Reproducibility of the EGFR immunohistochemistry scores for tumor samples from patients with advanced non-small cell lung cancer

ALEJANDRO AVILÉS-SALAS[1], SAÉ MUÑIZ-HERNÁNDEZ[2], HÉCTOR AQUILES MALDONADO-MARTÍNEZ[1], JOSÉ G. CHANONA-VILCHIS[1], LAURA-ALEJANDRA RAMÍREZ-TIRADO[2], NORMA HERNÁNDEZ-PEDRO[2], RITA DORANTES-HEREDIA[3], JOSÉ MANUEL RUÍZ-MORALES[4], DANIEL MOTOLA-KUBA[4] and OSCAR ARRIETA[2,5]

[1]Department of Pathology; [2]Experimental Oncology Laboratory, National Cancer Institute of Mexico (INCan), 14080 Mexico City; [3]Department of Pathology; [4]Oncology Center, Medica Sur Clinic and Foundation, 14050 Mexico City; [5]Thoracic Oncology Unit, National Cancer Institute of Mexico (INCan), 14080 Mexico City, Mexico

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Abstract. Epidermal growth factor receptor (EGFR) is overexpressed in >60% of non-small cell lung cancer (NSCLC) cases. In combination with radiotherapy or chemotherapy, first-line treatments with antibodies against EGFR, including cetuximab and necitumumab, have demonstrated benefits by increasing overall survival (OS), particularly in patients who overexpress EGFR. The present study evaluated the interobserver agreement among three senior pathologists, who were blinded to the clinical outcomes and assessed tumor samples from 85 patients with NSCLC using the H-score method. EGFR immunohistochemistry was performed using a qualitative immunohistochemical kit. The reported (mean ± standard deviation) H-scores from each pathologist were 111±102, 127±103 and 128.53±104.03. The patients with average H-scores ≥1, ≥100, ≥200 and between 250-300 were 85.9, 54.1, 28.2 and 12.9, respectively. Patients who had an average H-score >100 had a shorter OS time compared with those with lower scores. Furthermore, patients with EGFR mutations who were treated with EGFR-tyrosine kinase inhibitors (TKIs) and had an average H-score >100 had a longer OS time compared with those with an average H-score <100. The interobserver concordance for the total H-scores were 0.982, 0.980 and 0.988, and for a positive H-score ≥200, the interobserver concordance was 0.773, 0.710 and 0.675, respectively. The determination of EGFR expression by the H-score method is highly reproducible among pathologists and is a prognostic factor associated with a poor OS in all patients. Additionally, the results of the present study suggest that patients with EGFR mutations that are treated with EGFR-TKIs and present with a high H-score have a longer OS time.

Correspondence to: Dr Oscar Arrieta, Thoracic Oncology Unit, National Cancer Institute of Mexico (INCan), San Fernando 22, Section XVI, Tlalpan, 14080 Mexico City, Mexico
E-mail: ogar@unam.mx

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Introduction

Lung cancer (LC) accounts for 13% (1.6 million) and 18% (1.4 million) of the global cancer incidence and cancer-associated mortality, respectively, particularly in men (1). In females, these rates have increased in North America, representing the first cause of cancer-associated mortality and the second most prevalent type of cancer (1,2). Non-small cell LC (NSCLC) comprises 85% of all LC cases (3). At the time of diagnosis, ~60% of patients present with an advanced stage of the disease (3). In Mexico, <1% of NSCLC cases are diagnosed during early stages (3,4). The 1-year overall survival (OS) rate continues to be poor despite treatment (5). A total of 30-35% of patients respond to platinum-based chemotherapy, which improves the quality of life compared with the best supportive care (6). Other strategies have been evaluated to improve the survival rates of patients with advanced disease, including combining molecular targeted therapies and chemotherapy, but have produced contradictory results (7).

Certain cell surface proteins, such as the epidermal growth factor receptor (EGFR), are used as prognostic biomarkers and therapeutic targets that increase the response and OS rate of patients with NSCLC (8). EGFR is overexpressed in >60% of NSCLC cases (9). In combination with monoclonal antibodies against EGFR, including cetuximab and necitumumab, first-line chemotherapy has resulted in improved survival rates in patients with advanced-stage disease (10-12).

Immunohistochemistry (IHC) is a standard method used to identify the presence of EGFR. Currently, scoring systems assist in determining the EGFR expression levels in tumor samples using internationally validated antibodies (12-14). The FLEX study assessed EGFR expression using an IHC scoring

system according to the intensity of cell membrane staining (scale of 0-3) (15). In a subanalysis, the EGFR expression levels determined during IHC were tested as a biomarker to evaluate the efficacy of cisplatin and vinorelbine plus cetuximab (12). The EGFR expression data were used to generate IHC scores on a continuous scale of 0-300, and subsequently, the response data were used to select an outcome-based, discriminatory threshold for an IHC score for EGFR expression of 200 (10,13). The present study aimed to evaluate the interobserver agreement of the results of the H-score method for patients with advanced NSCLC among three senior pathologists using the same system as the FLEX study (12).

Materials and methods

Samples. Tumor tissue samples from 85 patients with NSCLC, who were treated at the Thoracic Oncology Unit, National Cancer Institute of Mexico (INCan) (Mexico City, Mexico) were reviewed. The samples were obtained via biopsy from patients treated between January 2008 and December 2012. All samples were histologically characterized. Three additional samples were used as internal controls for EGFR expression and one negative control was included. The general and clinical characteristics of each patient were retrieved from clinical records. The variables selected for analysis included age, gender, smoking history, wood-smoke and asbestos exposure, Eastern Cooperative Oncology Group (ECOG) and Karnofsky performance statuses, and therapy [platinum-based or tyrosine kinase inhibitors (TKIs)].

IHC protocols. All 85 tumor samples were fixed in 10% formalin for 10 h at room temperature and embedded in paraffin. Sections (3 μm thick) were prepared and mounted onto positively-charged glass slides. Immunostaining was performed with an automated immunostainer using the EGFR pharmDx™ kit (Dako, Glostrup, Denmark), which was performed as previously described by the FLEX study (15). EGFR expression was evaluated by three senior pathologists (Department of Pathology, INCan, Mexico City, Mexico), who were blinded to the clinical outcomes. EGFR expression was assessed by IHC using the DAKO EGFR pharmDx kit (Dako, Glostrup, Denmark), which was performed as previously described by the FLEX study (15). The tumor samples were scored according to the fraction of stained cells at each intensity. The staining intensity of the cell membrane was scored within a scale ranging from 0-3 and divided into 4 categories as follows: No staining, 0; weak staining, 1+ (light brown membrane staining); intermediate staining, 2+; and strong staining, 3+ (dark brown linear membrane staining). For more reliable scoring definitions, strong staining (3+) was clearly visible using a x4 objective lens, moderate staining (2+) required a x10 or x20 objective lens for clear observation, and weak staining (1+) required a x40 objective lens. The EGFR H-score was defined as a continuous variable with a scale ranging from 0-300 and was calculated using the following formula: 1 x (percentage of weakly stained cells, 1+) + 2 x (percentage of moderately stained cells staining, 2+) + 3 x (percentage of strongly stained cells, 3+). High and low scores of EGFR expression were defined using 200 as the threshold.

Statistical analysis. For the descriptive analysis, the general variables were summarized as the means and standard deviations or frequencies and proportions, according to the nature of the variable (continuous or categorical, respectively). The bivariate correlation coefficient was calculated between and within each pathologist's observations. The interobserver variations in the EGFR total score were established using the mean Pearson correlation test, whereas the interobserver variations in EGFR scores ≥200 were established using the mean Spearman correlation test. $P<0.05$ was considered to indicate a statistically significant difference. χ^2 or Fisher's exact tests were used to assess the significance among the clinical factors and the H-scores evaluated by each pathologist. OS time was analyzed by the Kaplan-Meier method, and comparisons among subgroups were analyzed by the log-rank test. For the survival curve analysis, all variables were dichotomized. The adjustment for potential confounders was performed using a multivariate Cox regression model, and hazard ratios (HRs) were calculated along with their corresponding 95% confidence intervals (CIs) as a measure of association. All statistical analyses were performed using SPSS v.20 (IBM SPSS, Armonk, NY, USA).

Results

Study population. A total of 85 patients diagnosed with NSCLC were included in the present study; 49 (57.6%) of them were female and 36 (42.4%) were male. The mean age at diagnosis was 61±12.97 years (range, 32-86 years). The majority of patients were smokers (60.0%), with 27.57 as the mean tobacco index, and 57% of patients were exposed to wood-smoke and 16.5% to asbestos. The most common histological type was adenocarcinoma (77.6%). A total of 82% of patients had stage IV cancer at diagnosis, 82.4% had a good ECOG performance status (0-1) and 61.2% had pleural effusion at diagnosis (Table I).

EGFR score evaluation. A total of 85 tumor tissue samples were analyzed according to the FLEX study methodology (12). Fig. 1 presents the differences in EGFR staining among the tissues histologically classified as adenocarcinoma. Each pathologist assessed all tumor samples; the means and standard deviations of the EGFR scores from pathologists A, B and C were 111±102, 127±103 and 128.53±104.03, respectively. When assessing the average EGFR scores from the three pathologists, EGFR scores ≥1, ≥100, ≥200 and between 250-300 were observed in 85.9, 54.1, 28.2 and 12.9% of the tumor samples, respectively (Table II).

Clinical characteristics associated with EGFR scores >100 and >200. The mean overall EGFR score was 125.45 (±96.36). No different was observed in the mean EGFR score in terms of any clinical characteristic. Furthermore, no differences were observed in the patients' age, tobacco smoking, exposure to wood-smoke or asbestos, histological type, disease stage, ECOG performance status or pleural effusion at diagnosis when patients with an EGFR score <200 were compared with patients with an EGFR score ≥200, or patients with an EGFR score <100 were compared with patients with an EGFR score ≥100. Female gender was the only clinical characteristic

Table I. Baseline general characteristics among all patients and by mean EGFR score index punctuation.

Characteristic	Overall (n=85)	Mean (± SD; mean EGFR) score=125.41±96.36	P-value	Mean EGFR-score index punctuation		P-value	Mean EGFR-score index punctuation		P-value
				<100 (n=39)	≥100 (n=46)		<200 (n=61)	≥200 (n=24)	
Gender			0.096			0.048[a]			0.123[a]
Male	42.4 (36/85)	105.09 (±88.18)		53.8 (21/39)	32.6 (15/46)		47.5 (29/61)	29.2 (7/24)	
Female	57.6 (49/85)	140.34 (±100.21)		46.2 (18/39)	67.4 (31/46)		52.5 (32/61)	70.8 (17/24)	
Mean age (± SD)	61.76 (12.97)	-	-	62.3 (13.88)	61.28 (12.28)	0.712[b]	62.54 (13.67)	59.79 (11.02)	0.382[a]
Median age, years			0.231			0.849[a]			0.113[a]
<60	44.7 (38/85)	138.38 (±97.82)		43.6 (17/39)	45.7 (21/46)		39.3 (24/61)	58.3 (14/24)	
≥60	55.3 (47/85)	114.11 (±94.69)		56.4 (22/39)	54.3 (25/46)		60.7 (37/61)	41.7 (10/24)	
Smoking exposure			0.252			0.288[a]			0.106[a]
Non-smoker	40.0 (34/85)	141.47 (±108.33)		33.3 (13/39)	45.7 (21/46)		34.4 (21/61)	54.2 (13/24)	
Smoker	60.0 (51/85)	116.86 (±86.44)		66.6 (26/39)	54.3 (25/46)		65.6 (40/61)	45.8 (11/24)	
Tobacco index						0.384[b]			0.696[b]
Mean (± SD)	27.57 (36.67)	-	-	22.99 (18.44)	32.16 (48.78)		26.48 (30.70)	31.45 (55.04)	
Wood-smoke exposure			0.488			0.514[a]			0.936[a]
Absent	57.6 (49/85)	131.66 (±88.74)		53.8 (21/39)	60.9 (28/46)		57.4 (35/61)	58.3 (14/24)	
Present	42.4 (36/85)	116.89 (±106.55)		46.2 (18/39)	39.1 (18/46)		42.6 (26/61)	41.7 (10/24)	
Wood-smoke index						0.193[b]			0.696[b]
Mean (± SD)	90.41 (100.68)	-	-	68.38 (72.94)	112.44 (120.50)		73.18 (81.17)	135.20 (134.28)	
Asbestos exposure			0.131			0.155[a]			0.976[a]
Absent	83.5 (71/85)	118.38 (±97.45)		89.7 (35/39)	78.3 (36/46)		83.6 (51/61)	83.3 (20/24)	
Present	16.5 (14/85)	161.07 (±84.99)		10.3 (4/39)	21.7 (10/46)		16.4 (10/61)	16.7 (4/24)	
Histology			0.974			0.883[a]			0.344[a]
Adenocarcinoma	77.6 (66/85)	125.22 (±97.14)		76.9 (30/39)	78.3 (36/46)		80.3 (49/61)	70.8 (17/24)	
Other	22.4 (19/85)	126.05 (±96.18)		23.1 (9/39)	21.7 (10/46)		19.7 (12/61)	29.2 (7/24)	
Disease stage			0.776			0.614[a]			0.882[a]
II-III	17.6 (15/85)	131.73 (±93.90)		15.4 (6/39)	19.6 (9/46)		18.0 (11/61)	16.7 (4/24)	
IV	82.4 (70/85)	124.02 (±97.48)		84.6 (33/39)	80.4 (37/46)		82.0 (50/61)	83.3 (20/24)	
ECOG PS			0.131			0.227[a]			0.158[a]
0-1	82.4 (70/85)	132.73 (±95.96)		76.9 (30/39)	87.0 (40/46		78.7 (48/61)	91.7 (22/24)	
2-3	17.6 (15/85)	91.22 (±93.80)		23.1 (9/39)	13.0 (6/46)		21.3 (13/61)	8.3 (2/24)	

Table I. Continued.

Characteristic	Overall (n=85)	Mean (±SD; mean EGFR) score=125.41±96.36	P-value	Mean EGFR-score index punctuation <100 (n=39)	Mean EGFR-score index punctuation ≥100 (n=46)	P-value	Mean EGFR-score index punctuation <200 (n=61)	Mean EGFR-score index punctuation ≥200 (n=24)	P-value
Pleural effusion			0.396			0.406[a]			0.515[a]
Yes	61.2 (52/33)	132.53 (±93.26)		56.4 (22/39)	65.2 (30/46)		59.0 (36/61)	66.7 (16/24)	
No	38.8 (33/85)	114.19 (±101.48)		43.6 (17/39)	38.4 (16/46)		41.0 (25/61)	33.3 (8/24)	
PB-CT			0.955			0.748[a]			0.969[a]
No	24.7 (21/85)	124.36 (±106.61)		23.1 (9/39)	26.1 (12/46)		24.6 (16/61)	25.0 (6/24)	
Yes	75.3 (64/85)	125.75 (±95.40)		76.9 (30/39)	73.9 (34/46)		75.4 (46/61)	75.0 (18/24)	
DCR with PB-CT			0.102			0.265[a]			0.697[a]
Yes	30.6 (26/64)	149.35 (±95.09)		33.3 (10/30)	47.1 (16/34)		39.1 (18/46)	44.4 (8/18)	
No	44.7 (38/64)	109.60 (±93.43)		66.7 (20/30)	52.9 (18/34)		60.9 (28/46)	55.6 (10/18)	
TKI			0.287			0.227[a]			0.158[a]
No	82.4 (70/85)	130.59 (±99.63)		76.9 (30/39)	87.0 (40/46)		78.7 (48/61)	91.7 (22/24)	
Yes	17.6 (15/85)	101.22 (±77.61)		23.1 (9/39)	13.0 (6/46)		21.3 (13/61)	8.3 (2/24)	
DCR with TKI			0.818			1.000[a]			0.283[a]
Yes	33.3 (5/15)	94.33 (±79.00)		33.3 (3/9)	33.3 (2/6)		38.5 (5/13)	0.0 (0/2)	
No	66.7 (10/15)	104.66 (±80.98)		66.7 (6/9)	66.7 (4/6)		61.5 (8/13)	100.0 (2/2)	

[a]Students *t*-test; [b]χ^2 test. Wood-smoke index=years exposed x no. of hours exposed. Tobacco smoke index=[(no. of cigarettes smoked per day) x (no. of years smoking)] / 20. EGFR, epidermal growth factor receptor; ECOG PS, eastern cooperative oncology group performance status; DCR, disease control rate; PB, platinum-based; CT, chemotherapy; TKI, tyrosine kinase inhibitor.

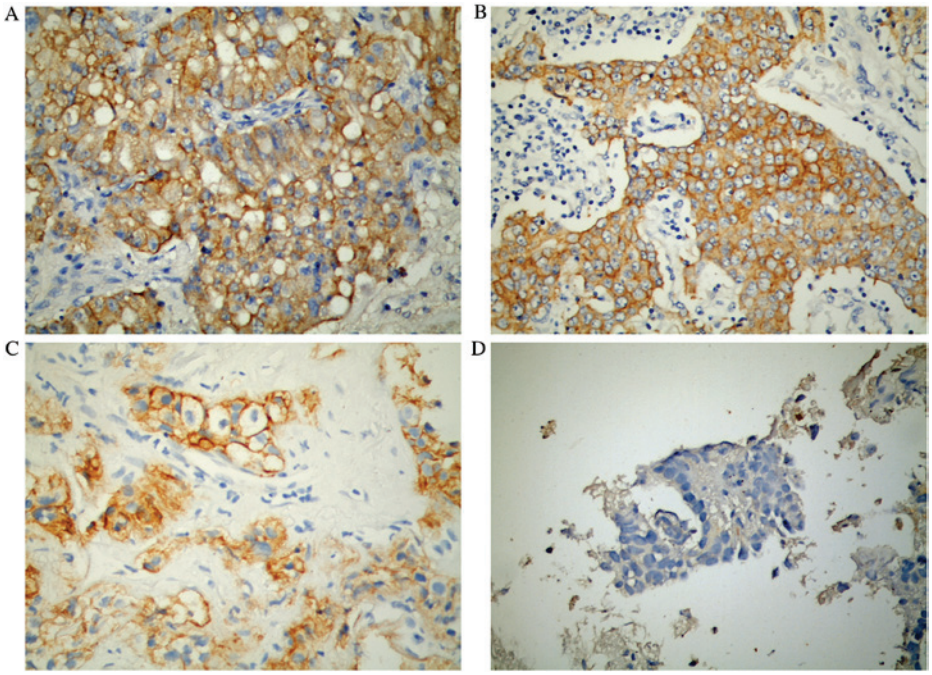

Figure 1. Representative examples of positive and negative immunohistochemical staining for EGFR. Membrane staining was scored as follows: (A) 3+ for dark staining of the linear membrane visible at a magnification of x100; (B) 2+ for intermediate staining visible at a magnification of x400; (C) 1+ for light staining only visible at a magnification of x400; and (D) 0 for no staining visible at a magnification of x400. EGFR, epidermal growth factor receptor.

that was associated with a higher frequency of patients with a mean EGFR score >100 (64.7 vs. 46.2%; P=0.048; Table I).

EGFR score correlation. Regarding EGFR score evaluation, the agreement contingency coefficient was 98%; the interobserver agreement between the high IHC EGFR scores (≥1, 100, 200 and 250-300) ranged from 0.487-0.604, 0.7.4-0.834, 0.675-0.773 and 0.541-0.635, respectively (Table II).

EGFR score and clinical outcomes. The median OS time among the study population was 21.81 months. No differences were observed in the OS time of patients with regard to their gender, age, tobacco smoking, wood-smoke or asbestos exposure, histological type, ECOG performance status, disease stage, pleural effusion at baseline or EGFR score (<200 vs. ≥200) (Fig. 2) in the univariate analysis. An EGFR score >100 was the only clinical characteristic associated with a shorter OS time (13.37 vs. 30.43 months for patients with an EGFR score <100; P=0.05) (Fig. 3). In the multivariate analysis, EGFR score (<100 vs. ≥100) was the only factor that was independently associated with OS time [HR, 2.56 (1.24-5.44); P=0.015] (Table III).

Discussion

EGFR, a gene that is frequently overexpressed in 40-80% of NSCLC cases, serves an important role in tumor cell survival and proliferation (16,17). Recent clinical trials with EGFR inhibitors have demonstrated positive results in patients with NSCLC, particularly by increasing the progression-free survival (PFS) time among patients harboring EGFR mutations compared with patients with wild-type EGFR (7-12). Meta-analyses regarding the use of TKIs in a population with mixed EGFR-activating mutations have only reported PFS and relative risk (RR) benefits, and no OS benefit (13-15). Previous studies have demonstrated the benefits of afatinib on patient OS using pooled data from the LUX-lung 3 and 6 clinical trials (11,12,18). Afatinib is hypothetically more effective at inhibiting EGFR signaling than reversible TKIs, due it forming stable covalent bonds and irreversibly inhibiting ATP from binding to the tyrosine kinase domain of EGFR (19); however, no randomized clinical trial or meta-analysis has demonstrated that afatinib is superior to erlotinib or gefitinib regarding OS. Irreversible TKIs have been reported to significantly improve the OS time of patients with exon 19 deletions (20,21).

Despite such findings, the association between the expression of EGFR mRNA and protein and treatment response is currently unclear, as is the optimal method for determining EGFR levels in tumors. A study of 183 tissue samples from patients with NSCLC assessing the correlation between protein expression and gene copy number by IHC observed that increased EGFR protein levels were correlated with a high gene copy number (13). The same study reported that a high copy number correlated with a poor prognosis and that this phenomenon was more frequently observed in patients with squamous cell carcinoma than in patients with other types of carcinoma (13). In addition, the correlation between the expression of ErbB receptor family proteins and different clinical outcomes and therapeutic responses to monoclonal antibodies has also been widely studied, although the results are more heterogeneous (22). EGFR expression is frequently observed in NSCLC patients with brain metastases (23). A previous study that performed IHC analysis of EGFR, human epidermal growth factor receptor (HER) 2 and HER3 expression in tissue microarrays of 131 NSCLC brain metastases identified that ErbB receptor family members were

Table II. EGFR immunohistochemistry scores interobserver agreement.

Score	Overall	Pathologist A	Pathologist B	Pathologist C
EGFR score				
Mean (± SD)	125.41 (96.36)	111 (102.08)	126.65 (103.05)	128.53 (104.037)
Contingency coefficient		0.982	0.980	0.988
EGFR score 1				
<1	14.1 (12/85)	31.8 (27/85)	30.6 (26/85)	14.1 (12/85)
≥1	85.9 (73/85)	68.2 (27/85)	69.4 (59/85)	85.9 (73/85)
Spearman's rho		0.487	0.495	0.604
κ measurement agreement		0.101	0.102	0.123
EGFR score 100				
<100	45.9 (39/85)	47.1 (40/85)	41.2 (35/85)	42.4 (36/85)
≥100	54.1 (46/85)	52.9 (45/85)	58.8 (50/85)	57.6 (49/85)
Spearman's rho		0.834	0.857	0.734
κ measurement agreement		0.201	0.193	0.082
EGFR score 200				
<200	71.8 (61/85)	74.1 (63/85)	62.4 (53/85)	58.8 (50/85)
≥200	28.2 (24/85)	25.9 (22/85)	37.6 (32/85)	41.2 (35/85)
Spearman's rho		0.773	0.71	0.675
κ measurement agreement		0.116	0.111	0.044
EGFR score 250-300				
<250	87.1 (74/85)	84.7 (72/85)	88.2 (75/85)	82.4 (70/85)
250-300	12.9 (11/85)	15.3 (13/85)	11.8 (10/85)	17.6 (15/85)
Spearman's rho		0.635	0.545	0.541
κ measurement agreement		0.057	0.056	0.021

EGFR, epidermal growth factor receptor; SD, standard deviation.

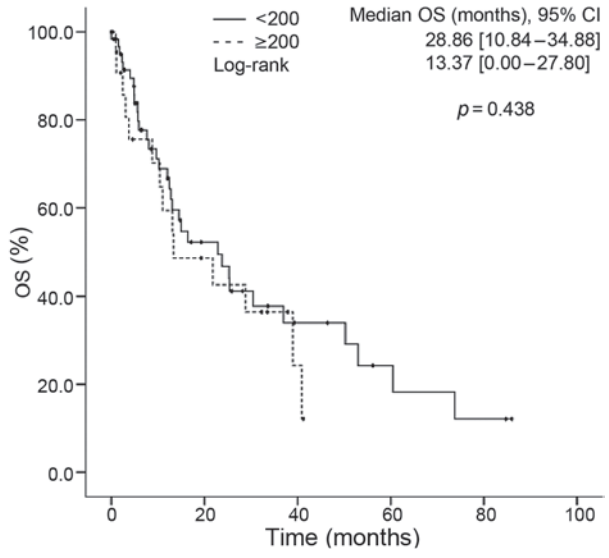

Figure 2. Kaplan-Meier curves by epidermal growth factor receptor score (<200 vs. ≥200). OS, overall survival; CI, confidence interval.

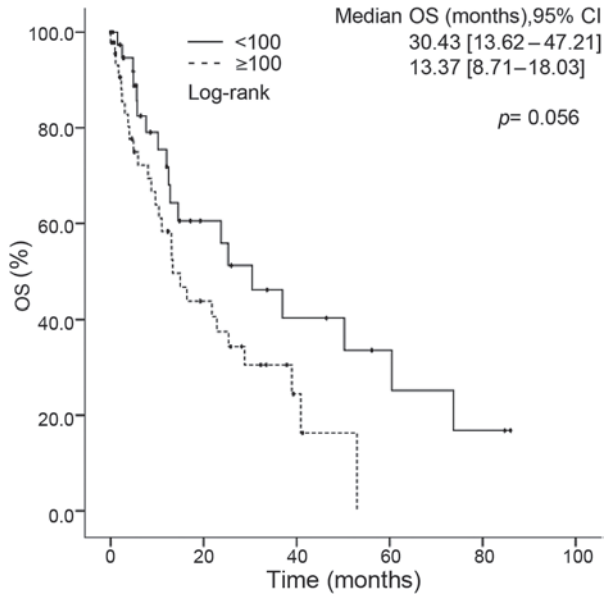

Figure 3. Kaplan-Meier curves by epidermal growth factor receptor score (<100 vs. ≥100). OS, overall survival; CI, confidence interval.

frequently overexpressed (23). However, no significant correlations between the overexpression of ErbB receptor family members and clinical pathological parameters, including OS time, were observed (24). By contrast, a prospective study assessing the development of brain metastasis in 293 patients with advanced NSCLC reported that EGFR expression (RR, 1.6; 95% CI, 1.4-1.9; P=0.012) was independently

Table III. Univariate and multivariate analysis.

	Overall survival					
	Univariate analysis			Multivariate analysis		
Characteristic	Median	95% CI	P-value	HR	95% CI	P-value
Overall	21.81	10.32-33.39				
Gender						
Female	25.29	7.63-18.58				
Male	13.1	11.19-39.40	0.195	0.49	0.21-1.12	0.091
Median age, years						
<60	28.84	16.78-40.90				
≥60	14.94	10.08-19.81	0.214	1.46	0.75-2.84	0.260
Smoking exposure						
Non-smoker	22.86	7.95-37.77				
Smoker	21.81	5.66-37.96	0.582	0.61	0.26-1.41	0.255
Wood-smoke exposure						
Absent	14.52	11.90-17.14				
Present	25.29	8.19-41.36	0.386	0.66	0.30-1.45	0.306
Asbestos exposure						
Absent	23.75	11.08-36.41				
Present	8.08	0.808-15.35	0.065	1.88	0.67-4.24	0.265
Histology						
Adenocarcinoma	22.86	11.5-34.23				
Other	13.1	2.87-23.37	0.634	1.55	0.65-3.67	0.313
Disease stage						
II-III	25.36	0.54-50.17				
IV	21.81	11.07-32.55	0.589	1.82	0.78-4.23	0.164
ECOG PS						
0-1	21.81	10.86-32.77				
2-3	30.42	0.00-70.76	0.740	1.4	0.53-3.68	0.485
Pleural effusion						
Yes	16.42	3.72-29.12				
No	25.36	4.06-46.44	0.778	1.05	0.53 -2.08	0.884
Mean EGFR score						
<100	30.43	13.62 -47.21				
≥100	13.37	8.701-18.03	0.056[a]	2.56	1.20-5.44	0.015[a]
Mean EGFR score						
<200	22.86	10.48-34.88				
≥200	13.37	0.00-27.80	0.438			

[a]P<0.05. Wood-smoke index=years exposed x no. of hours exposed. Tobacco smoking index=[(no. of cigarettes smoked per day) x (no. of years smoking)]/20. CI, confidence interval; HR, hazard ratio; ECOG PS, eastern cooperative oncology group performance status.

associated with a shorter OS time (25). Similarly, a report from a retrospective cohort of patients with NSCLC that assessed HER2 levels demonstrated a higher objective response rate among patients that overexpressed HER2 and were treated with trastuzumab (26). The median OS time of patients with a H-score ≥200 may be improved with the addition of cetuximab to their chemoradiation regimen (42 vs. 21 months), but cetuximab may be detrimental for patients with a H-score <200 (27). EGFR expression level is a predictive value for the response of patients with advanced NSCLC to chemotherapy plus cetuximab treatment (14). In addition to gemcitabine and cisplatin chemotherapy, a second generation of recombinant, human immunoglobulin G1 EGFR monoclonal antibodies, including necitumumab, improves OS time (28). Tissues were evaluated by IHC to determine the level of EGFR protein expression (28). EGFR expression was high (≥200) in 38% of the tissues and low (<200) in 62% of the tissues. The HR for OS for treatment with necitumumab/gemcitabine/cisplatin vs. gemcitabine/cisplatin alone was more favorable in patients bearing tumors with high EGFR expression (29). However,

there was no difference observed between the low and high H-score groups when assessing the PFS and OS time; in addition, an EGFR H-score ≥200 did not predict treatment efficacy in patients with NSCLC who received necitumumab plus cisplatin and pemetrexed (29).

Pirker *et al* (15) analyzed EGFR IHC data to investigate whether tumor EGFR expression level was predictive of the efficacy of chemotherapy plus cetuximab. EGFR expression data were used to generate IHC scores on a continuous scale of 0-300, and the response data was subsequently employed to select an outcome-based, discriminatory threshold IHC score for EGFR expression of 200 (10). Likewise, the phase III FLEX study demonstrated that the addition of cetuximab, an EGFR antibody, to cisplatin and vinorelbine significantly longer OS time compared with chemotherapy alone as a first-line treatment for patients with advanced NSCLC that overexpress EGFR (10). In this analysis, a total of 982 (90%) patients were evaluated by IHC, and EGFR expression was high (H-score ≥200) in 374 patients (38%) and low (H-score <200) in 608 patients (62%) (10). The HR for OS time for necitumumab plus gemcitabine/cisplatin vs. gemcitabine/cisplatin alone was more favorable in patients bearing tumors with high EGFR expression (HR, 0.75 [95% CI, 0.60-0.94]) than in those with low EGFR expression (HR, 0.90 [95% CI, 0.75-1.07]). This previous study allowed the current study to differentiate a patient subgroup that would derive a survival benefit from the addition of cetuximab to chemotherapy, which was associated with a score ≥200, compared with other subgroups that would receive little or no benefit and whose score was <200 (15).

Regarding the reproducibility of the H-score, Rüschoff *et al* (28) evaluated the interobserver reproducibility of this EGFR IHC scoring system. A high agreement was observed amongst the scores with an overall concordance rate of 90.9% and a mean coefficient of 0.812 (29). Specimens with reference scores <200 and ≥200 exhibited mean concordance rates of 94.7 and 85.6%, respectively (15). According to these studies, EGFR expression measured by IHC is a potential predictive biomarker for the response of patients with NSCLC to cetuximab, with the advantage that IHC is a well-established, widely used and low cost technique (15,29). The reproducibility and validation of these results in other populations has not been widely studied. According to Hirsch *et al* (24), a lower cut-off score for EGFR expression is able to better resolve positive and negative EGFR IHC results. When higher cut-off points were used to define positive staining, they did not improve the test's discrimination (24).

In the present study, a good interobserver agreement of 80-90% was observed with a mean coefficient of 0.983 among three pathologists, and the positivity in the samples was 70%, which is consistent with other studies (28,30). In the present study, a better concordance for the H-scores was observed when using a cut-off of 100 (73.4-83.4%); meanwhile, the concordance of the cut-off of 200 ranged from 67.5-77.3%. Samples with a reference EGFR H-score <200 and ≥200 demonstrated mean concordance rates of 94.7 and 85.6%, respectively (15). An important hallmark of the study was that the population was not selected based on IHC expression levels or treatment regimen.

In other forms of cancer, including breast and gastric cancer, IHC determination of molecular markers, such as HER2 overexpression, is important for the treatment strategy (31,32). Patients with high HER2-expressing tumors derive the greatest benefit from trastuzumab therapy (31). Additionally, it was previously determined that the interlaboratory reproducibility of HER2 expression in gastric cancer using two different antibodies was 48.3 vs. 75.9%, while the interobserver reproducibility was ~90% (32). Testing and scoring is important to ensure the accurate identification of patients who are eligible for treatment.

Finally, in the present study, an EGFR H-score >100 was frequently observed in women. Currently, the overall incidence of LC is increasing in females and Hispanic women present with a higher prevalence of EGFR mutations than men (36.9 vs. 18.5%, respectively) (4,33). Studies have demonstrated that EGFR expression is closely associated with poor survival in females who are undergoing conventional chemotherapy, with a higher mortality than breast and colorectal cancer combined (33). An EGFR expression rate of 50% has been reported in women with NSCLC, highlighting that EGFR may be used as an indicator of the increasing incidence, poor prognosis and disease progression in female patients with NSCLC (33,34). Conversely, alterations in the EGFR gene represent a better response to TKI treatment and OS time for women, who otherwise would have a poor prognosis in response to chemotherapy (35).

In conclusion, EGFR expression is a hallmark of several neoplasms, particularly LC, where it is a determinant for targeted treatment. The present study demonstrated that determination of EGFR expression levels by IHC is highly reproducible between pathologists. According to this data, high EGFR expression levels are associated with a poorer prognosis for patients with NSCLC; however, these levels may be associated with a better OS time in patients with EGFR mutations who undergo EGFR TKI treatment.

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