Association between brain natriuretic peptide and distant metastases in advanced non-small cell lung cancer patients

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Abstract. This study aimed to investigate the relationship between clinicopathological factors and plasma brain natriuretic peptide (BNP) levels in non-small cell lung cancer (NSCLC) patients. A total of 133 patients with advanced NSCLC were included in this study. The level of BNP was determined at the time of diagnosis. The BNP plasma concentration was measured using a chemiluminescent enzyme immunoassay kit. The univariate relationship between each independent clinicopathological variable and plasma BNP was examined using the Chi-square test. The survival curves were determined using the Kaplan-Meier method. According to the cut-off value of plasma BNP levels (11.5 and 22.4 pg/ml), plasma BNP negatively correlated with the presence of metastases (Chi-square test, p=0.0374 and p=0.0098, respectively). However, no significant association between patient survival time and plasma BNP levels was found. Reduced plasma BNP levels in advanced NSCLC patients with metastases were noted and the possibility was raised that BNP decreases distant metastases of advanced NSCLC patients.

Introduction

Lung cancer is a leading cause of cancer-related mortality worldwide and is expected to remain a major public health problem for the foreseeable future. Numerous phase III studies have been conducted aiming at validating therapeutic impact on patient survival among patients with advanced non-small cell lung cancer (NSCLC) (?).

The heart is a sophisticated endocrine gland that synthesizes a family of peptide hormones using three genes. These cardiac hormones are stored as three prohormones: atrial natriuretic peptide (ANP), brain natriuretic peptide (BNP) and C-type natriuretic peptide (1-4). The ANP prohormones comprise four peptide hormones: ANP, vessel dilator, kaliuretic peptide and long-acting natriuretic peptide. These peptides decrease up to 97% of human pancreatic, breast, colon, prostate, kidney and ovarian carcinomas as well as small-cell and squamous cell lung cancer cells within 24 h in cell culture (5). *In vivo* the four cardiac hormones eliminate up to 80% of human pancreatic adenocarcinomas, 66% of human breast cancers and up to 86% of human small-cell lung cancers in athymic mice. The anticancer mechanisms of these hormones target the Ras-MEK 1/2-ERK 1/2 kinase cascade in cancer cells. The four cardiac hormones also inhibit up to 95% of the basal activity of Ras, 98% of the phosphorylation of MEK 1/2 and 97% of the basal activity of ERK 1/2. They also block the activity of mitogens, such as the ability of epidermal growth factor to stimulate ERK (5).

ANP and BNP plasma concentrations were elevated in patients with congestive heart failure (6,7). The level of BNP was shown to be sufficiently accurate for diagnosing, monitoring and predicting prognosis in those patients (8). Of the six hormones, BNP had no antitumor effect against various tumor cell lines, including pancreatic, breast and small cell lung cancer (9,10). However, to the best of our knowledge, no reports exist on the prognostic effect of these peptide hormones or the relationship between clinicopathological factors and plasma levels in advanced NSCLC patients who are anticipated to have a high cardiac burden.

In this study, the concentration of plasma BNP levels in NSCLC patients was measured and the relationship between these levels and clinicopathological factors was investigated.

Patients and methods

Patients and methods. A total of 133 patients admitted to Kyoto University Hospital with advanced NSCLC between September 2007 and May 2009 were included in this study. Plasma samples were collected from all 133 patients.

The level of BNP was determined at the time of diagnosis. Venous blood (4 ml) was obtained from each patient and transferred to tubes containing aprotinin and ethylene-

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diamine-tetra-acetic acid, and stored at -20˚C until the measurements were taken. Plasma concentration of BNP was measured using a chemiluminescent enzyme immunoassay kit (MI02 Shionogi BNP; Shionogi Co. Ltd., Osaka, Japan) and an immunoassay system (MI02; A&T Co. Ltd., Yokohama, Japan). The minimum quantity of a human BNP detectable using this system is 4 pg/ml.

Statistical analysis. The univariate relationship between each independent clinicopathological variable and plasma BNP was examined using the Chi-square test. The survival curves were determined using the Kaplan-Meier method. The log-rank test was used to evaluate the differences between the survival curves. The data were analysed using JMP 6 software (SAS Institute, Cary, NC, USA).

Results

Patient characteristics. Table I shows the characteristics of the 133 advanced NSCLC patients who were admitted to our institution between June 2007 and May 2009. The patients were Japanese, and included 95 (71.4%) males and 38 (28.6%) females, with a median age of 61 years (range 34-88). The pathological diagnoses are listed in Table I and the number of dominant adenocarcinomas was 85 (63.9%). A total of 44 (33.1%) patients were non-smokers and 89 (66.9%) patients were former or current smokers. The Eastern Cooperative Oncology Group performance status was 0-1 for 104 patients and 2-3 for 29 patients. Of the 133 patients, 62 (46.6%) had been treated with platinum doublets, 30 (22.6%) with cytotoxic agent monotherapy, 7 (5.3%) with epidermal growth factor receptor tyrosine kinase inhibitors as the first-line regimen, 5 (3.8%) with chemo-radiotherapy, 3 (2.2%) with thoracic radiotherapy and 26 (19.5%) with best supportive care.

Table I. Patient characteristics and treatment (n=133).

Characteristics	n (%)
Age (years)	
Range	34-88
Median	61
Gender	
Female	38 (28.6)
Male	95 (71.4)
Smoking status	
Non-smoker	44 (33.1)
Smoker	89 (66.9)
Former	46 (34.6)
Current	43 (32.3)
Performance status	
0-1	104 (78.2)
>2	29 (21.8)
Tumor histology	
Adenocarcinoma	85 (63.9)
Squamous cell carcinoma	24 (18.0)
NSCLC	22 (16.5)
Large	2 (0.6)
Pathological stage	
IIIB	55 (41.4)
IV	78 (58.6)
Therapy	
Platinum doublet	62 (46.6)
Cytotoxic agent monotherapy	30 (22.6)
EGFR TKIs	7 (5.3)
Chemo-radiotherapy	5 (3.8)
Radiation	3 (2.2)
Best supportive care	26 (19.5)

NSCLC, non-small cell lung cancer; EGFR TKIs, epidermal growth factor receptor tyrosine kinase inhibitors.

Plasma brain natriuretic peptide. The mean and median concentration of plasma BNP was 11.5 and 22.4 pg/ml, respectively. A statistical comparison of the plasma BNP in our study population showed significantly lower levels in patients with metastasis compared to those without metastasis (two-tailed t=1.969, df=9.177, p=0.05, Fig. 1). According to the cut-off level of plasma BNP (11.5 and 22.4 pg/ml), plasma BNP negativity correlates with the presence of metastases (Table II-A and B) (Chi-square test, p=0.0374 and p=0.0098, respectively).

Plasma brain natriuretic peptide and survivals. Positive or negative plasma BNP was determined by the cut-off values of 11.5 and 22.4 pg/ml, which are the 25 and 50% percentiles, respectively. No significant association between patient survival time and plasma BNP level was noted (Figs. 2 and 3).

Discussion

In this study, reduced plasma BNP levels were noted in advanced NSCLC patients with metastases. We also found that plasma BNP levels did not affect the survival of patients with or without chemotherapy.

The most recently-discovered property of the cardiac natriuretic peptides is their ability to inhibit the growth of cancers *in vitro* and *in vivo* (9-13). In any cancer cell line that has been examined *in vitro* (9-11,13) and *in vivo* (12), including that of human pancreatic adenocarcinoma cells, human breast adenocarcinoma, human squamous cell lung cancer and human small lung cancer cells, vessel dilator has had the strongest anticancer effects, whereas BNP had no effect on the cancer cells. However, in our study, the plasma BNP level was significantly lower in patients with metastasis, indicating that BNP decreases distant metastases in patients with NSCLC.

We hypothesized that plasma BNP inhibits the profibrotic action of tumor growth factor-β (TGF-β) (14), thereby decreasing distant metastases. TGF-β promotes an epithelial to mesenchymal transition that is associated with increased tumor cell motility, invasion and metastasis (15,16).

Moreover, BNP promotes vessel growth by increasing the number of endothelial progenitors and enhancing their functional properties (17). The provasculogenic properties of

Table II. Correlation between metastases and serum BNP according to the cut-off BNP levels.

A, 11.5 pg/ml

	No. of cases	Metastases	
		Positive	Negative
Negative (<11.5 pg/ml)	34	25	9
Positive (>11.5 pg/ml)	99	53	46
Total	133	78	55

Chi-square test, p=0.0374.

B, 22.4 pg/ml

	No. of cases	Metastases	
		Positive	Negative
Negative (<22.4 pg/ml)	66	46	20
Positive (>22.4 pg/ml)	67	32	35
Total	133	78	55

Chi-square test, p=0.0098; BNP, brain natriuretic peptide.

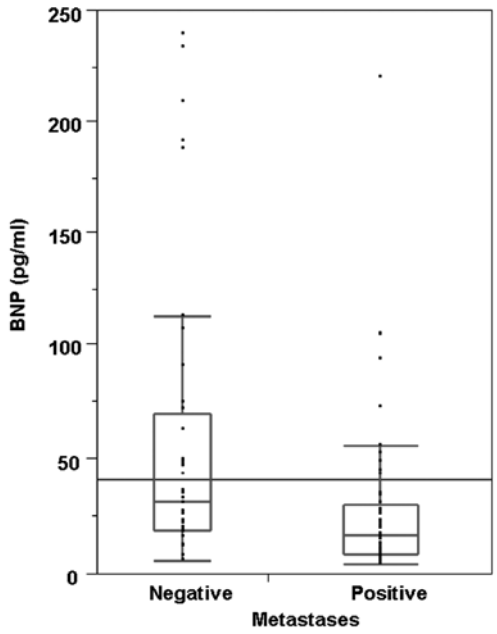

Figure 1. Serum levels of brain natriuretic peptide in relation to the presence of metastases in the patients (two-tailed t=1.969, df=9.177, p=0.05).

BNP may account for certain beneficial effects in NSCLC patients in terms of normal vasculogenesis in the tumor bed which differs from plasma vascular endothelial growth factor (VEGF). Subsequently, the association between plasma BNP and VEGF *in vivo* should be investigated.

No prognostic significance of plasma BNP was found whether or not chemotherapy was administered. The level

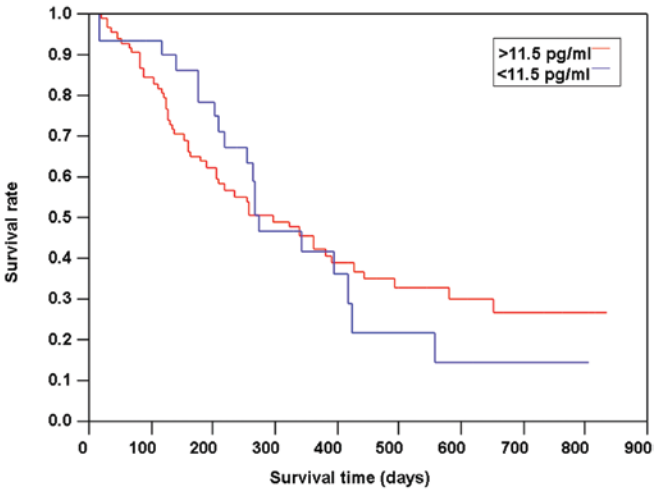

Figure 2. Kaplan-Meier analysis of the survival of 133 patients according to the serum brain natriuretic peptide levels. The differences between the groups were evaluated using the log-rank test (p=0.9196).

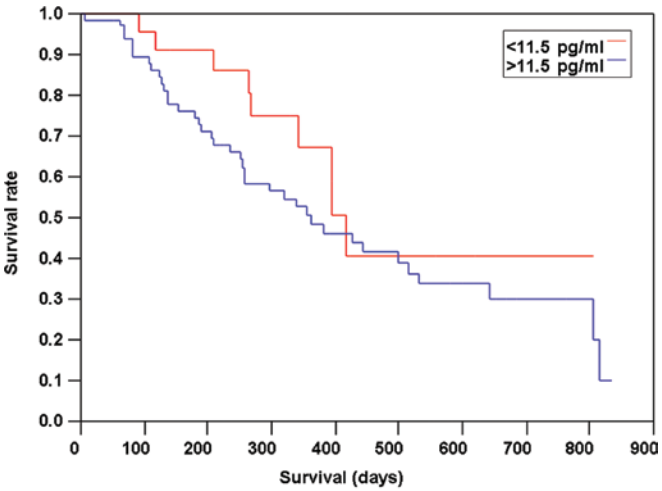

Figure 3. Kaplan-Meier analysis of the survival of 113 patients who received chemotherapy according to the serum brain natriuretic peptide levels. The differences between the groups were evaluated using the log-rank test (p=0.9919).

of BNP has been shown to be sufficiently accurate to predict prognosis in those patients (7). To the best of our knowledge, no reports exist regarding the relationship between prognosis and the levels of cardiac peptide hormones, including BNP, although advanced NSCLC patients anticipate to have a high cardiac burden. The reason is that in many cohorts dealing with advanced NSCLC patients, clinical stage is not always considered to be a prognostic factor.

The limitations of our study include the small sample size, the heterogeneity of the treatment regimens and its retrospective nature. However, we showed reduced plasma BNP levels in advanced NSCLC patients with metastases, suggesting that BNP decreases the distant metastases of advanced NSCLC patients. In conclusion, whether BNP is a potential therapeutic option for inhibiting distant metastases of advanced NSCLC patients remains to be determined.

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