Diagnostic Accuracy of CYFRA21-1 in the Differential Diagnosis of Pleural Effusions

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Abstract. *Background/Aim:* Approximately 20% of pleural effusions are associated with cancer; about 50% require invasive procedures to perform diagnosis. Determination of the concentration of soluble cytokeratin 19-fragments (CYFRA21-1) may help identify patients with malignant effusions. However, pathologies other than cancer can increase its concentration. The identification of these possible false positives with routine tests CRP, ADA, % polymorphonuclear cells (PN) may improve diagnostic accuracy. This study aimed to determine the diagnostic accuracy of CYFRA21-1 in the detection of malignant pleural effusions and the possible false positives. *Materials and Methods:* Analysis of CYFRA21-1, adenosine deaminase (ADA), C-reactive protein (CRP), and the percentage of polymorphonuclear leukocytes (PN%) in the fluid from 643 consecutive undiagnosed pleural effusions was performed. *Results:* CYFRA21-1 showed 38.7% sensitivity and 97.3% specificity at 175 ng/ml cut-off. Effusions not suspicious of a false-positive showed 39.0% sensitivity and 98.2% specificity, while effusions suspicious of false positive showed lower sensitivity (36.4%) and specificity (95.0%). *Conclusion:* The diagnostic accuracy of CYFRA21-1 in pleural effusions

can be improved by classification according to the possibility of false positives.

Pleural effusions can be caused by a wide variety of diseases. Among them, cancer is one of the most frequent; with rates between 15% and 27% (1, 2). Differential diagnosis of pleural effusions is not easy. Cytology is the gold standard test for ruling out cancer, but its sensitivity is only moderate (between 45% and 70%) (2, 3). This means that more invasive tests are sometimes necessary, increasing morbidity and raising the economic cost.

Soluble cytokeratin 19-fragments (CYFRA21-1) have been evaluated in the differential diagnosis of cancer in pleural effusions. The results vary widely from study to study; the specificity ranges between 7% and 100% and the sensitivity between 25% and 90%, using discriminant values between 3.3 ng/ml and 175 ng/ml (4-10). Most studies applying highly discriminant values (55-175 ng/ml) obtained sensitivities between 24% and 70% and specificities above 95% (6, 10-13). However, in diseases such as tuberculosis, empyema and parapneumonic effusions, as well as in inflammatory processes of the surrounding tissues, high concentrations of this tumor marker can be found in the pleural fluid (11, 14, 15). Some biomarkers suggest a benign diagnosis: examples are adenosine deaminase (ADA) in tuberculosis and empyema, white blood cell count and the percentage of polymorphonuclear leukocytes (PN%) in empyema and complicated parapneumonic effusions, and C-reactive protein (CRP) in all three of the above conditions, and in the process of necrosis or inflammation of the surrounding tissues.

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Table I. Etiology of the effusions included in the study.

	Group A (n=502) (78.1%)	Group B (n=141) (21.9%)	All effusions (n=643)
Neoplastic	163 (88.1%)	22 (11.9%)	185
Lung cancer	70 (92.1%)	6 (7.9%)	76
Breast cancer	22 (91.7%)	2 (8.3%)	24
Mesothelioma	18 (81.8%)	4 (18.2%)	22
CUP ¹	19 (90.5%)	2 (9.5%)	21
Ovarian cancer	9 (90.0%)	1 (10.0%)	10
Lymphoma	5 (50.0%)	5 (50.0%)	10
Bladder cancer	5 (100.0%)	0 (0.0%)	5
Others ²	15 (88.2%)	2 (11.8%)	17
Cardiogenic	109 (87.2%)	16 (12.8%)	125
Parapneumonic			
Non-complicated	44 (89.8%)	5 (10.2%)	49
Complicated	5 (16.1%)	26 (83.9%)	31
Empyema	2 (8.3%)	22 (91.7%)	24
Tuberculosis	0 (0.0%)	22 (100%)	22
Rheumatoid arthritis	3 (33.3%)	6 (66.7%)	9
Cirrhosis	15 (100.0%)	0 (0.0%)	15
Paraneoplastic	44 (81.5%)	10 (18.5%)	54
PTE ³	10 (90.9%)	1 (9.1%)	11
Post traumatic	13 (100.0%)	0 (0.0%)	13
Pneumonitis	6 (54.5%)	5 (45.5%)	11
Asbestosis, nephrotic syndrome, ARDS ⁴ , pancreatitis, hypothyroidism, uremia, others	88 (93.6%)	6 (6.4%)	94

¹Cancer of known primary site; ²Pancreatic, gastric, colon, endometrial, cervix, sarcoma, multiple myeloma, melanoma, cholangiocarcinoma, hypernephroma. ³pulmonary thromboembolism; ⁴adult respiratory distress syndrome.

In a previous work by our group, classifying the effusions with the aid of these biomarkers indicating a benign status, enabled us to differentiate between two groups of patients, presenting pleural effusions with regard to cancer prevalence. Those that were negative for all the benign biomarkers presented higher cancer prevalence and greater diagnostic sensitivity of malignancy (16).

The aim of this study was to determine the diagnostic accuracy of CYFRA21-1 in detecting malignant pleural effusions and to establish the extent to which the identification of possible false positives using benign biomarkers (ADA, CRP and PN%) improves diagnostic accuracy.

Patients and Methods

From January 2005 to December 2012, pleural effusion fluid samples were collected from consecutive patients of all medical specialties at our hospital who presented pleural effusions.

The reference method was pathological confirmation of cancer in serous pleural effusions, or definitive diagnosis assessed during the three months following the determination of CYFRA 21-1. Serous pleural effusions were defined as malignant when the presence of neoplastic cells was detected by cytology, biopsy or autopsy. Paramalignant pleural effusions were defined as effusions

in which no neoplastic cells were detected by any of the methods described above in patients diagnosed with cancer. Diagnostic procedures were performed by assessors who were blinded to the study data.

In order to identify benign pleural effusions, the following tests were performed in fluid and/or serum: protein, albumin, N-terminal-pro-brain natriuretic peptide (Nt-ProBNP), lactate dehydrogenase (LDH), microbiological cultures, and if necessary antinuclear antibodies, anti-cyclic citrullinated peptide, rheumatoid factor, thyrotropin, and serological tests for viruses, bacteria and fungi.

Effusion fluid samples were collected and analyzed on the same day. CYFRA21-1 was determined using an electrochemiluminescence method on a Cobas 601 analyzer (Roche Diagnostics, Barcelona, Spain). The analytical variation of CYFRA21-1 expressed as the between-assay coefficient of variation was 3.0% at concentrations of 3.2 ng/ml.

The criteria used to suggest that an effusion may be a false positive (*i.e.*, empyema, complicated parapneumonic effusion or tuberculosis) were PN%>90, CRP>5.0 mg/dl (to convert to milligrams per liter multiply per 10) or ADA>45 U/l (16, 17). The use of these biomarkers identified two groups of effusions: group A, effusions with all biomarkers below the cut-off point, and group B, effusions with at least one positive biomarker. ADA (EC3.5.4.4) (ITC Diagnostics, Barcelona, Spain) and CRP (Tina-quant CRP latex, Roche Diagnostics, Barcelona, Spain) were determined in a LX-20 autoanalyzer (Beckman Coulter, Madrid, Spain). Leukocyte

Table II. Diagnostic accuracy of CYFRA 21-1 at 175 µg/l cut-off.

	Whole group			Negative cytology		
	All effusions (n=643)	Group A (n=502)	Group B (n=141)	All effusion (n=561)	Group A (n=424)	Group B (n=137)
Prevalence of malignant effusions	28.9 (25.5-32.6)	32.7 (28.6-37.0)	15.6 (10.2-22.9)	18.6 (15.5-22.1)	20.3 (16.7-24.6)	13.2 (8.4-20.4)
Sensitivity (%)	38.7 (31.7-46.1)	39.0 (31.6-47.0)	36.4 (18.0-59.2)	30.8 (22.3-40.7)	29.1 (20.0-40.0)	38.9 (18.3-63.9)
Specificity (%)	97.3 (95.3-98.6)	98.2 (96.0-99.3)	95.0 (88.9-97.9)	97.4 (95.3-98.6)	98.2 (96.0-99.3)	95.0 (88.9-97.9)
NPV (%)	79.6 (76.0-82.8)	76.9 (72.5-80.7)	89.0 (81.9-97.9)	86.0 (82.6-88.8)	84.4 (80.4-87.8)	91.1 (84.2-95.2)
PPV (%)	85.7 (76.0-92.1)	91.4 (81.6-96.5)	57.1 (29.6-81.2)	72.7 (57.0-84.5)	80.7 (61.9-91.9)	53.8 (26.1-79.6)
PLR	14.7 (8.2-26.5)	22.0 (9.7-49.7)	7.2 (2.8-18.8)	11.7 (6.2-21.9)	16.3 (6.0-38.6)	7.6 (2.9-20.2)
NLR	0.63 (0.56-0.71)	0.62 (0.55-0.70)	0.67 (0.49-0.92)	0.71 (0.62-0.81)	0.72 (0.63-0.83)	0.64 (0.44-0.93)

95%: Confidence interval.

Table III. Sensitivity of CYFRA 21-1 at 100% specificity and fluid concentrations (µg/l) in the different groups.

	Cytology (95%CI)	Sensitivity at 100% specificity (co)	Benign effusions Median (IQR)	Malignant effusions Median (IQR)
All effusions (n=643)	44.1% (36.9-51.5)	22.6% 42/186 (447)	13.2 (23.5) ^a	92.6 (354.6)
Group A (n=502)	47.6% (39.8-55.5)	25.6% 42/164 (324)	11.4 (19.9) ^a	96.7 (299.9)
Group B (n=141)	18.2% (6-41)	36.4% 8/22 (447)	22.4 (39.5) ^{a,b}	58.2 (1053.9)
Using specific cut-off		26.9% 50/186	-	-

95%CI: 95% Confidence interval; IQR: interquartile range; co: Cut-off; ^asignificant difference ($p=0.001$) between benign and malignant effusions; ^bsignificant difference ($p=0.001$) between benign effusions from groups A and B.

count was performed in a Neubauer chamber using May-Grünwald-Giemsa stain. The analytical variation, expressed as the between-assay coefficient of variation, was 7.4% for ADA and 2.3% for CRP at concentrations of 10.3 U/l and 7.66 mg/dl respectively.

Statistical analysis. We used the cut-off of 175 µg/l for CYFRA21-1 as described previously by Porcel *et al.* (7) and by our group in pleural effusions and ascites (13). ROC analysis was used to establish a cut-off for CYFRA21-1 at a specificity of 100%. Sensitivity, specificity, negative predictive values (NPV), positive predictive values (PPV), negative likelihood ratio (NLR) and positive likelihood ratio (PLR) were calculated for CYFRA21-1. The parameters of diagnostic accuracy are shown together with their 95% confidence intervals (CI). A two-sided 5% significance level was assumed. All statistical analyses were performed using IBM® SPSS® Statistics for Windows v.22 (IBM Corporation, Armonk, NY, USA) and Stata® v.14 (StataCorp LP, College Station, TX, USA).

Results

Six hundred forty-three consecutive pleural effusions were included, 253 from women (39.3%) and 390 from men (60.7%), with ages ranging from 11 to 97 years (mean=71.6; SD=14.8). Table I shows the patients' characteristics. The effusions were classified into two groups: group A (78.1%), effusions with all benign biomarkers (ADA, CRP and %PN) below the proposed

cut-off point, and group B (21.9%) effusions with at least one of the benign biomarkers above the cut-off.

Table II shows the diagnostic accuracy of CYFRA 21-1 for the whole group and for group A and group B. For a cut-off of 175 µg/l in group A, six false positives (6/502; 1.2%) were found: two parapneumonic effusions, two paramalignant effusions, one asbestosis, and one non-affiliated effusion. In group B, there were 6/141 (4.2%) false positives: four empyema, one complicated parapneumonic effusion, and one paramalignant effusion. Figure 1 shows the ROC analysis for whole group, group A and group B. Table III shows the sensitivity of cytology, the diagnostic performance of CYFRA 21-1 at 100% specificity and the concentrations of CYFRA 21-1 in the different groups of pleural effusions.

Table IV shows the sensitivity of CYFRA21-1 according to tumor type.

Discussion

The data reported for sensitivity and specificity using 175 µg/l as a cut-off point for CYFRA21-1 is in concordance with Porcel *et al.* (7) who found 35% sensitivity at maximum specificity. Using 150 µg/l as cut-off point, Ferrer *et al.* (11) obtained a sensitivity of 22.6%. With the same cut-off value,

Figure 1. ROC for CYFRA21-1 in different groups of study.

Table IV. Sensitivity and concentrations of CYFRA 21.1 according to tumor type.

	% CYFRA 21-1>175 µg/l	Mean; Median (IQR) µg/l
Lung cancer	47.2	550; 140 (360.6)
Breast cancer	37.4	322; 136 (365.8)
Ovarian cancer	60.0	839; 207 (523.3)
Digestive (pancreas, stomach, colon)	25.0	578; 40 (1623)
Cancer of unknown primary site	42.9	427; 126 (368.8)
Mesothelioma	31.8	1062; 54 (893.1)
Lymphoma, sarcoma, hypernephroma	0.0	11; 7 (13.1)

IQR: Interquartile range.

our group (13) obtained 50% sensitivity at maximum specificity in pleural and ascitic effusions, and in our 2015 study, using the same cut-off, we reported sensitivity and specificity of 50% and 97.6% in ascitic effusions (18). With a cut-off point of 163 µg/l, Miédougé *et al.* (12) have reported 42.8% sensitivity at 99% specificity. The majority of authors that use cut-offs above 100 µg/l have found sensitivities between 2% and 50% at a specificity of more than 95%; other authors who have used cut-offs below 100 µg/l obtained higher sensitivity, but specificity fell below 90% (7, 9, 19, 20). The current study showed that non-malignant effusions with benign positive biomarkers have higher concentrations of CYFRA21-1 than non-malignant effusions with negative benign biomarkers. These results are similar to those obtained previously by our group for CEA, CA15-3, CA19-9 and CA72-4, where the majority of false positive cases were included (16). In group B, the prevalence of empyema, complicated parapneumonic and tuberculous effusions was approximately 50%, but the figure did not reach 2% in group A. This strategy, used previously by our group for other tumor markers, enabled us to identify most of the false positives in the group with positive benign biomarkers. This seems to be a valid way to improve the sensitivity of tumor markers in effusion fluid; the group with benign biomarkers has low prevalence of malignancy and a high prevalence of false positives.

Most of the tumors shown to have increased CYFRA21-1 were epithelial cancers. Increased CYFRA21-1 was found in 44.7% of lung cancers, 60% of ovarian cancers, and 37.5% of breast cancers, and among non-epithelial tumors it was recorded in 31.8% of mesotheliomas and in one multiple myeloma.

The differences between NPV, PPV and PLR in groups A and B should be noted. In group A, PPV was above 90% and PLR+ above 20, indicating that the impact on post-test probability is large; in group B, this impact was only moderate.

In conclusion, the determination of CYFRA 21-1 in pleural effusion shows a high specificity but moderate sensitivity. With our subclassification into two groups, diagnostic performance increased in the group with negative benign biomarkers, and was poorer in the group with at least one positive benign biomarker. These results indicate that CYFRA 21-1 is most effective in effusions with negative benign biomarkers.

Conflicts of Interest

The Authors declare that they have no conflicts of interests regarding this study.

Authors' Contributions

Conception and design: JT, RM; Analysis and interpretation: JT, JM, AA, MS, JL, CG; Histological examination FS; Acquisition data: OB, EM DP, SR, EE, RP, JA, SC, MD, DR, MB, JO; Critical revisions: All Authors. All Authors read and approved the final manuscript.

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