European Review for Medical and Pharmacological Sciences 2014; 18: 398-403

Prognostic and predictive significance of plasma hepatocyte growth factor and carcinoembryonic antigen in non-small lung cancer after surgery

M.-Y. FANG, S.-Y. WANG, Y.-B. ZHENG, L.-Y. GONG, W.-L. BAO, D.-L. GU, W.-M. MAO

Department of Integrated Chinese Traditional Medicine and Western Medicine, Zhejiang Cancer Hospital, Zhejiang Thoracic Oncology Institute, Zhejiang Cancer Center, Banshanqiao District, Hangzhou, People's Republic of China

Abstract. – OBJECTIVES: Scatter factor, also known as hepatocyte growth factor (SF/HGF), is a polypeptide growth factor with a number of biologic activities, including cell scattering, stimulation of cell motility, mitogenesis, morphogenesis, angiogenesis, and cellular invasiveness, it is thought to be important in the growth and spread of several carcinomas. We assessed whether preoperative plasma levels of HGF and carcinoembryonic antigen (CEA) can enhance the accuracy of standard models for predicting pathologic features and clinical outcomes.

PATIENTS AND METHODS: The study comprised 45 consecutive patients treated with surgery for clinically localized non-small-cell lung cancer. HGF and CEA were measured using the commercially available immunoassay. Multivariate logistic regression was used to assess the relationship between plasma HGF/CEA and pathologic features. Multivariate Cox regression was used to predict disease recurrence.

RESULTS: Patients with lung squamous cell cancer (SCC) more frequently had higher plasma HGF, whereas CEA levels were significantly elevated in patients with non-SCC histology. Preoperative plasma HGF and CEA levels were not the independent predictors of overall survival.

CONCLUSIONS: Preoperative plasma levels of HGF and CEA are not the independent predictors of non-small lung cancer disease recurrence and metastasis after surgery; HGF is a predictor of lung squamous cell cancer. Use of HGF may help in therapeutic decision-making and estimate the histological type of NSCLC.

Key Words:
Hepatocyte growth factor, Lung cancer, Surgery, Prognosis.

Introduction

Lung cancer is the first most common cancer worldwide, the age-adjusted incidence rates for men and women of all races are more than 150.0 and 50.0 per 100,000 people per year, respectively[1]. It is considered one of the most common forms of cancer, with a high death incidence ratio, making it the leading cause of cancer death in the world. This high rate of fatality is largely due to late presentation; only 30-40% of cases of lung cancer are diagnosed at a localized stage, whereas more than 60% are diagnosed at an advanced stage[2]. The corresponding 5-year survival rates decrease significantly from 60-80% for patients with Stage 1 disease to less than 10% for the advanced stage, and the median survival time is about 8 months for patients with Stage 4 (metastasis) disease[3]. There are no tumor markers that are sufficiently useful for detecting lung cancer at a stage where the patients can be cured completely. Therefore, accurate detection and diagnosis of lung cancer is imperative and may provide a survival advantage.

Presenting signs and symptoms can be nonspecific and vague, especially in patients with chronic pneumonia (COPD). Likewise, serologic tests for identifying and measuring biomarkers released into the blood can be normal and nondiagnostic. Among these biomarkers, the most extensively studied is CEA. However, CEA levels are known to be elevated in a number of benign and non pulmonary malignant conditions, which diminishes this biomarker's value in establishing the diagnosis of lung cancer[4].

Corresponding Author: Mei-Yu Fang, MD; e-mail: fangjade2004@yahoo.com.cn

Hepatocyte growth factor (HGF), a cytokine produced mainly by mesenchymal cells, has been implicated along with its receptor (c-MET), a proto-oncogenic product, is expressed on most epidermal cells and a wide variety of cancer, involved in cancer proliferation, invasion, and angiogenesis[5,6]. Clinical studies have shown an association between the concentration of HGF in serum or cancer tissue and the progression of the disease in various cancer types, including breast, gastric, bladder, colorectal, and small cell lung cancer, myeloma, and synovial sarcoma[7].

It is proved that the c-Met receptor is frequently over expressed in non-small lung cancer cells and have shown that it may be a useful biomarker for diagnosing and predicting the prognosis of advanced pulmonary tumors[8,9] which is secreted by tumor-associated fibroblasts binding to c-Met in a paracrine function[10]. Few studies (to our knowledge) have investigated the clinical value of HGF measurement in patients with localized lung cancer treated by radical surgery. Therefore, we conducted a pilot study to investigate the utility of plasma HGF level in diagnosing early stage non small lung cancer after radical surgery, and we compared its diagnostic accuracy with serum CEA immunoassay. We also evaluated the relationship of the preoperational HGF and CEA levels and the survival to determine their prognostic value.

Patients and Methods

Between Jan 2010 and Feb 2010, 47 cases of NSCLC consecutively treated in our Department, 45 patients were agree to sign the written informed consent and participated in the study. The study protocol was approved by the Ethics Committee of our Hospital. The diagnosis was based on clinical, biochemical, and radiological investigations that included a liver function test (LFT), a triple-phase helical computed tomographic (CT) scan of the chest, fiber bronchoscope. The diagnosis was further confirmed by histological examinations of the resected tissues, fine needle aspiration, or core biopsies. Histological diagnosis was confirmed in all patients with NSCLC. The type of surgical procedure was determined by the underlying disease; procedures included Wedge resection, sublobar resection, segmentectomy, Video-assisted thoracic surgery (VATS), Sleeve lobectomy and the pneumonectomy.

Preoperative laboratory and histological data were obtained from a computerized database of medical record.

Sample Collection

Standardized plasma collection, isolation, and storage procedures were used. Serum samples were obtained immediately before the start of treatment and 4 weeks later of the operation after informed consent was obtained.

Peripheral blood samples were collected in 5 to 10 ml EDTA tubes pretreatment. In general, tumor resection was performed 1-2 weeks after the full preoperative preparation. In patients who underwent surgical resection, blood samples were obtained 4 weeks after the procedure. Plasma samples were separated by centrifugation at 1,500 rpm (Revolutions Per Minute) for 30 min and then stored at 80°C until they could be analyzed.

HGF Assay

The concentration of HGF was carried out with a modified ELISA system (R&D Systems, Minneapolis, MN, USA) in which Assay Diluent GF2 (Meso Scale Discovery) was used to ensure assay linearity[11].

To set the standard curve of the HGF level, HGF was measured in duplicate by using five dilutions. Plasma samples were assayed in duplicate by using different dilutions of the original plasma sample from each patient. The assay is sensitive to final HGF concentrations as low as 40 pg/ml. The cutoff value was at 1,000 pg/ml.

CEA Assay

CEA was measured by using an AXSYM CEA kit (Abbott Laboratories, Chicago, IL, USA), which is based on micro particle enzyme immunoassay technology. Results were expressed in nanograms per milliliter (ng/ml). The selected cut-off value was 5.0 ng/ml for CEA. The assay was performed according to the manufacturer's instructions.

Statistical Analysis

Values are presented as the mean standard deviation or median and range. The Student *t*-test was used to analyze differences between subgroups of patients. Chi-squared test or, when appropriate, the Fisher exact test was used to compare categorical data. Repeated measures analysis of variance was used to analyze changes in variables over time. Non-parametric Spearman R correlation coefficients were computed to analyze the relationship between HGF and different clinical parameters (e.g. CEA, tumor stage and grade).

The Kaplan-Meier method was used to estimate OS (overall survival) curves. The groups

were compared with respect to OS, using the log-rank test and Cox proportional regression hazards model, adjusting for previously identified prognostic factors described above. A *p*-value of < 0.05 was considered significant. All *p* values are 2-sided. Statistical analyses were conducted with the SPSS statistical package version 10.0 (SPSS Inc., Chicago, IL, USA).

Results

Table I shows the clinical profiles of the 45 patients, and the HGF and CEA levels in each group. The 45 patients who underwent surgical resection comprised 23 (51.1%) patients with SCC, 17 (37.8%) patients with adenocarcinoma, 3 (6.7%) patients with the large-cell carcinoma cancer and 2 (4.4%) patients with other types.

The median follow-up time was 30 months (range 9-30). The levels of HGF detected in plasma samples of various patients were in the range of 850-1394 pg/ml for the pre-treatment and 790-1400 pg/ml for the post-operation. The mean pre-treatment HGF level in the 45 patients was 1089.56 pg/ml. In this study, we evaluated the significance of the plasma HGF level in patients with NSCLC. Although the mean plasma HGF level was significantly higher in SCC patients than in the other histological types ($p = 0.043$), we could not find a role for HGF as a useful tumor marker for the early diagnosis of NSCLC because of low sensitivity.

The CEA levels detected in the plasma samples from the 45 patients were in the range of 1.01-8.53 pg/ml. The median CEA level was 2.24 pg/ml, with the average being 2.89 pg/ml. The CEA concentrations were significantly higher in lung non-squamous cell cancer patients.

Patients with squamous cell cancer (SCC) had significantly higher baseline plasma levels of HGF than patients with adenocarcinoma. High HGF level was significantly associated with higher T-stage (T3-4) and COPD co-existence; whereas high CEA was associated with non-squamous cell cancer. There was no difference between the patients at early stage (I-II).

Table I. Patient characteristics and the mean HGF and CEA serum concentrations before the treatment.

Characteristic	N total (n = 45)	%	HGF1 (pg/ml)	*p*	CEA	*p*
Age (y)						
< 45	6	13.3	1099.83	0.869	3.39	0.31
≥ 45	39	86.7	1087.97		2.81	
Gender						
male	27	60	1159.48	0.000	2.97	0.57
female	18	40	984.67		2.75	
Smoking						
Yes	23	51.1	1187.23	0.000	3.06	0.10
No	22	48.9	996.13		2.70	
COPD						
Yes	6	13.3	1190.67	0.005	3.12	0.12
No	39	86.7	1073.43		2.41	
Histology						
SCC	23	51.1	1228.70	0.000	2.34	0.00
Adeno	17	37.8	929.82		2.80	
Large-cell	3	6.7	932.00		4.74	
Others	2	4.4	1083.50		6.86	
TNM stage						
T1	20	44.4	1022.10	0.003	2.98	0.14
T2	12	26.7	1058.00		1.82	
T3	8	17.8	1224.25		3.36	
T4	5	11.1	1219.60		4.32	
N0	20	40.0	1040.11	0.132	2.68	0.57
N1	12	33.3	1082.40		2.92	
N2	8	24.4	1161.09		3.36	
N3	5	2.2	1300.00		1.83	
M0	40	88.9	1081.88	0.373	2.81	0.24
M1	5	11.1	1151.00		3.49	

p values are calculated using the Fisher's exact test for the variables.

Tumor marker CEA was reported to be a prognostic factor for patients with surgically resected NSCLC[11]. However, the CEA level was not associated with the overall survival rate in our study (Figure 1). The media survival time of patients according the CEA levels were as follow: 27 months for CEA above the cut-off limit and 28 months for the others, there was a trend for worse overall survival (OS) with high CEA level but the difference was not significant ($p = 0.06$).

The relationship between the HGF and CEA levels in the sera from the patients was examined. It showed that the HGF levels were not correlated with the CEA concentrations (Pearson correlation coefficient by rank test: r = –0.117; p = 0.443).

When examining if the different HGF levels affecting the overall survivals, there was no apparent difference in OS by HGF levels. The median survival time was 33 months for patients with HGF above 1000 pg/ml and 34 months for the others. The data showed that HGF level was not associated with survival, $p = 0.71$ (Figure 2).

There was a difference was confirmed in the survival rate between patients with disease at stages I-II and III-IV ($p = 0.0005$). The analyzed patients included 13 patients at stage I, 12 at stage II, 15 at stage III and 5 at stage IV. Since the patients with disease at stage II was principally underwent the same surgery as in patients at stage I, the patients at stages I and II were analyzed together.The T status (pT factor) was also a strong prognostic factor, $p = 0.04$.

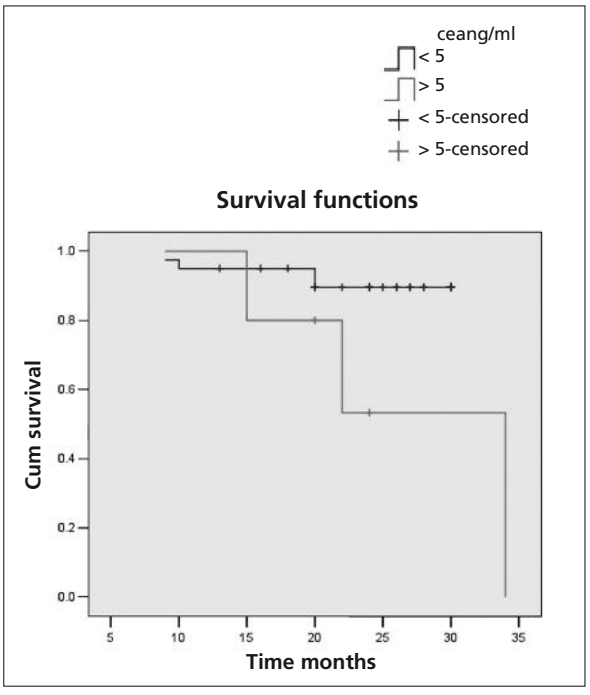

Figure 1. Kaplan-Mayer survival curve of 45 patients by pretreatment CEA levels, $p = 0.06$.

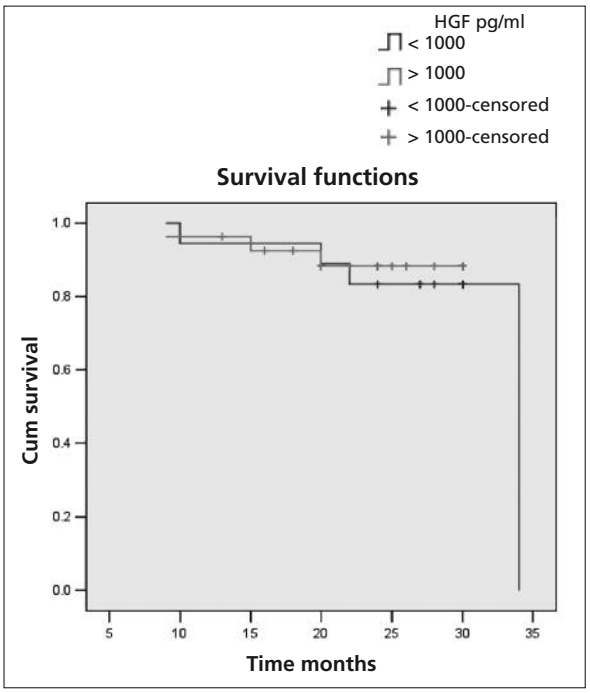

Figure 2. Overall Survival for 45 patients by pretreatment HGF levels, $p = 0.71$.

In this study, the prognosis of patients with adenocarcinoma was better than that of patients with other types of NSCLC ($p = 0.01$).

In the univariate analysis, a statistically significant prognostic impact on survival from the landmark date for disease stage was observed. CEA and HGF baseline values were not correlated with survival, independent of any cutoff selected, whereas their reduction during treatment was a favorable prognostic factor. It was found that stage, histological type, pT, M status contributed to the overall survival rate. With regard to histological subtypes, the median survival was 34 months for patients with adenocarcinoma and 32 months for SCC ($p = .17$).

On univariate analysis of the overall survival rate, gender, age and histological subtypes were not the prognostic factors. Both pretreatment HGF and CEA levels were not related to the survival.

Results from the Cox regression analysis showed, the independent prognostic role of TNM stage were confirmed.

Four patients had early tumor recurrence within 9 months after resection. The mean preoperative HGF levels were higher in these patients (1,114 pg /ml) than in patients without tumor recurrence (1054 pg /ml), but this difference was not statistically significant ($p = 0.062$).

Among all 17 patients who underwent recurrence, preoperative HGF levels did not correlate significantly with the histologic stage and grade of resected tumors (Pearson correlation coefficient by rank test: r = 0.27; $p = 0.07$; r = 0.17; $p = 0.21$, respectively).

Discussion

The first objective of our pilot study was to determine the sensitivity and specificity of plasma HGF measurement in diagnosing NSCLC. We found that plasma HGF was not sensitive for the diagnosis of early stage NSCLC (stage I-II). The sensitivity of HGF level was significantly higher in SCC patients at stage III-IV, whereas plasma CEA level was elevated in non-SCC patients.

Although the HGF assay was accurate in discriminating SCC patients from adenocarcinoma patients, it was not accurate enough to discriminate between stage stages I and II patients. Further studies involving larger numbers of patients with lung cancer could be done to ascertain the role that plasma HGF level might play in the differential diagnosis of lung neoplasm, including small cell lung cancer.

The second objective of our study was to analyze the factors that affect preoperative plasma HGF levels. Previous studies have shown that the circulating level of HGF are elevated in several inflammatory diseases such as chronic hepatitis and pancreatitis[12,13].

We also found that plasma HGF/SF level was increased in patients with chronic pneumonia. We found the correlation between HGF levels and the results of the COPD. HGF is a cytokine that promotes invasion and metastasis[1,14]. *In vivo* studies have shown that activation of HGF-cMET signaling promotes cell invasiveness and triggers metastases through direct involvement of angiogenic pathways[15]. Both the degree of differentiation and the stage of tumors were found to correlate with plasma levels of HGF.

The third and final objective of this study was to monitor long-term changes in HGF and CEA values after resection in patients with NSCLC to determine the prognostic values of plasma HGF and CEA.

Several researchers have observed the dynamic changes in plasma HGF level in the immediate period after liver resection and found that the serum level increases significantly after hepatectomy[16,17] and that both plasma HGF and vascular endothelial growth factor (VEGF) levels were elevated during the first 10 days after liver and pancreatic resection[18]. It is supposed that these higher than baseline values reflect a systemic response to surgical trauma. Reports by Belizon et al[19] and Shantha Kumara et al[20] have shown that HGF promotes expression of angiogenic factors such as VEGF and IL-8 during the immediate postoperative period.

To avoid such trauma response, we collected the sample before the resection. In our study, patients with COPD plasma HGF levels were significantly higher than the others; it was in accord with previous studies and reflect the inflammatory process[20].

The proangiogenic potential of HGF may account for the accelerated growth in patients of metastatic tumors that we were unable to detect during the preoperative staging investigations. Although HGF levels were higher in these patients than in patients who did not have early recurrence, but the difference did not reach statistical significance, probably because only a few patients had metastatic tumors. Therefore, further studies involving larger numbers of patients are warranted to ascertain.

The present work demonstrated that HGF was not correlated with CEA. Both of the preoperative CEA and HGF levels could not predict the prognosis of NSCLC.

Conclusions

We have shown that baseline plasma HGF levels are significantly higher in patients with SCC than in patients with adenocarcinoma, while the plasma CEA levels will be biological marker of non-squamous lung cancer.

However, we are aware that the sensitivity and specificity of HGF testing depend largely on when and how many specimens are collected for analysis. Therefore, it will be necessary to corroborate our initial results with further studies that involve larger numbers of patients with both early and advanced disease to assess the potential use of HGF measurement in screening patients at risk of NSCLC. However, we do believe, HGF measurement may increase the accu-

racy of NSCLC diagnosis when combined with other clinical parameters.

In conclusion, plasma HGF levels were increased in patients with advanced stage SCC, but this increase was unrelated to the survival. Measurement of plasma HGF level as a tumor marker for SCC might be clinically useful.

Conflict of Interest

The Authors declare that there are no conflicts of interest.

References

1) Naishadham D, Jemal A. Cancer Statistics, 2012. CA Cancer J Clin 2012; 62: 10-29.
2) Ries LAG, Young JL, Keel GE, Eisner MP, Lin YD, Horner M-J (editors). "Cancer of the lung" SEER Survival Monograph: Cancer Survival Among Adults: U.S. SEER Program, 1988-2001, NIH. Pub. No. 07-6215, Bethesda, MD, 2007; pp. 73-80.
3) Rami-Porta R, Crowley JJ, Goldstraw P. The revised TNM staging system for lung cancer. Ann Thorac Cardiovasc Surg 2009; 15: 4-9.
4) Molina R, Filella X, Augé JM, Fuentes R, Bover I, Rifa J, Moreno V, Canals E, Viñolas N, Marquez A, Barreiro E, Borras J, Viladiu P. Tumor Markers (CEA, CA125, CYFRA 21-1, SCC and NSE) in patients with non-small-cell lung cancer as an aid in histological diagnosis and prognosis. Comparison with the main clinical and pathological prognostic factors. Tumour Biol 2003; 24: 209-218.
5) Gao CF, Vande Woude GF. HGF/SF-Met signaling in tumor progression. Cell Res 2005; 15: 49-51.
6) Peruzzi B, Bottaro DP. Targeting the c-Met signaling pathway in cancer. Clin Cancer Res 2006; 12: 3657-3660.
7) Ujiie H, Tomida M, Akiyama H, Nakajima Y, Okada D, Yoshino N, Takiguchi Y, Tanzawa H. Serum hepatocyte growth factor and interleukin-6 are effective prognostic markers for non-small cell lung cancer? Anticancer Res 2012; 32: 3251-3258.
8) Han JY, Kim JY, Lee SH, Yoo NJ, Choi BG. Association between plasma hepatocyte growth factor and gefitinib resistance in patients with advanced non-small cell lung cancer. Lung Cancer 2011; 74: 293-299.
9) Kasahara K, Arao T, Sakai K, Matsumoto K, Sakai A, Kimura H, Sone T, Horiike A, Nishio M, Ohira T, Ikeda N, Yamanaka T, Saijo N, Nishio K. Impact of serum hepatocyte growth factor on treatment response to epidermal growth factor receptor tyrosine kinase inhibitors in patients with non-small cell lung adenocarcinoma. Clin Cancer Res 2010; 16: 4616-4624.
10) Chau GY, Lui WY, Chi CW, Chau YP, Li AF, Kao HL, Wu CW. Significance of serum hepatocyte growth factor levels in patients with hepatocellular carcinoma undergoing hepatic resection. Eur J Surg Oncol 2008; 34: 333-338.
11) Gordon MS, Sweeney CS, Mendelson DS, Eckhardt SG, Anderson A, Beaupre DM, Branstetter D, Burgess TL, Coxon A, Deng H, Kaplan-Lefko P, Leitch IM, Oliner KS, Yan L, Zhu M, Gore L. Safety, pharmacokinetics, and pharmacodynamics of AMG 102, a fully human hepatocyte growth factorneutralizing monoclonal antibody, in a first-in-human study of patients with advanced solid tumors. Clin Cancer Res 2010; 16: 699-710.
12) Tomita M, Shimizu T, Ayabe T, Onitsuka T. Maximum SUV on positron emission tomography and serum CEA level as prognostic factors after curative resection for non-small cell lung cancer. Asia Pac J Clin Oncol 2012; 8: 244-247.
13) Otte JM, Schwenger M, Brunke G, Sparmann G, Emmrich J, Schmitz F, Fölsch UR, Herzig KH. Expression of hepatocyte growth factor, keratinocyte growth factor and their receptors in experimental chronic pancreatitis. Eur J Clin Invest 2001; 31: 865-875.
14) Funakoshi H, Nakamura T. Hepatocyte growth factor: from diagnosis to clinical applications. Clin Chim Acta 2003; 327: 1-23.
15) Puri N, Salgia R. Synergism of EGFR and c-Met pathways, cross-talk and inhibition, in non small cell lung cancer. J Carcinog 2008; 7: 9.
16) Christensen JG, Burrows J, Salgia R. C-Met as a target for human cancer and characterization of inhibitors for therapeutic intervention. Cancer Lett 2005; 225: 1-26.
17) Barakat O, Rodriguez GC, Raijman I, Allison PM, Nieto J, Ozaki CF, Wood RP, Engler DA. Clinical value of plasma hepatocyte growth factor measurement for the diagnosis of periampullary cancer and prognosis after pancreaticoduodenectomy. J Surg Oncol 2010; 102: 816-820.
18) Justinger C, Schluter C, Oliviera-Frick V, Kopp B, Rubie C, Schilling MK. Increased growth factor expression after hepatic and pancreatic resection. Oncol Rep 2008; 20: 1527-1531.
19) Belizon A, Balik E, Feingold DL, Bessler M, Arnell TD, Forde KA, Horst PK, Jain S, Cekic V, Kirman I, Whelan RL. Major abdominal surgery increases plasma levels of vascular endothelial growth factor: open more so than minimally invasive methods. Ann Surg 2006; 244: 792-798.
20) Shantha Kumara HM, Hoffman A, Kim IY, Feingold D, Dujovny N, Kalady M, Luchtefeld M, Whelan RL. Colorectal resection, both open and laparoscopic-assisted, in patients with benign indications is associated with proangiogenic changes in plasma angiopoietin 1 and 2 levels. Surg Endosc 2009; 23: 409-415.