Serum hepatocyte growth factor and Interleukin-6 are effective prognostic markers for non-small cell lung cancer

（非小細胞肺癌における、血中 HGF (Hepatocyte Growth Factor)　および、IL-6（Interleukin-6）の予後マーカーとしての有用性の検討）

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Abstract

Aim: We surveyed prognostic biomarkers for resectable non-small cell lung cancer (NSCLC). Patients and Methods: We obtained preoperative serum from 109 patients, and measured the levels of hepatocyte growth factor (HGF), interleukin-6 (IL-6), and nicotinamide N-methytransferase (NNMT) in the sera. Results: The median HGF and IL-6 contents were 860 pg/ml and 2.7 pg/ml, respectively. Analysis of survival curves indicated that an HGF or IL-6 level higher than the median was associated with poor overall survival (HGF, p=0.019; IL-6, p=0.002). In addition, we analyzed stage III lung cancer alone. Higher HGF and IL-6 levels were associated with poor overall survival (HGF, p=0.016; IL-6, p=0.013). Disease-free survival was not statistically significantly affected by these cytokine contents. The tumor status (pT factor) and nodal status (pN factor) were not associated with the survival of stage III patients. Conclusion: The levels of HGF and IL-6 in serum could be useful prognostic indicators of the survival of patients with stage III NSCLC undergoing surgery and chemotherapy.

Introduction

Lung cancer is a fatal malignant tumor that develops at high frequency in most countries at present. There are no tumor markers that are sufficiently useful for detecting lung cancer at a stage where the patients can be cured completely. We previously examined whether or not nicotinamide *N*-methytransferase (NNMT) is a potential biomarker of non-small cell lung cancer (NSCLC) (1, 2). The serum levels of NNMT were significantly higher in patients with lung cancer than in healthy donors and in patients with non-neoplastic disease.

In the present study, we analyzed prognostic factors in 109 cancer patients. Surgical resection of the tumor is the principle form of treatment for patients with stage I or stage II lung cancer. However, treatment of patients with stage III disease is not as simple. Some patients with stage III disease undergo surgery for tumor resection. Another treatment option is preoperative chemoradiotherapy and, if a response is seen, application of follow-up resectioning of any remaining tumor. The remaining patients are not surgical candidates. Therefore, predictors of the prognosis in stage III NSCLC would be useful for selection of the most appropriate treatment (3).

Hepatocyte growth factor (HGF) was originally found as a blood-derived factor released during regeneration of the liver (4, 5). At present, it is recognized that this factor is involved in the development of various organs during embryogenesis and tissue regeneration. Although HGF is produced mainly by mesenchymal cells, it acts on epidermal- and endothelial-derived cells (6, 7). It is also involved in cancer growth and metastasis by enhancing the motility of cancer cells and by stimulating angiogenesis (8, 9). The hepatocyte growth factor receptor (*c-MET*), proto-oncogene product, is expressed on most epidermal cells and a wide variety of cancer cells (7). 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 (10, 11), gastric (12), bladder (13), colorectal (14), and small cell lung (15) cancer, myeloma (16, 17), and synovial sarcoma (18). For NSCLC, the intratumoral HGF level was reported to be a prognostic indicator (19 - 21), but the significance of the serum HGF level was not reported until fairly recently (22, 23). HGF is mainly produced by stromal fibroblasts (24). We previously showed that the *HGF* gene in cancer cells is transcriptionally activated by leukemia inhibitory factor (LIF) through the signal transducers and activators of transcription 3 (STAT3) (25). Furthermore, the expression of HGF in human lung fibroblasts and MRC-5 cells, derived from lung fibroblasts is correlated with that of IL-6. Expression of the *HGF* gene in MRC-5 cells was suppressed by treatment with curcumin, an inhibitor of STAT3 (unpublished results). Therefore, cytokines, such as LIF and IL-6, that activate STAT3 might stimulate stromal cells to produce HGF.

The inflammatory cytokine IL-6 exhibits multiple functions and stimulates the progression of various kinds of cancers (17, 26, 27). Constitutive activation of the STAT3 pathway in alveolar epithelial cells induces inflammation and adenocarcinomas in mouse lungs (28). In human lung adenocarcinoma, mutant epidermal growth factor receptor (EGFR) activates the STAT3 pathway through IL-6 up-regulation (29). It has been reported that increased serum IL-6 levels were associated with poor survival in patients with NSCLC (30, 31).

In this study, we analyzed the relationship between the levels of HGF, IL-6 and NNMT in sera, and the survival of patients with NSCLC.

Patients and Methods

Serum samples. This study and the use of blood samples collected after obtaining informed consent, were approved by the Ethical Committee of the Saitama Cancer Center (1). As preoperative blood samples, serum was collected from 109 patients undergoing radical pulmonary resection at the Department of Thoracic Surgery of Saitama Cancer Center Hospital during the period November 2006 to November 2007. The blood was maintained at room temperature for 20 min before centrifugation. The serum was separated, and then frozen at −70°C.

The 109 lung cancer patients included 79 with adenocarcinoma, 26 with squamous cell carcinoma (SCC), and 4 with other non-small cell lung cancer (Table I). For the examined tumors, the pathological stage was determined based on the standard criteria, UICC 7th edition (32).

Enzyme linked immunosorbent assay (ELISA) of cytokines and NNMT. ELISA assays for HGF and IL-6 were performed using a Quantikine ELISA kit (R&D Systems, Minneapolis, MN, USA), according to the manufacturer's instructions. The levels of NNMT were measured by a method developed by Tomida *et al.* 2009 (1). The limit of detection for this method is 30 pg NNMT. The levels of carcinoembryonic antigen (CEA) were measured by routine examination at our hospital.

Statistical analysis. The relationships between the clinical outcome, and the HGF and IL-6 contents were analyzed by means of the Mann Whitney or Kruskal Wallis test. The correlation was examined by means of Spearman's rank correlation test. Regarding the total survival period and disease-free period, we prepared a Kaplan Meier survival curve (33). Univariate analysis of prognostic factors was performed by means of the log-rank test. Multivariate analysis was performed using the Cox proportional-hazards model (34). Statistical calculations were performed using the SPSS software (ver.17.0) (IBM, Chicago, IL, USA). When the two-sided *p*-value was lower than 0.05, statistical significance was considered.

Results

Table I shows the clinical profiles of the 109 patients, and the HGF and IL-6 levels in each group. The levels of HGF detected in serum samples of various patients were in the range of 215-2,000 pg/ml. The median HGF level in the 109 patients was 860 pg/ml, with the average being 875 pg/ml. The HGF levels were not significantly different between the groups.

The IL-6 levels detected in the serum samples from the 109 patients were in the range of 0-62 pg/ml. The median IL-6 level was 2.7 pg/ml, with the average being 5.0 pg/ml. The IL-6 concentrations were significantly higher in older patients and in patients with SCC.

The relationship between the HGF and IL-6 levels in the sera from the patients was examined. As shown in Figure 1, the HGF levels were correlated with the IL-6 concentrations (Spearman's correlation coefficient by rank test: r=0.435; $p<0.0001$).

For the analysis of overall survival of the 109 patients, the median HGF value (860 pg/ml) was used as the cut-off. As shown in Figure 2a, patients with low HGF levels had a significantly better overall survival than ones with elevated HGF levels ($p=0.019$). For the patients who died during the follow-up observation period, the median HGF level was 1,025 pg/ml. On the other hand, the median HGF level in the surviving patients was 738 pg/ml, with there being a significant difference ($p=0.036$).

The IL-6 level was also associated with overall survival when the median IL-6 level (2.7 pg/ml) was used as the cut-off value ($p=0.002$) (Figure 2b). The median IL-6 level, 4.0 pg/ml, in the patients who died was higher than that of 2.3 pg/ml in the survivors ($p=0.027$).

Tumor marker CEA was reported to be a prognostic factor for patients with surgically resected NSCLC (3). We analyzed CEA and NNMT, which we reported as candidate tumor markers for NSCLC (1). However, the CEA and NNMT levels were not associated with the overall survival rate (Figure 2c and d).

When examining the overall survival rate of patients at individual disease stages, a difference was confirmed in the survival rate between patients with disease at stages I-II and III (p=0.0005). The analyzed patients included 50 patients at stage I, 17 at stage II, and 38 at stage III. Since the number of patients with disease at stage II was small, and they principally underwent the same surgery as in patients at stage I, the patients at stages I and II were analyzed together (Table I). The T status (pT factor) was also a strong prognostic factor.

In this study, the prognosis of patients with adenocarcinoma was better than that of patients with other types of NSCLC (p=0.007). The prognosis of female patients was also better than that of male ones (p=0.023). On univariate analysis, it was found that gender, stage, histological type, HGF level, IL-6 level, pT, and pN contributed to the overall survival rate (Table II).

On multivariate analysis of the overall survival rate, IL-6 (p=0.034, hazard ratio HR=3.46), pN factor (p=0.004, HR=3.71), and pT factor (p=0.001, HR=4.41) were statistically significant (Table II).

On analysis of the disease-free survival period, histological type, stage, pN factor and pT factor were found to be significant on univariate analysis, and histological type (p=0.02, HR=2.40) and pN factor (p=0.036, HR 2.41) to be significant on multivariate analysis (Table II).

Next, we analyzed the patients with stage III disease separately (Table III). Elevated HGF and IL-6 levels were associated with poor overall survival (Figure 3b and d). Disease-free survival was

also affected by these cytokines, but not statistically significantly (Figure 3a and c). The pT and pN factors were not related to overall or disease-free survival of patients with stage III disease.

The results of multivariate analysis of overall survival of stage III patients are shown in Table III. Because there is a correlation between the IL-6 and HGF levels, each HR is significant only when the other factor is omitted from the analysis. A poorer prognosis was associated with higher HGF levels (p=0.038, HR=3.97) and higher IL-6 levels (p=0.045 HR=4.76). The prognosis of patients with NSCLC other than adenocarcinoma was poorer than that of those with adenocarcinoma, but not statistically significantly when IL-6 was included in the analysis, since the histological types affected IL-6 level (Table I).

On the other hand, the HGF and IL-6 levels were not associated with overall or disease-free survival of patients with stage I-II disease (Figure 3e and f).

Furthermore, we analyzed the overall survival of 109 patients based on combinations of the HGF and IL-6 levels (Figure 4). In patients with low HGF and low IL-6 levels, prognosis was favorable and the survival rate was 93%. On the other hand, the survival rate in patients with high levels of both cytokines, who had the poorest prognosis, was 56%. The survival rates in patients with high HGF and low IL-6 levels, and those with low HGF and high IL-6 levels, were 86% and 78%, respectively. Therefore, it would be effective in regard to prediction of the survival rate to use a combination of two biomarkers (p=0.0011).

Discussion

The results found in this study suggest that the HGF and IL-6 levels in blood are useful as predictors of the aggressive characteristics of stage III NSCLC. Since it is known that HGF is a

stimulating factor for infiltration, and that it induces cell division and angiogenesis, a high HGF level in blood may be a marker suggesting latent metastasis. It is also suggested that cytokines, such as IL-6, released from a tumor and inflammatory cells stimulate fibroblasts in the lungs to produce HGF (24, 25). In addition, IL-6 directly stimulates the progression of lung tumor (29). It was reported that the IL-6 level would increase with increasing NSCLC stage, suggesting a correlation between high efficacy of chemotherapy and low IL-6 level in blood (34, 35). If tumor cells move from the primary tumor site and then induce local release of IL-6 and HGF, this will contribute to aggravation of the disease. Our study results showed a correlation between HGF and IL-6 levels, suggesting that IL-6 levels would reflect the HGF levels to a certain extent.

Disease-free survival was affected by these cytokines, but the correlation between relapse and these cytokines was not statistically significant. Chemotherapy may affect overall survival. Recently, the serum HGF levels in patients with advanced NSCLC were analyzed (22, 23). An association between HGF and gefitinib resistance was found in accordance with previous studies showing that the HGF/MET pathway played a role in the development of gefitinib resistance in NSCLC with an *EGFR* gene mutation (37, 38). It has also been reported that IL-6 is a biomarker of resistance to multitargeted receptor tyrosine kinase inhibitors in prostate cancer (39). In our study, patients with resectable stage III cancer were subjected to adjuvant chemotherapy using paclitaxel and carboplatin, but not gefitinib. Our results suggest that HGF and IL-6 generally stimulate resistance to chemotherapy. It has been reported that IL-6 reduces the sensitivity of cancer cells to chemotherapeutic agents, such as paclitaxel and cisplatin, by activating the PI3K/AKT and STAT3 pathways in cells (40); HGF has similar actions (41).

All these studies show that HGF and IL-6 are good molecular targets for cancer therapy (42 - 44). Our findings suggest that patients with stage III NSCLC who have low levels of HGF and IL-6 should be considered to be surgical candidates.

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Disclosure Statement

The Authors have no conflict of interest.

Table I. *Clinical profile of 109 patients, and serum levels of hepatocyte growth factor (HGF) and interleukin-6 (IL-6).*

Factor	n	HGF (pg/ml)		IL-6 (pg/ml)	
		Median (range)	*p*-Value	Median (range)	*p*-Value
Age, years					
>66	53	895 (360-2000)	0.109	3.7 (1.1-62)	0.001
≤66	56	753 (215-1625)		2.0 (0-24)	
Gender					
Male	67	895 (215-2000)	0.103	2.7 (0-26)	0.278
Female	42	700 (230-1500)		2.6 (0-62)	
Stage					
I-II	67	775 (215-2000)	0.584	2.4 (0-62)	0.115
III	38	875 (230-1575)		2.7 (1.5-23)	
IV	2	795 (440-1150)		0.9 (0-1.7)	
Histology					
ADC	79	730 (215-1625)	0.086	2.4 (0-26)	0.001
SCC	26	1000 (360-2000)		4.2 (1.5-62)	
Other	4	1088 (675-1525)		4.6 (3.9-5.7)	
CEA (ng/ml)					
≥4.6	34	875 (360-2000)	0.631	3.4 (1.6-62)	0.088
<4.6	75	775 (215-1575)		2.3 (0-26)	
NNMT (pg/ml)					
≥710	27	875 (230-2000)	0.352	2.7 (0-62)	0.136
<710	82	730 (215-1625)		2.4 (0-24)	
pN factor					
N (–)	72	820 (215-2000)	0.645	2.4 (0-62)	0.796
N (+)	31	912 (295-1625)		2.7 (1.6-10.3)	
pT factor					
T1-2	77	820 (215-2000)	0.381	2.3 (0-62)	0.103
T3-4	28	830 (230-1550)		3.4 (1.5-24)	

ADC: Adenocarcinoma; SCC: squamous cell carcinoma; CEA: carcinoembryonic antigen; NNMT: nicotinamide *N*-methytransferase.

Table II. *Univariate and multivariate analyses of overall and disease-free survival of 109 patients.*

Factor	n	Overall survival			Disease-free survival		
		Univariate	Multivariate (Cox regression model)		Univariate	Multivariate (Cox regression model)	
		p-Value	Hazard ratio (95% CI)	*p*-Value	*p*-Value	Hazard ratio (95% CI)	*p*-Value
Age, years							
>66	53	0.057			0.519		
≤66	56						
Gender							
Male	67	0.023			0.806		
Female	42						
Stage							
I-II	67	<0.001			<0.001	2.67 (0.96-7.46)	0.061
III	38						
Histology							
ADC	79	0.007	2.35 (0.94-5.84)	0.067	0.035	2.40 (1.15-5.02)	0.02
Other	30						
CEA (ng/ml)							
≥4.6	34	0.327			0.765		
<4.6	75						
NNMT (pg/ml)							
≥710	27	0.924			0.956		
<710	82						
HGF (pg/ml)							
≥860	53	0.019			0.439		
<860	56						
IL-6 (pg/ml)							
≥2.7	55	0.002	3.46 (1.10-10.8)	0.034	0.052		
<2.7	54						
pN factor							
N0	72	0.026	3.71 (1.51-9.12)	0.004	0.014	2.41 (1.06-5.54)	0.036
N1-2	31						
pT factor							
T1-2	77	<0.001	4.41 (1.80-10.9)	0.001	<0.001	2.35 (0.85-6.49)	0.099
T3-4	28						

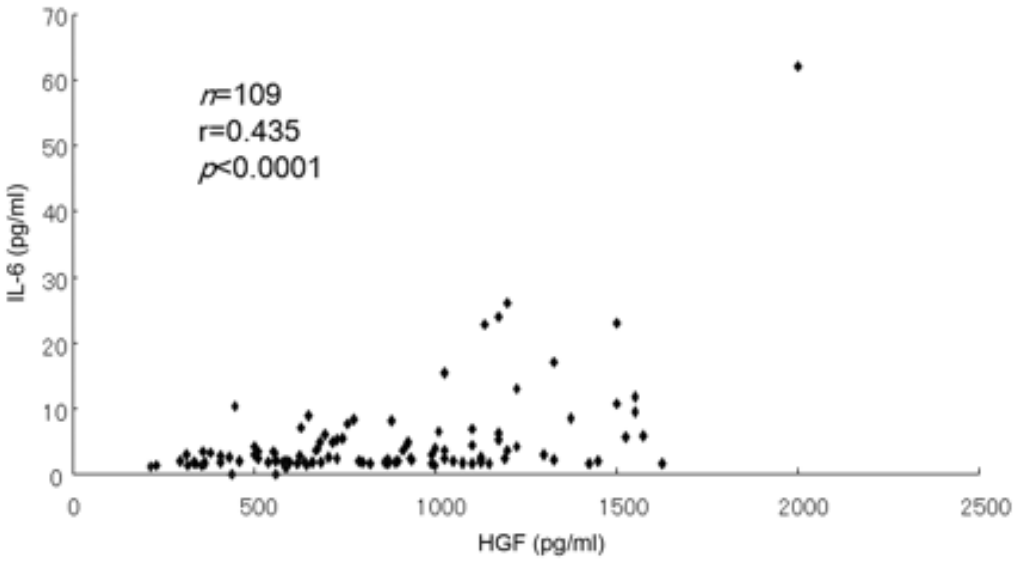
ADC: Adenocarcinoma; CEA: carcinoembryonic antigen; NNMT: nicotinamide *N*-methytransferase; IL-6: interleukin-6; HGF: hepatocyte growth factor; CI: confidence interval.

Table III. *Univariate and multivariate analyses of overall survival of patients with stage III disease.*

Factor	n	Univariate	Multivariate (Cox regression model)	
		p-Value	Hazard ratio (95% CI)	*p*-Value
Age				
>66	18	0.255		
≤66	20			
Gender				
Male	26	0.12	2.89 (0.63-13.3)[a]	0.172
Female	12		3.17 (0.70-14.3)[b]	0.133
Histology				
ADC	29	0.007	4.07 (1.32-12.5)[a]	0.015
Other	9		2.73 (0.92-8.16)[b]	0.072
CEA (ng/ml)				
≥4.6	15	0.249		
<4.6	23			
NNMT (pg/ml)				
≥710	11	0.517		
<710	27			
HGF (pg/ml)				
≥860	20	0.016	3.97 (1.08-14.6)[a]	0.038
<860	18			
IL-6 (pg/ml)				
≥2.7	23	0.013	4.76 (1.03-21.9)[b]	0.045
<2.7	15			
pN factor				
N (–)	14	0.455		
N (+)	21			

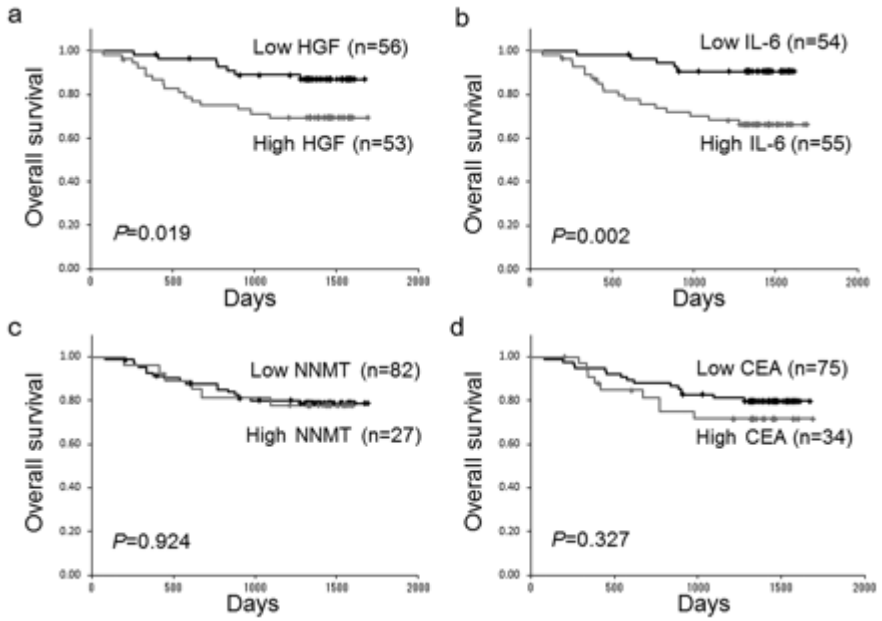
ADC: Adenocarcinoma; CEA: carcinoembryonic antigen; NNMT: nicotinamide *N*-methytransferase; HGF: hepatocyte growth factor; IL-6: interleukin-6; CI: confidence interval. [a]IL-6 as a factor was excluded from the variables; [b]HGF as a factor was excluded from the variables.

Figure 1.



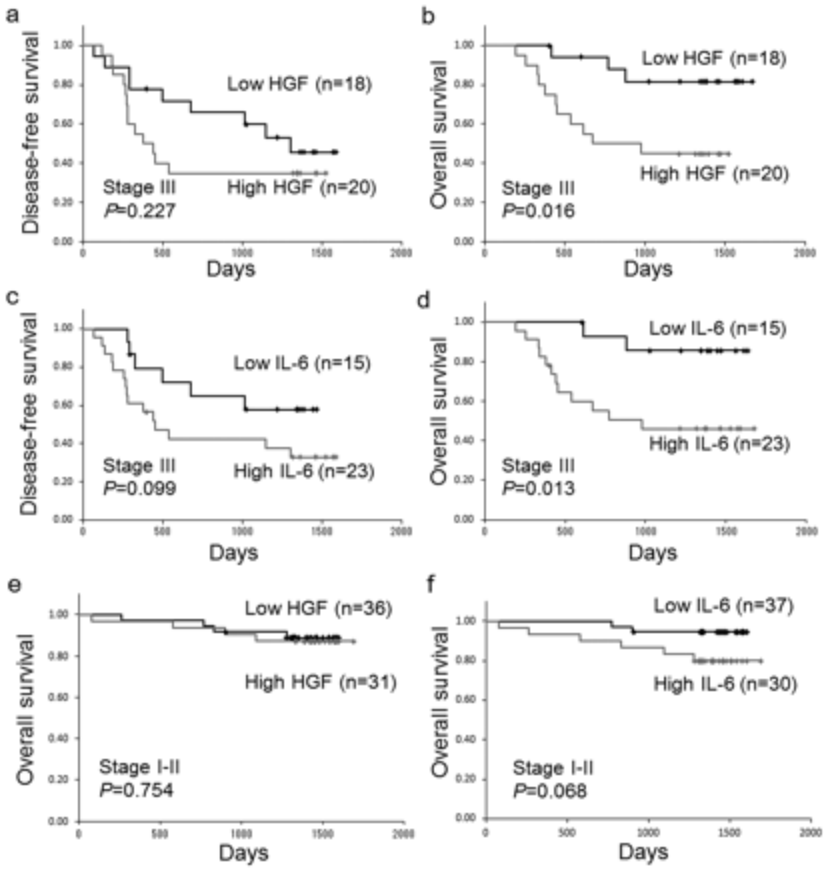
The serum levels of hepatocyte growth factor (HGF) in patients with lung cancer were correlated with those of interleukin-6 (IL-6). The correlation was examined by means of Spearman's rank-correlation test.

Figure 2.



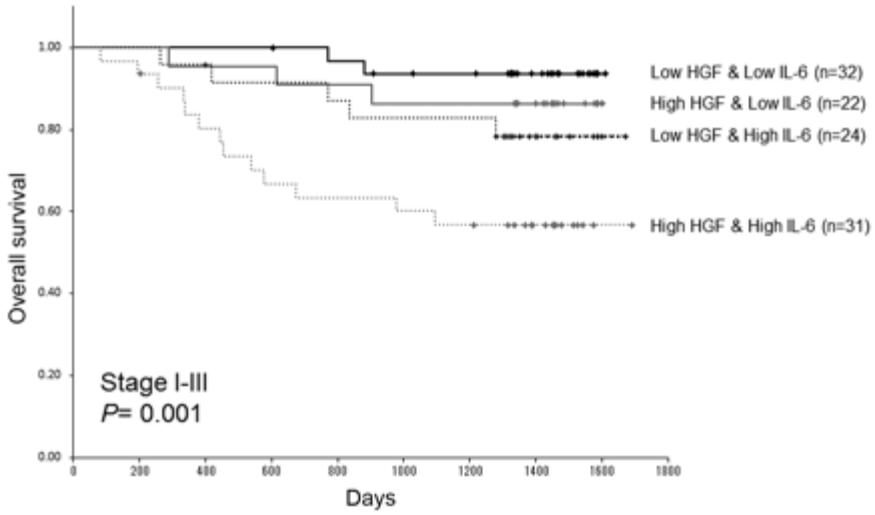
Overall survival curves for 109 lung cancer patients, according to the hepatocyte growth factor (HGF) (a), interleukin-6 (IL-6) (b), nicotinamide N–methytransferase (NNMT) (c), and carcinoembryonic antigen (CEA) (d) levels. The cut-off values are shown in Table II.

Figure 3.



Disease-free and overall survival curves for patienrs with stage III (a-d) and stage I-II (e-f) disease, according to the hepatocyte growth factor (HGF) (a, b, e) and interleukin-6 (IL-6) (c, d, f) levels.

Figure 4.



Overall survival curves for 109 lung cancer patients, according to the combination of hepatocyte growth factor (HGF) and interleukin-6 (IL-6) levels.

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