neuron-specific enolase, as reported by other authors.

Keywords

ProGRP, small cell lung cancer, tumor marker, differential diagnosis

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Introduction

At the start of 20th century, lung cancer was a rare disease, but slowly it has become the leading cause of cancer death in the world. In 2008 lung cancer was diagnosed in about 1.6 million people. The first cause of lung cancer has been identified as cigarette smoking. Tobacco smoke indeed contains many carcinogenic compounds, such as polyaromatic hydrocarbons. An increase in air pollution and the occupational exposure to asbestos, arsenic, nickel, and chromium are other factors that can affect the increase in lung cancer.[1–3]

Lung cancer can be divided into two categories according to its histology: small cell lung cancer (SCLC) and non-small cell lung cancer (NSCLC), with different treatments and prognosis. SCLC is diagnosed in about 15% to 20% of all lung cancer patients, and in contrast to NSCLC it is highly sensitive to chemotherapy and radiotherapy. Unfortunately, SCLC is usually discovered late in patients, who, at the time of diagnosis, already have metastatic lesions in regional lymph nodes or distant organs.[4]

A simple and economical tool in the management of lung cancer patients for prognosis and follow-up is represented by tumor markers. A number of serum components have been proposed as markers for this malignancy: carcinoembryonic antigen (CEA), squamous cell carcinoma (SCC) antigen, tissue polypeptide antigen (TPA), and cytokeratin 19 fragment (CYFRA 21-1) have been investigated in NSCLC and neuron-specific enolase (NSE) in SCLC.[5–8] None of these markers is specific for lung cancer and there is no clear relationship with the histological type. Some studies demonstrate CYFRA 21-1 is a prognostic and predictive marker in NSCLC on the contrary NSE in SCLC. NSE alone has a low sensitivity, especially in patients with limited disease, therefore it is frequently combined with other tumor markers, such as CEA and CYFRA 21-1.[7–10]

In recent years, studies have been focused on a new marker: gastrin releasing peptide (GRP), a bombesin-like peptide present in the adult human gastrointestinal and respiratory tract. GRP is a neuropeptide hormone originally isolated from porcine gastric tissue. Because of its short half-life, which is about 2 minutes, GRP is not suitable in laboratory practice. On the other hand, ProGRP, a serum precursor peptide of GRP, is stable in serum and may be used as a possible tumor marker of SCLC.[11,12] Only few data are available concerning the utility of ProGRP as a marker for monitoring the disease and for the detection of recurrences.

The aims of the present study were:

- to establish the added value of quantitative determination of ProGRP in the differential diagnosis of patients with clinical suspicion of lung cancer;
- to evaluate the usefulness of NSE, CEA, and CYFRA 21-1 as tumor markers in lung cancer patients.

Material and methods

Patients

From September 2009 to March 2012 we enrolled 390 patients with a suspicion of lung cancer, attending the Division of Thoracic Surgery at the European Institute of Oncology. The study was approved by the Ethical Committee of the European Institute of Oncology. Every patient was informed about the aims and the importance of the study; they signed an informed consent before inclusion.

Since an impaired kidney function can affect the levels of ProGRP, no patients with levels of creatinine greater than 1.30 mg/dL were enrolled in our group, as well as patients with other tumors of the neuroendocrine system that may also impact on ProGRP levels, and patients who had received previous treatment.

Histological tumor typing, the gold-standard method for diagnosis, and information about other diagnostic procedures (x-rays, bronchoscopy, computed tomography), were available for all the patients.

Laboratory analysis

Besides ProGRP, NSE, CEA and CYFRA 21-1 were determined in all the patients. Serum samples, obtained by venous puncture, were centrifuged at 3000 rpm for 10 minutes and then separated and stored at −80°C until the execution of the tests.

Serum determination of ProGRP, CEA, and CYFRA 21-1 were performed using the chemiluminescent microparticle immunoassay (CMIA) on the Architect i2000SR analyzer (Abbott Laboratories, Abbott Park, IL, US), while the LIAISON system (DiaSorin, Saluggia, Vercelli, Italy) was used for NSE. The upper limits of normal concentrations of ProGRP, NSE, CEA, CYFRA 21-1, and lactate dehydrogenase were set according to the corresponding manufacturers' suggestions: 63 pg/mL (ProGRP), 18.3 ng/mL (NSE), 5 ng/mL (CEA) and 3.3 ng/mL (CYFRA 21-1). According to previous studies[13–15] that used different cut-offs to obtain an improvement in specificity, 97 pg/mL was also considered an alternative cut-off point for ProGRP, which was derived from the group of our patients with non-malignant lung disease (NMLD; e.g. 95th percentile).

Statistical methods

Patient characteristics were summarized and tabulated either as counts and percentage or median, minimum and maximum for categorical and continuous variables. Baseline values for ProGRP, CEA, CYFRA 21-1, and NSE were summarized and tabulated by histology. Pairwise comparisons for continuous variables were done by the Wilcoxon two-sample test. Categorical variables were tested by the chi-square test or Fisher's exact test as appropriate. A receiver operating characteristic (ROC) analysis was done

in order to compare the performance of each biomarker in discriminating the SCLC and SCC/adenocarcinoma (ADK) lung cancer types with respect to the non-malignant lung disease (NMLD). A secondary ROC analysis on the same biomarkers was performed, which aimed to compare their performance in discriminating SCLC versus SCC/ADK. Results of the ROC analysis were tabulated as odds ratios, sensitivity, specificity, and accuracy (e.g. the area under the curve (AUC)) with 95% confidence intervals. ROC curves were also produced and plotted. Statistical tests were two-tailed and considered significant at the 5% level. All analyses were done using SAS 9.3 and STATA/MP 14.0.

Results

Table 1 shows the characteristics of the 390 patients enrolled in this study (267 males and 123 females). The overall median age of the patients was 65 years. Patients with non-malignant lung disease were significantly younger ($P<0.001$) with respect to patients with malignant disease, but there was no difference between SCLC, ADK, and SCC ($P=0.056$). The histological types included 212 ADK (54.3 %), 77 SCC (19.7%), 29 SCLC (7.4 %) and 72 benign cases (18.4%).

According to the tumor node metastasis (TNM) staging, stage IA–IB disease was diagnosed in 139 (48.6%) patients, 42 (14.7%) were in stage IIA–IIB and stage IIIA–IIIB–IV disease was diagnosed in 105 (36.7%) patients.

The median, minimum and maximum values of ProGRP, CEA, CYFRA 21-1 and NSE for lung cancer patients are shown in Table 2. Tables 3 and 4 show the results of the ROC analysis and the performance of different biomarkers in discriminating SCLC with respect to NMLD and SCC/ADK, respectively. A significant risk (odds ratio) of being classified as SCLC or NSCLC with respect to NMLD was observed for any marker except ProGRP and NSE, neither of which discriminated between the SCC/ADK types with NMLD (Table 3, $P= 0.24$ and $P= 0.27$, respectively). Table 4 shows that only CEA and CYFRA 21-1 failed to discriminate between SCLC and SCC/ADK.

Using a cut-off level of 63 pg/mL for serum ProGRP, sensitivity and specificity were 55.2% and 87.5% respectively; while using a cut-off level of 97 pg/mL the sensitivity and specificity were 48.3% and 97.2%, respectively (Table 5). The corresponding values for NSE, using a cut-off level of 18.3 ng/mL, were 55.6 and 98.6%. The values for the other markers are shown in Table 5. In particular, CEA and CYFRA 21-1 showed a sensitivity of 34.8% and 34.5% with a specificity of 93.5% and 97.2%, respectively, in discriminating SCLC with respect to NMLD. In order to quantify the added value of ProGRP to other conventional markers we ran a multivariable logistic regression analysis and the results are presented in Table 3.

Table 4 shows the results of the multivariable analysis of ProGRP and NSE in discriminating SCLC with respect to SCC/ADK. The added value of NSE to ProGRP does not statistically increase the accuracy in the diagnosis of SCLC.

Discussion

Lung cancer presents as one of the most serious problems of modern oncology and reducing its mortality remains an important issue. Most lung cancer patients, despite the continuous improvement and development of diagnostic methods, are diagnosed in advanced stages; therefore, surgical therapy with an intent of cure is not feasible.[16]

Serum tumor markers have been widely studied in lung cancer, mainly for the prediction of tumor recurrence and the early detection of treatment failure. However, their roles are still controversial either because they have a low sensitivity or because a clear relationship with the histological type is still lacking.[4]

In the present study, four tumor markers were evaluated. In line with previous reports,[7,17,19] our results confirm that patients with lung cancer have increased serum levels of CEA, CYFRA 21-1, ProGRP, and NSE compared to patients with benign disease. Moreover, we found a strong association among the SCLC patients and high levels of ProGRP and NSE. This relationship between these tumor markers and the histological type suggests their possible utility as a support in the diagnostic work-up in case of a suspicious lung mass.

NSE is a glycolytic enzyme widely expressed in neuroendocrine tumors, especially in SCLC. It has been considered the tumor marker of choice for the diagnosis, therapy, monitoring, and prognosis in SCLC.[17,19,20] However, NSE has a low sensitivity and this has led to the evaluation of the performance of other markers. ProGRP is a new tumor marker, and preliminary results seem to indicate its utility in the follow-up of SCLC patients.[7,15–19]

We found that the sensitivity of ProGRP was almost identical to that of NSE—a result that contradicts previous reports.[17,19,21–23] In fact, ProGRP was higher than the cut-off point of 63 pg/mL only in 16 patients out of 29 with SCLC, achieving a diagnostic sensitivity of 55.2% in discriminating SCLC from SCC/ADK, and is practically identical to the sensitivity found for NSE (55.6%). Also, in contrast to other studies,[15,24] the combination of ProGRP and NSE determine an increase in accuracy, which is not significant (Table 4.2). We also evaluated ProGRP in patients with NSCLC and found increased levels (>63 pg/mL) of the marker in 13.8% of patients; this agrees with other studies, ranging from 14% to 26%.[4,24]

The very small target group of patients with SCLC is a limitation of this study and it can explain why ProGRP alone does not show a sensitivity higher than NSE, as reported by other authors.

We also considered an alternative cut-off of 97 pg/mL for ProGRP, which was derived from the group of our

patients with non-malignant lung disease (95th percentile). As expected, this approach determined a substantial increase in specificity (97.2%) coupled with a substantial decrease in sensitivity (48.3%). At this cut-off only 4 out of 289 patients with NSCLC had high ProGRP plasma concentrations. Different cut-offs were also evaluated by other authors. For example, Molina et al.[4] evaluated the performance of two different cut-offs, 50 pg/mL and 300 pg/mL, achieving a sensitivity of 73% and 39%, respectively, with a specificity of 100% at a cut-off of 300 pg/mL. In a meta-analysis including 5146 subjects in which different ProGRP methods were used with cut-offs ranging from 29.1 ng/L to 87 ng/L, the pooled sensitivity and specificity for ProGRP were 71.6% and 92.1%, respectively.[22] Better results were observed in two more recent studies. Yang et al.,[24] using a cut-off of 300 pg/mL, found a sensitivity of 75% with a specificity of 100%; Nisman et al.,[25] considering a cut-off of 140 pg/mL, found a sensitivity of 84% and a specificity of 96.3%. In this last study, the better results were probably due to the utilization of a new ARCHITECT plasma ProGRP assay, confirming the previous study of Kim et al.,[26] and demonstrating that the diagnostic performance of ProGRP is improved with the use of plasma instead of serum.

We also tested the discriminatory capacity of CEA and CYFRA 21-1 in patients with a suspicion of lung cancer. CEA, which is a widely used serum tumor marker in colon cancer,[27] has also been tested in patients with adenocarcinoma of the lung,[5,28] while CYFRA 21-1, the soluble fragment of cytokeratin 19, has been proposed for SCC.[5,8] The results of our study in a large group of patients confirmed that CEA and CYFRA 21-1 displayed the best performance in discriminating SCC/ADK types with respect to NMLD, which is in agreement with previous publications.[29–33]

However, both CEA and CYFRA 21-1 added individually to ProGRP did not increase the accuracy in diagnosis of SCLC with respect to NMLD. In conclusion, the present study shows that high serum levels of ProGRP and NSE predict SCLC histology with good accuracy; however, the small number of patients with SCLC determined a low sensitivity of ProGRP, which is similar to that of NSE. The other markers analyzed confirmed that their determination might be of help in directing the suspicion toward a specific type of lung cancer histology.

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Supplemental material

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