Diagnostic value of SCC, CEA and CYFRA 21.1 in lung cancer: a Bayesian analysis

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Diagnostic value of SCC, CEA and CYFRA 21.1 in lung cancer: a Bayesian analysis. A. Pastor, R. Menéndez, Ma.J. Cremades, V. Pastor, R. Llopis, J. Aznar. ©ERS Journals Ltd 1997.

ABSTRACT: The aim of this study was to evaluate the diagnostic value of three tumour markers, squamous cell carcinoma (SCC) antigen, carcinoembryonic antigen (CEA) and CYFRA 21.1, in lung cancer using a Bayesian analysis to obtain the predictive values for different pretest probabilities or prevalences.

A cross-sectional study included 94 patients with lung cancer, 40 with benign lung disease, and 40 healthy controls. SCC antigen and CEA were measured in blood samples by microparticle enzyme immunoassay (MEIA), and CYFRA by enzyme-linked immunosorbent assay (ELISA).

The results of tumour marker determinations were expressed as percentiles, and showed significantly higher levels in the cancer group than in the two control groups. Taking the 95th percentile of benign lung diseases as the cut-off point (specificity 95%), the following sensitivities were found: SCC 41%, CEA 31% and CYFRA 79%. After a Bayesian analysis, the best results for the three tumour markers were found in prevalences of 30–40%. The highest incremental gain was obtained by CYFRA (at prevalence of 36%, positive and negative predictive value approximately 90%). The three tumour markers were included in a stepwise regression analysis to predict lung cancer, and CYFRA was the only selected variable.

We conclude that CYFRA 21.1 may be a useful marker in lung cancer when there is an intermediate pretest probability of disease.
Eur Respir J 1997; 10: 603–609.

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In the last few decades, tumour markers have been evaluated for their diagnostic and prognostic value in malignant diseases, such as lung cancer. The best known and most widely studied tumour markers are carcinoembryonic antigen (CEA) and squamous cell carcinoma (SCC) antigen. Many adenocarcinomas are known to have high serum levels of CEA [1, 2]. SCC antigen, a purified subfraction of tumoral antigen TA-4 obtained from squamous cell cancer tissue of the uterine cervix [3], is also present at high concentrations in neoplasias with this type of cell histology [4, 5]. However, measurement of these tumour markers has a limited diagnostic value due to the lack of specificity, demonstrated by the increased levels of these tumour markers in several benign diseases. A recently commercialized monoclonal antibody (CYFRA 21.1) to the human variant of cytokeratin 19 has been reported to achieve higher sensitivity and specificity than other tumour markers in lung cancer.

Cytokeratins (class I and II intermediate filaments) represent a family of cytoskeletal proteins of 40–68 kDa, that are present in epithelial tissue [6] and have proved to be clinically useful in diagnosing poorly-differentiated tumours, whose histological appearance alone does not allow a distinction between lineages [7]. Various studies have shown the existence of high levels of cytokeratins 7, 8, 18 and, especially, 19 in all histological types of lung cancer [6, 8]. Although cytokeratins are part of the cytoskeleton, some fragments might be released into the serum owing to cell lysis or tumour necrosis. For these reasons, the cytokeratin subunit 19 fragment can be treated as a tumour marker for lung cancer.

Most of the numerous studies on the diagnostic value of tumour markers, focus only on sensitivity and specificity, the two intrinsic characteristics of the test. However, assessment of these does not, *per se*, permit accurate interpretation of the test results. Therefore, the pretest probability or prevalence might help ascertain how much information is gained by the test result. Bayes' theorem [9–11] makes it possible to calculate predictive values at several pretest probabilities and may help physicians to know where the test is most useful [12].

The aim of the present study was, firstly, to quantify the diagnostic value of SCC antigen, CEA and CYFRA 21.1 (hereafter, simply called CYFRA) by calculating their sensitivity, specificity and then their predictive value according to the formulae of the Bayes' theorem; and, secondly, to analyse the behaviour of the three histological lineages: squamous cell carcinoma, adenocarcinoma and small cell lung carcinoma.

Patients and methods

Subjects

A cross-sectional study was designed for three groups of subjects: patients with lung cancer (LC); patients with benign lung diseases (BD); and healthy controls (HC). The consecutive type of sampling technique was used.

The LC group (n=94; mean age 64 yrs) consisted of patients hospitalized in our Pneumology Service from March 1992 to August 1993, with histologically confirmed lung cancer. Routine pretreatment staging procedures included physical examination, blood chemistry profile, chest radiography, computed tomography (CT) of the chest and abdomen, ultrasonography of the liver, and bronchoscopy with biopsy. In addition, CT of the brain, bone scan and bone marrow biopsy, were performed to evaluate patients with small cell lung carcinoma (SCLC), and these additional tests were used with the other cell types of LC when the symptoms and laboratory data demanded. Histological diagnosis was performed according to the guidelines of the World Health Organization (WHO) (Histological Classification of Lung Tumours; Geneva, 1981), and was based on the predominant cell type [13]. The distribution among these subjects was: 42 squamous cell carcinomas (SCC), 29 adenocarcinomas (AC), 15 small cell lung carcinomas (SCLC), and eight undifferentiated large cell lung carcinomas. All patients were staged according to the guidelines of the American Joint Committee on Cancer (AJCC 1988) [14].

The BD group comprised 40 patients (mean age 65 yrs) hospitalized in our Pneumology Service, who had benign lung disease of known aetiology (chronic obstructive lung disease, acute infectious diseases, tuberculosis, asthma and diffuse noninfectious interstitial diseases). Patients with a case history of malignant disease, or who had digestive or kidney disease, or two or more concomitant lung diseases were excluded from the study.

Finally, the HC group (mean age 64 yrs) comprised 40 blood donors from the blood bank at our hospital and lung disease out-patients called in for follow-up of an already cured acute disease.

A venous blood sample was collected from each subject into a sterile tube containing 3.8% sodium citrate, after fasting for 12 h or more. The samples were then centrifuged at 2,500 rpm for 20 min. The plasma supernatant was fractionated into three aliquots, which were frozen at -80°C. Samples were labelled with a numerical code unknown to laboratory personnel. Once frozen the aliquots were stored at -20°C until processed when the study had ended.

Tumour markers assays

The Enzymun-test CYFRA 21-1 (Boehringer-Mannheim, Germany) is based on enzyme immunoassay technology, following a typical sandwich protocol. Briefly, in the first step of the assay, standards or samples were incubated with biotinylated antibodies (MAK 19.1) in streptavidin-coated polystyrene tubes for 30 min. After aspiration and washing, the antibody-horseradish peroxidase (HRP) conjugate (MAK 19.21) was added and incubated for 30 min. After further aspiration and washing, ABTS (2,2'-azino-bis-[3-ethyl-benzthiazoline-sulphonic acid-(6)] diammonium salt)-substrate solution was added and incubated for another 60 min. Absorbance was recorded at 422 nm. The CYFRA 21.1 concentration was calculated from the standard curve. The test was performed at 25°C on the multibatch analyser ES 300. The sensitivity of the assay was 0.30 ng·mL⁻¹.

SCC antigen and CEA concentrations were measured using the IMx Abbott (microparticle enzyme immunoassay (MEIA)). The sensitivity of the assays was of 0.10 ng·mL⁻¹.

Data analysis

For the statistical evaluations, the Kolmogorov-Smirnov test was employed to evaluate the distribution of the variables studied. The Chi-squared test was used for the qualitative comparisons (sex and smoking habits), and one-way analysis of variance (ANOVA) for quantitative comparison (age), since this variable showed a Gaussian distribution. Data on tumour markers are expressed as percentiles because they were not normally distributed. Therefore, nonparametric statistical analyses were used to compare the results of the different groups. Differences between more than two groups were determined by means of Kruskal-Wallis one-way ANOVA. A probability level less than 0.05 was considered significant. Subsequently, when the result was positive, differences between two independent groups were determined by the Mann-Whitney U-test, and the significance level for multiple comparisons was corrected using the Bonferroni test [15] (α corrected = α/n; where n is the number of comparisons). Spearman's rank correlation test was used to evaluate a possible association between levels of tumour marker and tumour, node, metastases (TNM) stage.

The sensitivity and specificity of the tumour markers were determined [11]. Sensitivity (S) is defined as the probability of testing positive if the disease is truly present, whereas specificity (Sp) is the probability of screening negative if the disease is absent. $S=TP/(TP+FN)$ and $Sp=TN/(TN+FP)$, where TP is true-positive, TN is true-negative, FP is false-positive and FN is false-negative. These values (S and Sp) were calculated in each tumour marker for several percentiles of group BD, in order to obtain receiver operating characteristic (ROC) curves [11]. These curves were graphically constructed by plotting sensitivity against the false-positive rate (1-specificity) for each value obtained. The areas under ROC curves were calculated with the program, RSCORE II. Using the 95th percentile of the levels obtained in benign lung disease as the cut-off point (specificity of 95%), a Bayesian analysis [9–11] was performed to obtain positive and negative predictive values according to the following formulae:

$$\text{PPV: } (Pr \times S)/[Pr \times S] + (1-Pr) \times (1-Sp)]$$
$$\text{NPV: } [(1-Pr) \times Sp]/[(1-Pr) \times Sp + Pr \times (1-S)]$$

In these equations, Pr is prevalence or pretest probability, S is sensitivity, and Sp is specificity. PPV represents positive predictive value (probability that a person given positive on the test actually has the disease), and NPV represents negative predictive value (probability

of testing negative and, in fact, not having the disease). The difference between PPV or 1-NPV and the pretest probability is the positive (G+) or negative (G-) final diagnostic gain of the test. The security interval (SI) of the test is the pretest probability range when PPV and NPV are greater than 50%.

Finally, a stepwise logistic regression analysis was performed with the three tumour markers simultaneously (independent variables) to predict the presence of lung cancer or not (Y=dependent variable). The maximum likelihood estimation method was used to obtain the coefficient of selected variables. Its mathematical expression is:

$$P(Y=1) = 1/[1 + \exp - (\alpha + \beta_1 x_1 + ... \beta_k x_k)]$$

where, $x_1 ... x_k$ take the value of the selected independent variables, $\beta_1 ... \beta_k$ are the coefficients of each variable, and α is the constant.

Results

Descriptive and analytic statistics

The three groups of subjects (LC, BD and HC) were homogeneous for the variables sex, age and smoking habits.

Table 1 summarizes the results of simultaneously performed SCC antigen, CEA and CYFRA measurements in patients with lung cancer prior to therapy, in patients with benign lung diseases of various aetiologies, and in healthy controls. For patients with lung cancer, the marker concentrations were stratified according to different histological cell types and TNM stage.

The concentrations of the three tumour markers were significantly higher in the LC patient group (p<0.025) than in the BD and HC groups. In the LC group, the levels of the three tumour markers were evaluated in three histological types (SCC, AC and SCLC). SCC antigen was higher in patients with SCC, although the only statistically significant differences were between this histological type and AC (p<0.0125). The highest

concentrations of CEA corresponded to patients with AC, and there were statistically significant differences (p<0.0125) between AC and SCC and between SCC and SCLC, but not between AC and SCLC. The highest levels of CYFRA corresponded to patients with SCC, but the differences between this and other histological types were not significant.

The correlation between levels of tumour markers and TNM stage was only significant for CYFRA (p<0.01), with a correlation coefficient 0.42.

Diagnostic value

Sensitivity and specificity. In the group of LC patients (n=94), the sensitivities obtained for a specificity of 95% were: SCC antigen 41% (cut-off 2.10 ng·mL⁻¹), CEA 33% (cut-off 5.90 ng·mL⁻¹) and CYFRA 79% (cut-off 2.10 ng·mL⁻¹). The 95% confidence interval (95% CI) for the sensitivities obtained were: SCC antigen 31– 51%, CEA 22–40%, and CYFRA 71–87%.

The sensitivity of the tumour marker tested in three histological types was calculated at the same specificity (95%). In patients with SCC (n=42) the sensitivities recorded were: SCC antigen (61%), CEA (15%) and CYFRA (83%). In the AC group (n=29) they were: SCC antigen (41%), CEA (57%) and CYFRA (79%). And in SCLC patients (n=15) the sensitivities observed were: SCC antigen (21%), CEA (31%) and CYFRA (79%).

ROC curves. Another method for evaluating the usefulness of these tumour markers in diagnosing lung cancer is to calculate the ROC curves using different plasma levels (percentiles 5, 25, 40, 50, 60, 75 and 95) of the benign lung disease patients as the cut-off point. Figure 1a shows the ROC curve in the LC patient group. As can be observed, the largest area under the curve correspond to CYFRA (0.92±SEM 0.02) (mean±SEM), which differs significantly from those of SCC antigen (p<0.002) and CEA (p<0.001).

For the same purpose, the ROC curves for the three histological types were calculated using the same cut-off point. Figure 1b shows the ROC curves obtained for SCC patients. As can be seen, although the areas under

Table 1. – Several percentiles (P5, P50 and P95) for plasma concentrations of SCC antigen, CEA and CYFRA in patients with lung cancer of different histology, subjects with benign lung disease and healthy controls

Group	Pts n	SCC Ag (ng·mL⁻¹)			CEA (ng·mL⁻¹)			CYFRA (ng·mL⁻¹)		
		P5	P50	P95	P5	P50	P95	P5	P50	P95
Total LC	94	0.25	1.50	8.90	0.26	2.90	119	1.21	5.62	60.0
AC	29	0.20	1.25	5.70	0.72	6.60	176	1.28	3.84	60.0
SCC	42	0.57	2.40	8.90	0.20	2.10	12.0	1.20	5.68	61.0
SCLC	15	0.14	0.60	11.0	0.26	4.00	143	0.79	3.86	34.0
TNM I	13	0.52	1.10	4.60	0.13	2.20	26.0	0.79	2.20	12.0
II	5	0.18	1.00	2.90	0.08	5.20	16.0	0.33	2.40	4.90
IIIa	15	0.15	2.05	4.70	0.08	2.10	16.0	0.90	3.95	34.0
IIIb	27	0.20	1.90	8.00	0.27	2.25	19.0	1.12	6.56	33.0
IV	34	0.26	1.50	10.0	0.27	5.55	195	1.34	11.3	64.0
BD	40	0.40	0.80	2.10	0.60	1.50	5.90	0.34	1.17	2.10
HC	40	0.10	0.50	1.80	0.20	0.60	2.30	0.90	1.19	1.90

SCC Ag: squamous cell carcinoma antigen; CEA: carcinoembryonic antigen; CYFRA: a recently commercialized monoclonal antibody, CYFRA 21.1; Pts: patients; LC: lung cancer; AC: adenocarcinoma; SCC: squamous cell carcinoma; SCLC: small cell lung cancer; TNM: tumour, node, metastases staging; BD: benign lung disease; HC: healthy controls.
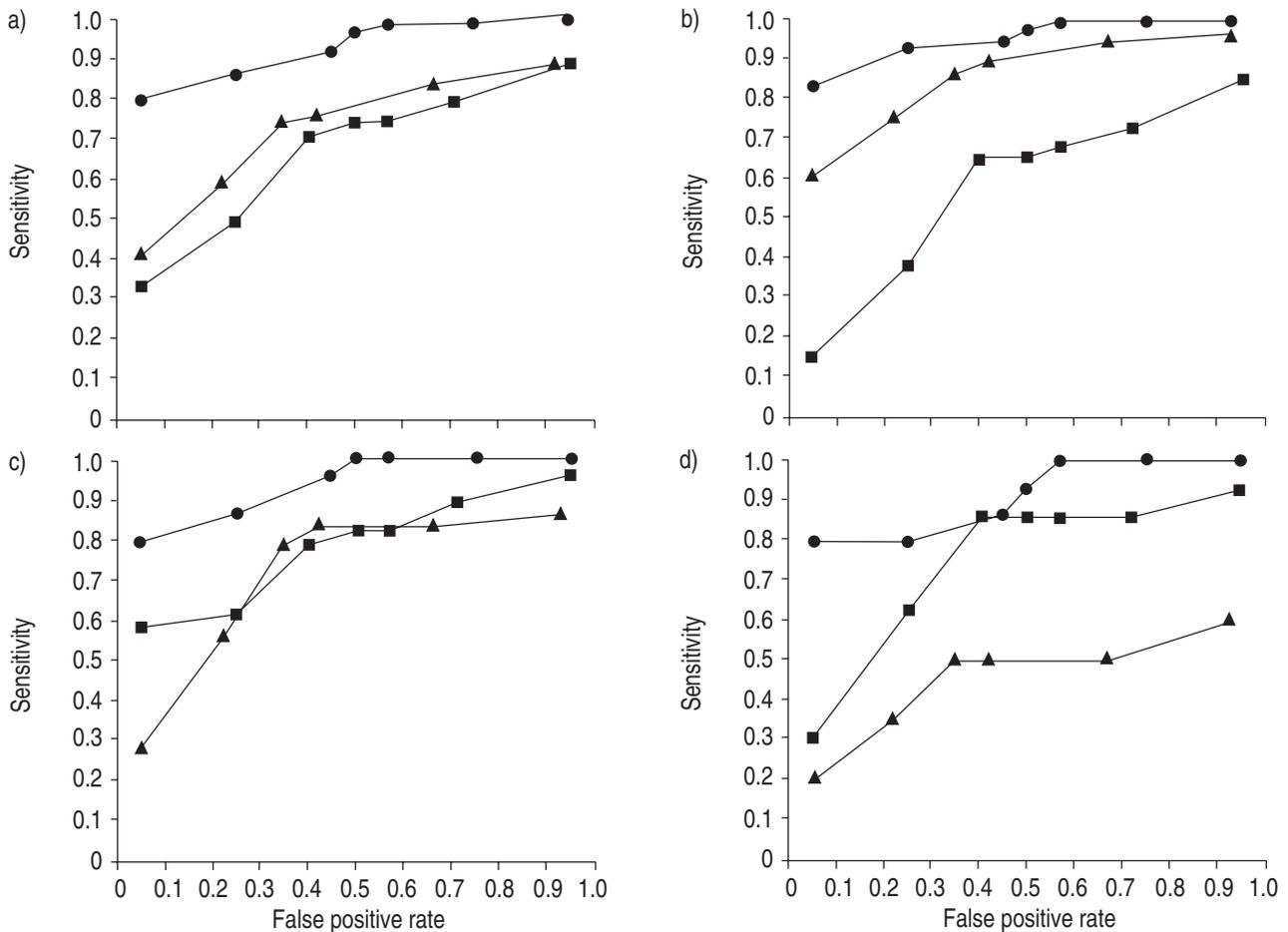
Fig. 1. – Receiver operating characteristic (ROC) curves in the LC group (patients with lung diseases) and according to the histological types *versus* benign lung diseases for SCC antigen, CEA and CYFRA. a) ROC curves of all LC: the areas under ROC curves were 0.79±0.04, 0.76±0.06 and 0.92±0.02, respectively. b) ROC curves of squamous cell carcinoma (SCC): the areas under ROC curves were 0.87±0.03, 0.57±0.06 and 0.93±0.03, respectively. c) ROC curves of adenocarcinoma (AC): the areas under ROC curves were 0.77±0.06, 0.81±0.06 and 0.92±0.06, respectively. d) ROC curves of small cell lung cancer (SCLC): the areas under ROC curves were 0.49±0.09, 0.79±0.07 and 0.89±0.04, respectively. CEA: carcinoembryonic antigen; CYFRA: a recently commercialized monoclonal antibody, CYFRA 21.1. ——▲——: SCC antigen; ——■——: CEA; ——●——: CYFRA.

the curves for CYFRA (0.93±0.03) and SCC antigen (0.87 ±0.03) do not differ greatly, the best sensitivities when the specificity increases correspond to CYFRA.

The ROC curves obtained in AC patients are presented in figure 1c. Although CEA obtained its best area under the curve (0.81±0.06) and, therefore, its highest sensitivities in this histology, the best results are again those of CYFRA (area under ROC curve 0.92±0.06).

Finally, figure 1d gives the ROC curves obtained for SCC antigen, CEA and CYFRA in SCLC patients. The largest area under the curve again corresponds to CYFRA (0.89±0.04).

Bayes' theorem. The sensitivity of each tumour marker at a specificity of 95% was employed to calculate the post-test probability of the person having or not having cancer for all the pretest probabilities or prevalences. Figure 2 shows the curves of post-test probability (CPTP) obtained in the LC group for SCC antigen, CEA and CYFRA. The graph shows clearly that the best predictive values, both positive and negative, correspond to CYFRA. For this tumour marker, the maximum gain for a positive result (G+) on the test is obtained with
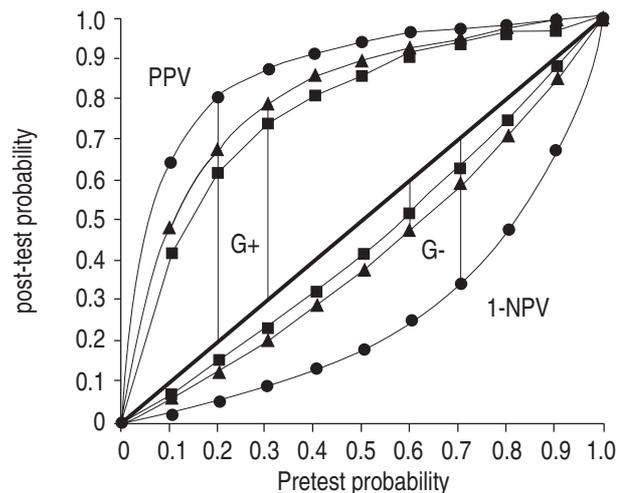

Fig. 2. – Curves of post-test probability (CPTP) in the LC group (patients with lung cancer) for SCC antigen, CEA and CYFRA. G+: maximal positive diagnostic gain of the test; G-: maximal negative diagnostic gain of the test; PPV: positive predictive value; NPV: negative predictive value. ——●——: CYFRA; ——▲——: SCC antigen; ——■——: CEA. For definitions see legend to figure 1.
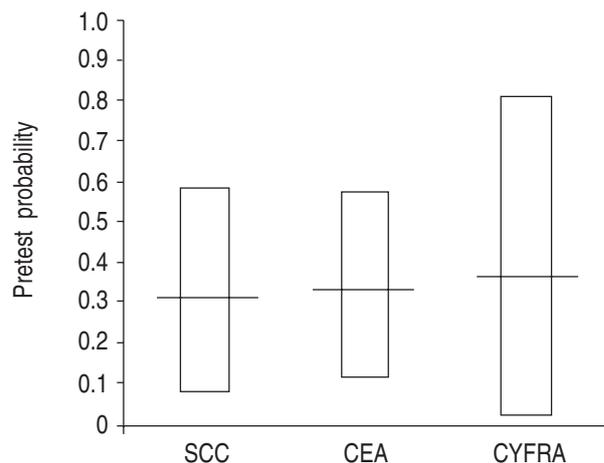
Fig. 3. – Security intervals in boxes and the best results of the tests (best positive and negative predictive value) in horizontal lines for SCC antigen, CEA and CYFRA in the LC group (patients with lung cancer). For definitions see legend to figure 1.

pretest probabilities of 0.20 (PPV 0.80). In the case of SCC antigen and CEA, the maximum G+ is observed with somewhat higher pretest probabilities (0.30) and somewhat lower PPV (0.78 and 0.74, respectively).

Finally, figure 3 presents the security intervals (SI) of the tests and pretest probabilities or prevalences that give the best overall results (best PPV and NPV) for the three tumour markers in the LC group. The broadest SI belongs to CYFRA. The best test results for the three tumour markers were obtained with pretest probabilities of 0.30–0.40, but the predictive values were higher for CYFRA. Thus, the maximum overall gain for this tumour marker is obtained at a pretest probability of approximately 0.36, and predictive values of practically 0.90.

Logistic regression analysis. After applying the logistic regression analysis, CYFRA was the selected variable to diagnose lung cancer. The mathematical model calculated was: $\alpha = -2.82$; $\beta = 1.63$ and $Exp(\beta) = 5.1$.

Discussion

The main finding in this study was that at identical specificity, CYFRA shows a higher sensitivity than SCC antigen and CEA for diagnosing lung cancer in the three histological types studied. Moreover, when a Bayesian analysis was used, CYFRA obtained the maximal diagnostic gain (both in PPV and NPV) at prevalence or pretest probabilities of 0.30–0.40, and the largest security interval.

Traditionally, tumour markers measured prior to treatment in lung cancer patients have shown higher levels than in control groups, although a lack of specificity due to increased levels in benign diseases has also been reported. In the present study, we also found significantly higher SCC antigen, CEA and CYFRA levels in the lung cancer group than in the two control groups. The three study groups were carefully matched for age, sex and smoking habits. It has been reported that these variables may influence the levels of tumour markers, especially for CEA [16, 17]. Differences according to

the histological types were detected in SCC antigen and in CEA. Although some statistical differences between certain histological types were found, the levels of tumour markers are not specific to one histological type, and they are not useful in distinguishing one from another.

When considering the TNM stage of cancer, the results for SCC antigen, CEA and CYFRA differed. A significant correlation between tumour marker levels and TNM stage was found for CYFRA, but not for SCC antigen or CEA. However, several authors [2, 4, 16, 18] suggest that there seems to be a correlation between the serum level of SCC antigen, the stage of the disease and the therapeutic response. Nevertheless, a stage identification which does not include systematic assessment of the endothoracic extension has certain limitations, as do all pre- and postoperative clinical staging procedures. In this context, MATTHEWS *et al.* [19] demonstrated that, in their autopsies, 35% of the patients who died after curative resection showed metastases not previously detected. Nevertheless, CYFRA levels correlated with TNM stage in the present study, as in those reported in the literature [20–24].

Previous studies on the diagnostic value of tumour markers have reported the sensitivity and specificity of SCC antigen, CEA and CYFRA in lung cancer, with some variations due to the different cut-off points and control groups selected. To select a cut-off point and to obtain the ROC curves only benign lung diseases and cancer groups were analysed. The reason is that a diagnostic test is useful when it closely resembles clinical practice, *i.e.* when it distinguishes between cancer (in the present study) and diseases that might mimic cancer or may coexist (as often happens with chronic obstructive pneumopathies and lung cancer). A healthy control group was, therefore, not included in order to avoid an overestimation of specificity. The 95th percentile of benign lung diseases was selected as the cut-off point (specificity 95%), for two reasons: firstly, in screening tests a high sensitivity is preferable and in diagnostic tests a high specificity is selected; and secondly, in malignant disease a high specificity is recommended. Although ROC curves can be obtained to select the cut-off point, they were calculated in the present study, in order to compare the diagnostic value of different tests by examining the area under each curve.

Analysis of the ROC curves in the LC group clearly shows that the largest area under the curves and the highest sensitivity correspond to CYFRA. In the present study the sensitivity obtained by CYFRA is higher than in other studies [20–25]. The basic reason for this is the cut-off point. If 3.3 ng·mL^{-1} had been used as the cut-off point, the sensitivity would have gone down to 61.9%, similar to that obtained by EBERT *et al.* (60.9%) [20].

In the SCC group, the largest area under the curve and the best sensitivity at a specificity of 95% also belong to CYFRA. With the exception of EBERT *et al.* [20], who are in agreement with us in finding no differences between the various histological types of LC, and who, moreover, obtained the highest CYFRA values in SCLC patients, most of the studies reported observe significantly higher CYFRA values in SCC patients. This discrepancy has several possible explanations. One is a difference in the composition of the populations

studied. Another is related to the histopathological classification. It is well-known that 30–50% of all lung tumours are made up of heterogeneous cell populations [26, 27], but are classified according to the dominant cell type [13]. Finally, it must be remembered that MOLL *et al.* [6], using immunohistochemical techniques, demonstrated the presence of cytokeratin 19 in all lung cancer types, including SCLC. The sensitivity observed for SCC antigen (61%) is similar to that reported by NIKLINSKI *et al.* [28] (69%), and although it is lower than that of CYFRA, it is much higher than that observed for the other histological types. The analysis of the areas under the ROC curve obtained in the present study for this histological type is similar to that of WIESKOPF *et al.* [24].

In the group of patients with adenocarcinoma, the best sensitivity again corresponded to CYFRA and is the same as in the LC group (79%). We, like other authors, found the best sensitivities for CEA in this histological type of LC [16–18, 29].

In the patients with SCLC, the largest area under the ROC curve and the best sensitivity (79%) both correspond to CYFRA and, in the latter case, was the same as in LC and AC patients.

However, a diagnostic test cannot be interpreted properly with information only about sensitivity and specificity. The prior estimate of the likelihood of the disease, the pretest probability or prevalence, must also be taken into account [12]. This can be done using a Bayesian analysis. In the present study, the predictive values (PPV and NPV) were calculated at several pretest probabilities for SCC antigen, CEA and CYFRA in the LC group, and the post-test probability of cancer was represented in curves. The distance between the post-test curves and the diagonal expresses the positive and negative gains after the test results, *i.e.* the precise contribution of the test to diagnosing lung cancer.

Bayesian analysis [9–11] confirms that CYFRA is the best of the three tumour markers analysed. Our findings show that this tumour marker can only be considered if prior probability of cancer is established between 0.05 and 0.80. If pretest probability is lower than 0.05, the post-test probability of cancer is lower than 0.50, which is the least reliable probability accepted. Likewise, if pretest probability is higher than 0.80, the test result does not significantly increase the prior probability of disease. The best utility of the test is obtained with an intermediate pretest probability (approximately 0.36, with predictive values near to 0.90), *i.e.* in clinical conditions when there is an inconclusive diagnosis. Predictive values for SCC antigen and CEA were lower than those of CYFRA.

In the present study, the pretest probability employed for the Bayesian analysis was numerical, with values between 0 and 1; however, another common method used to estimate the pretest probability by clinicians is to express it in words such as "low", "intermediate" or "high". In fact, a moderate error in the pretest estimate of the likelihood of disease has relatively limited implications for test interpretations [12].

Finally, the three tumour markers were included in a stepwise logistic regression analysis to predict lung cancer, and CYFRA was the only selected variable; *i.e.* levels of CYFRA alone are enough and, moreover, it is possible to calculate with them the likelihood ratio of

having lung cancer. This mathematical model also permits to calculate the post-test probabilities without using a pre-established cut-off point [30].

In summary, of the three tumour markers studied, CYFRA 21.1 showed the best diagnostic value in the three histological types. Its plasma concentration correlated with the stage of the disease, and at the same specificity it gave the best sensitivity and predictive values, both positive and negative. As a diagnostic test, it is recommended when there is an intermediate pretest probability, and it reaches its maximum performance at a probability of 0.30–0.40.

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