The Radiotherapeutic Significance of Serum NSE Level in Non-Small Cell Lung Cancers(NSCLC)

Sang Mo Yun, M.D., Sang Bo Kim, M.D., In Kyu Park, M.D. and Tae Hoon Jung, M.D.*

Department of Radiation Oncology and Internal Medicine,*
School of Medicine, Kyungpook National University, Taegu, Korea

From December 1989 to February 1993, 108 patients with Non-Small Cell Lung Cancers(NSCLC) were studied retrospectively to evaluate radiotherapeutic significance of serum levels of NSE. We considered elevated serum neuron specific enolase(S-NSE) level as one of the neuroendocrine features in NSCLC. Histopathologic evaluation revealed 86 squamous cell carcinomas, 11 adenocarcinomas, 3 large cell carcinomas, 3 mucoepidermoid carcinomas, and 5 unknown pathology. Eight patients had stage I, 40 stage IIIA, and 60 stage IIIB. S-NSE level greater than 15 ng/ml was considered as elevated, and below this considered as normal. All patients recieved radiotherapy as primary treatment modality. The responders to radiotherapy had significantly higher mean S-NSE level than non-responders (28.5 ng /ml vs 20 ng/ml, p=0.01). Overall 2-year survival rate(YSR) was 23.6%. According to radiotherapy response, 2 YSR for patients with CR, PR, and NR were 39.2%, 28.6 %, and 6.2% respectively(p=0.001). 2 YSR for patients with elevated and normal S-NSE were 14.6% and 31.7%(p=0.02). The patients with NR showed no difference in survival according to S-NSE level. When we considered all patients, S-NSE level showed no significant impact on response. But for squamous cell carcinomas alone, patients with elevated S-NSE had more responders(80% vs 61%, p=0.05). There was no correlation between tumor characteristics and S-NSE level. But the patients with elevated S-NSE had more patients with higher nodal stage. Based on our and other datas, NSCLC with neuroendocrine features have different response to treatment and clinical behavior compared to other NSCLC. Thus, this subgroup may need different treatment modality, and S-NSE level may have prognostic significance.

Key Words : Non-Small Cell Lung Cancers, Neuron-specific enolase, Radiotherapy

INTRODUCTION

Neuroendocrine carcinomas of the lung includes carcinoid tumors and small cell carcinomas as listed in Table 1. These tumors secrete many markers and these markers were used in predicting response to treatment and survival in small cell cacinoma of the lung(SCLC)[5-7]. Non-small cell lung cancers(NSCLC) also secrete these markers and some of them, particulary adenocarcinoma and large cell carcinoma, show neuroendocrine differentation. It has been suggested that the biology of these "neuroendocrine" NSCLC might resemble that of SCLC, more malignant part of NSCLC. The percentage of expression of these markers in SCLC and NSCLC are listed in Table 2. Generally, neuroendocrine phenotype is

Table 1. Classifications of Neuroendocrine(NE) Carcinomas of the Lung

WHO[1]	Paladugh[2]	Gould[3]	Travis[4]
Carcinoid	KCC* 1	Carcinoid	Carcinoid
Atypical Carcinoid	KCC 2	Well differentiated NE carcinoma	Atypical carcinoid
		NE carcinoma of intermediate cell type	Large cell NE carcinoma
Small cell carcinoma	KCC 3	NE carcinoma of Small cell type	Small cell carcinoma

* Kulschitsky cell carcinoma.

Table 2. Neuroendocrine Markers in Lung Cancer Tumors and Cell Lines[8]

Marker	SCLC	NSCLC
Chromogranin-A	48- 93%	0-28%
Neuron-specific enolase	93-100%	27-57%
Leu-7	59- 89%	16-44%
L-dopa decarboxylase	48- 82%	12-33%
Bombesin	20- 69%	1-17%
Synaptophysin	43- 88%	10-28%

defined as expression of two or more of these markers, By this definition, 75% of SCLCs abd 20 % to 25% of NSCLCs have the neuroendocrine phenotype[9-11].In NSCLC, some studies have shown that neuroendocrine phenotype was better responded to chemotherapy and improved survival[10,12,13], but others have shown no improved response or survival[14].

There is few published clinical or laboratory data about radiotherapeutic significance of neuroendocrine differentiation in NSCLC. We used only serum neuron-specific enolase(S-NSE) as neuroendocrine marker because of our laboratoty limitations. We report retrospective study to evaluate radiotherapeutic significance of serum levels of NSE in patients with NSCLC.

METHOD AND MATERIAL

From December 1989 to February 1993, 108 patients with NSCLC entered this study. The patients and tumor characteristics are listed in Table 3. Histopathologic classification according to World Health Organization(WHO)[1] criteria was done, 86 squamous cell carcinomas, 11 adenocarcinomas, 3 large cell carcinomas, 3

Table 3. Patients Characteristics

	S-NSE level	
	Normal (Below 15ng/ml)	Elevated (Above 15ng/ml)
No. of patients	51	57
Age(yrs)		
mean	61	63
Range	30-73	47-82
Sex		
Male	47	52
Female	4	5
Performance status		
0	34	38
1	9	11
2	6	7
3	2	0
4	0	1
Histology		
Squamous cell	41	45
Adenocarcinoma	6	5
Large cell	1	2
Mucoepidermoid	1	2
Unknown	2	3
Stage		
I	6	2
IIIA	18	22
IIIB	27	33
Tumor stage		
1	1	1
2	20	19
3	13	12
4	17	25
Nodal stage		
0	14	8
2	25	28
3	12	21

mucoepidermoid carcinomas, and 5 unknown histology. According to AJCC criteria, 8 patients had stage I, 40 patients stage IIIA, and 60 patients stage IIIB. Diagnostic and staging work-up included medical history, physical examination, CBC, LFT, chest x-rays, bronchoscopy, and computed tomography(CT) of the chest. Additional staging work-up including bone scan, liver scan, and brain CT scan were only done if clinical signs or results of routine laboratory investigations were suggestive of distant metastasis.

S-NSE Measurements

S-NSE level was checked before radiation therapy during staging procedures. S-NSE levels were determined by radioimmunoassay. Normal mean levels are 11.5ng/ml for male and 10.4ng/ml for female, and 2 SD are 5.24 and 3.0 respectively. The serum level greater than 15ng/ml was considered as elevated, about 2 SD above the mean, and below this level considered as normal. The patients were grouped in two as in Table 3. At the end of radiotherapy, S-NSE measured once again in 43 patients.

Treatment and Follow-up

Radiation field included the primary mass with 1.5-2㎝ margin and mediastinal lymph nodes. If the lesion was in the upper lobe or had positive supraclavicular lymph node, ipsilateral or bilateral supraclavicular lymph nodes were included in the radiation field. Total dose ranged from 4000 cGy -6600 cGy with daily 180 cGy-200 cGy. Response to the treatment was assessed at 2-3 months after the end of treatment by chest x-ray with or without chest CT scan. Response was judged as complete(CR) when all clinical evidence of tumor disappeared; and partial(PR) when there was a reduction of 50% or more in the sum of all measurable tumor masses. Lesser degree of tumor reduction were regarded as no response(NR).

Follow-up study was done with 1 month interval for 1 year, 3 months for 2 years, and after then, 6 months interval. The minimum and maximum follow-up time were 4 months and 42 months, respectively. After about 1 year, half of the patients were lost to follow-up. At each visit, chest x-rays were checked, and 1 month after radiation therapy, computed tomography of chest was done, If patients' complaints were suspected of distant metastasis, appropriate studies were done.

Statistical Method

We analyzed the data by non-parametric methods. Chi-Square test was used to compare differences between groups of patients, and T-test was used to compare differences of mean S-NSE level. Survival curves were estimated by the method of Kaplan-Meier, and comparison carried out by the Peto-Wilcoxon test. The p-values less than 0.05 were considered as significant.

RESULTS

S-NSE Level before Treatment

We evaluated the mean S-NSE level in patients with elevated S-NSE. When comparison was done for overall stage, T(tumor) stage, and N (nodal) stage, there was no significant differences in mean S-NSE level. According to radiotherapy response, there noted statistically significant difference in mean S-NSE level. Mean S-NSE level in responders(CR and PR, 28.5ng/ml) was significantly higher than mean S-NSE level in non-responders(NR, 20 ng/ml) ($p=0.01$)

Survival

Overall 2 YSR was 23.6%. According to radiotherapy response, 2 YSR for CR, PR, and NR were 39.2%, 28.6%, and 6.2% respectively ($p=0.0001$). Overall stage, T stage, and N stage showed no statistically significant difference in survival. 2 YSR for patients with elevated S-NSE and normal S-NSE were 14.6% and 31.7%($p=0.07$, Fig. 1).

In the responding patients, S-NSE level showed statistically significant impact on survi-

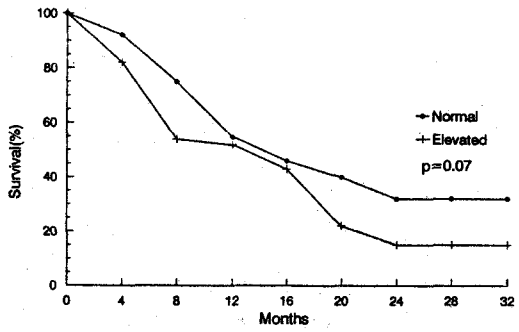

Fig. 1. Overall survival according to S-NSE level.

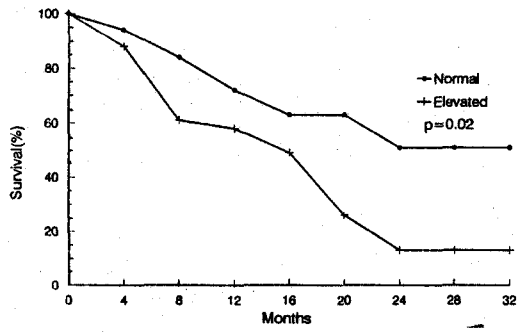

Fig. 2. Overall survival according to S-NSE level in responders(CR and PR) to radiotherapy.

val as noted in Fig. 2, revealing poorer prognosis in patients with elevated S-NSE(2 YSR 12% vs 50.7%, p=0.02). But in the non-responders, S-NSE level showed no impact on survival. According to T and N stage, S-NSE level also showed no difference in survival.

S-NSE level vs response and tumor characteristics

When comparison was done for all patients, response to treatment had no correlation with S-NSE level. But for squamous cell carcinoma, patient group with elevated S-NSE level responded more frequently than patient group with normal S-NSE (80% vs 61%, p=0.05) (Table 4).

According to T and N stage, patients with elevated S-NSE level were more frequently in higher nodal stage, although there was no statistical significance(Table 5).

We also analyzed the correlation between response and degree of decrease in S-NSE level after treatment. But, we could not find relationship between these.

Table 4. S-NSE Level vs Radiotherapy Response for Squamous Cell Carcinoma

	Normal	Elevated	
Responder	25(61%)	36(80%)	p=0.05
Non-responder	16(39%)	9(20%)	

Table 5. S-NSE Level vs N Stage

	Normal	Elevated	
N stage 0	14(27.5%)	8(14)	
2	25(49%)	28(49.1%)	p=0.14
3	12(23.5%)	21(36.8%)	

Patterns of Failure

The first sites of failure for patients with elevated S-NSE were; primary in 3, brain in 3, bone in 2, contralateral lung in 2, and liver in 1 patient. For patients with normal S-NSE, the first sites of failure were; brain in 5, bone in 3, neck in 3, primary in 1, pleura in 1, and adrenal gland in 1 patient.

We cannot say the difference in failure patterns between these two groups according to S-NSE level, because about half of patients were lost to follow-up.

DISCUSSION

It is clear from both clinical and in vitro data that SCLC has distinct neuroendocrine properties. SCLC is characterized by early and widespread metastasis. Therefore, the possibility of a curative resection is extremely rare. However, most patients benefit from chemotherapy with or without radiotherapy. In NSCLC, main treatment modalities are surgery or radiotherapy, but curative resection can be performed in a selected group of patients. Only a subgroup of NSCLC patients benefits from chemotherapeutic treatment and it is not possible to identify this

subgroup by normal histopathological procedures.

Non-small cell neuroendocrine lung cancers, which by light microscopy had been diagnosed as large cell or squamous cell carcinomas of adenocarcinomas, was firstly noted in 1981, when McDowell et al[15] described seven cases. McDowell labelled them "Atypical endocrine tumors of the lung". Electron microscopy subsequently showed dense core neurosecretory granules. The tumors were argyrophilic and contained 5-hydroxytryptamine, confirming endocrine differentiation at the level of light microscopy. Berger et al[16] reported that biochemical differences between the major histologic types were quantitative rather than qualitative and probably reflect the fact that the major forms of lung cancer represent a continuum of differentiation within a common cell lineage.

More recent data suggests that evaluation of endocrine markers in NSCLC may be of clinical value[10,13,14,17]. In several studies of fresh biopsies of NSCLC, especially adenocarcinomas, endocrine markers including L-dopa decarboxylase, NSE, etc, have been detected in up to 20% of tumors[16]. In addition, elevated serum levels of NSE are detected in up to 15% of patients with NSCLC[17]. Preliminary data suggested that such NSCLC with endocrine markers are more sensitive to cytotoxic therapy than nonendocrine NSCLC. Ariyoshi et al[17] reported that NSCLC patients with elevated serum NSE had response to cytotoxic therapy which were similar to those of patients with SCLC. Such clinical observations are supported by the demonstration that cell lines of NSCLC which express neuroendocrine markers have a pattern of in vitro chemosensitivity like SCLC cell lines in contrast to the usual pattern of resistance observed among other NSCLC cell lines[12,18]. And it has been suggested that the biology of these neuroendocrine NSCLC might resemble that of SCLC, representing therefore the more malignant part of NSCLC [19,20].

Graziano et al[10] in their retrospective analysis reported differences in response to cisplatin combination chemotherapy and survival between neuroendocrine markers positive and negative NSCLC. They used immunoperoxidase staining for NSE, Leu-7, and chromogranin A. In the patients group, adenocarcinoma were most common, comprising 81% in responders and 65% in nonresponders. Two markers were positive in 38 % of responders and 0% of nonresponders. Responders with two or more markers showed superior survival compared with responders with fewer than two positive markers and nonresponder(median, 79 vs 38 weeks, $p=0.02$). In contrast to this report, Berendsen et al[21] reported that the patients with neuroendocrine differentiation had worse prognosis in their multivariate analysis. In their report, squamous cell carcinoma accounts for 60.9 %, adenocarcinoma for 28%, and large cell carcinoma for 10%. They used monoclonal antibodies that reacted with SCLC-associated antigens. Linnoila et al[22] described a group of NSCLC patients receiving chemotherapy. More responders were noted in the group in which a neuroendocrine differentiation was recognized. However, survival was equal in both groups, with or without neuroendocrine differentiation. Eastern Cooperative Oncology Group(ECOG) studies showed that presence of NSE was associated with chemotherapy response, whereas Leu 7 and CEA expression were correlated with longer survival in NSCLC[13]. In our study, primary treatment modality is radiotherapy. Histopathologically, squamous cell carcinoma accounts for 79.6 %, adenocarcinoma for 10%, and large cell carcinoma and mucoepidermoid carcinoma for 5.5%. We only used S-NSE level to detect NSCLC with neuroendocrine feature. In patients with elevated S-NSE, responders had significantly higher mean S-NSE level than non-responders (28.5 ng/ml vs 20 ng/ml, $p=0.01$). Our results showed that patients with elevated S-NSE had worse prognosis in responders, 2 YSR 12.9% vs 50.7%($=0.02$). There noted no relationship between radiotherapy response and S-NSE level. But when

analysis is done for squamous cell carcinoma only, patients with elevated S-NSE have more responders(80% vs 61%, $p=0.05$). These differences of results between authors may be caused by different patients population and methods of detecting and quantifying neuroendocrine markers.

Sundaresan et al[14] described in their surgically treated NSCLC that there was significant correlation between nodal status and neuroendocrine differentiation, and disease stage and neuroendocrine differentiation, They concluded that neuroendocrine NSCLC were more metastatic. However, there was no correlation between neuroendocrine differentiation and survival. In our study, patients with elevated S-NSE showed higher nodal stage, but statistically not significant.

Some authors have suggested the change of chemotherapy regimen in neuroendocrinologically differentiated NSCLC. Terence Chorba et al[23] reported that NE(neuroendocrine)-NSCLC was sensitive to radiotherapy and to 5-FU + Streptozotocin, but resistant to CAV and Vp-16+ platinum. Neal et al[24] also reported that NE-NSCLC had dramatic response to same regimen. Thus, they proposed change of chemotherapy regimen in NE-NSCLC.

The clinical relevance of a separate group of non-small cell neuroendocrine carcinomas remains to be determined because of most studies are small and lack of follow-up. Also some consider that the markers lack specificity for neuroendocrine tumors[25,26]. However, our and other datas suggest that NSCLC with neuroendocrine features have different response to treatment and clinical behavior compared to other NSCLC. Thus, this subgroup may need different treatment modality, and neuroendocrine features in NSCLC may have prognostic significance. To confirm these findings, prospective randomized studies are needed.

ACKNOWLEDGMENT

We thank Department of Preventive Medicine, School of Medicine, Kyungpook National University, for their exellent statistical analysis.

REFERENCES

1. **World Health Organization.** Histological typing of lung tumours. 2nd ed. Geneva. Who, 1981
2. **Paladugh RR, Benfield JR, Pak HY.:** Bronchopulmonary Kulschitzky cell cadrcinoma. Cancer 55:1303, 1985
3. **Gould VE, Linnoila RI, Memoli VA, et al:** Biology of disease;neuroendocrine components of the bronchopulmonary tract. Lab Inves 49:519, 1983
4. **Travis WD, Linnoila RI, Tsokos MG, et al:** Neuroendocrine tumors of the lung with proposed criteria for large cell neuroendocrine carcinoma. Am J Surg Pathol 15:529, 1991
5. **Adi F. Gazdar, Carney DN, Marangos PJ, et al:** Serum neuron specific enolase; a marker for disease extent and response to therapy for small cell lung cancers. Lancet 1:583, 1982
6. **Akoun GM, Scrana HM, Milleron BJ, et al:** Serum neuron specific enolase. Chest 87:39, 1985
7. **Burghuber OC, Worofka B, Schernthanner G, et al:** Serum neuron specific enolase is a useful tumor marker for small cell lung cancers. Cancer 65:1386, 1990
8. **Richardson GE and Johnson BE:** The biology of lung cancer. Semi oncol 20:105, 1993
9. **Adi F. Gazdar, Helman LJ, Israel MA, et al:** Expression of neuroendocrine cell markers in human tumors of endocrine and nonendocrine origin. Cancer Research 48:4078, 1988
10. **Graziano SL, Mazid R, Newman N, et al:** The use of neuroendocrine immunoperoxidase markers to predict chemotherapy response in patients with non-small cell lung cancers. J Clin Oncol 7: 1398, 1989
11. **Jensen SM, Adi F. Gazdar, Frank Cuttitta, et al:** A comparison of Synaptophysin, Chromogranin, and L-dopa decarboxylase as markers for neuroendocrine differentiation in lung cancer cell lines. Cancer research 50:6068, 1990
12. **Mulshine J, Ihde D, Linnoila RI, et al:** Preliminary report of a prospective trial of neuroendocrine marker analysis and in vitro drug sensitivity test-

ing in patients with non–small cell lung cancers. Proc Am Soc Clin Oncol 6:181, 1987

13. **Ruckdeschel JC, Linnoila RI, Mulshine JL, et al:** The impact of neuroendocrine and epithelial differentiation on response and survival in lung cancers.; ECOG experience. Proc Am Soc Clin Oncol 10:248, 1991
14. **Sundaresan V, Reeve JG, Stenning S, et al:** Neuroendocrine differentiation and clinical behaviour in non–small cell lung tumors. Br J Cancer 64: 333, 1991
15. **McDowell EM, VetMed B, Wilson TS, et al:** Atypical endocrine tumors of the lung. Arch Pathol Lab Med 105:20, 1981
16. **Berger CL, Goodwin G, Mendelsohn G, et al:** Endocrine–related biochemistry in the spectrum of human lung cancer. J Clin Endocrinol Metab 53:422, 1981
17. **Ariyoshi Y, Kato K, Sugiura T, et al:** Therapeutic significance of neuron specific enolase in lung cancer. Proc Am Soc Oncol 5:23, 1986
18. **Gazdar AF, Tsai CM, Park TG, et al:** Relative chemosensitivity of non–small cell lung cancer expressing neuroendocrine cell properties. Proc Am Soc Clin Oncol 7:200, 1988
19. **Mooi WG, Dewar A, Springall D, et al:** Non–small cell lung carcinomas with neuroendocrine features. Histopathology 13:329, 1988
20. **Hammond ME and Sause WT:** Large cell neuroendocrine tumors of the lung. Cancer 56: 1624, 1985
21. **Berendsen HH, Lou de Leij, Sibrand Poppema, et al:** Clinical characterization of non–small cell lung cancer showing neuroendocrine differentiation features. J Clin Oncol 7:1614, 1989
22. **Linnoila RI, Jensen S, Steinverg S, et al:** Neuroendocrine differentiation corrlates with favorable response to chemotherapy in patients with non–small cell lung cancers. Fifth World Conference on Lung Cancer, 3.08, 1988(abstr).
23. **Terence Chobra, Orenstein JM, Haristiadis L, et al:** An atypical endocrine tumor of the lung responsive to radiation therapy and 5–fluorouracil–Streptozotocin. Cancer 53:2430, 1984
24. **Neal MH, Kosinski R, Cohen P, et al:** Atypical endocrine tumors of the lung. Hum Pathol 17: 1264, 1986
25. **Leader M, Collins M, Patel J, et al:** Antineuron specific enolase staining reactions in sarcomas and carcinomas. J Clin Pathol 39:1186, 1986
26. **Reeve JG, Stewart J, Watson JV, et al:** Neuron specific enolase expression in carcinoma of the lung. Br J Cancer 53:519, 1986

=국문초록=

비 소세포성 폐암의 방사선 치료에서 혈청내 NSE 치의 중요성

경북대학교 의과대학, 치료 방사선과학교실, 내과학교실*

윤 상 모·김 상 보·박 인 규·정 태 훈*

저자들은 1989년 12월부터 1993년 2월까지 108명의 비 소세포성 폐암환자를 대상으로 혈청내 NSE치가 방사선 치료에 미치는 영향을 평가하기위해 본 후향성 조사를 시행하였다. 병리학적으로 편평상피세포암이 86명으로 대부분을 차지했으며, 그외 3명의 점막 표피성암(mucoepidermoid carcinomas), 11명의 선암, 3명의 대 세포성암, 그리고 5명에서는 병리 조직형을 증명하지 못했다. 병기별로는 stage I에 8명, stage IIIA에 40명, stage IIIB에 60명이 속해 있었다. 환자는 혈청내 NSE(neuron specific enolase)치에 따라 두군으로 나뉘었다. NSE치가 15ng/ml 이상은 증가군, 그 미만은 정상군으로 하였다. 모든 환자에서 주 치료는 방사선 치료이었다. NSE가 증가된 군에서 NSE측정치를 보면, 치료에 반응하는 환자에서 평균 NSE치는 반응이 없는 군보다 통계학적으로 유의하게 높은 수치를 보였다(28.5ng/ml vs 20ng/ml, p=0.01). 전체적으로 볼때 2년 생존율은 23.6%였다. 방사선 치료 반응에 따라 보면, 완전 관해, 부분 관해, 그리고 반응이 없는 군에서 2년 생존율은 각각 39.2%, 28.6%, 그리고 6.2%로 나타났다(p=0.001). NSE치가 증가된 군에서 2년 생존율은 14.6%, 정상인 군에서는 31.7%로 나타났다(=0.07). 치료에 대한 반응이 있는 환자만 고려했을 경우는 NSE치에 따른 생존율 차이가 더 현격했다. 전체적으로 볼때, NSE치는 치료에 대한 반응과는 별 상관관계가 없었다. 그러나, 편평 상피세포암만 고려했을 경우, NSE치가 증가된 군에서 치료에 반응이 있는 환자가 더 많았다 (80% vs 61%, p=0.05). NSE치와 전체 병기, 원발 종양의 병기, 그리고 임파선 병기는 통계학적으로 상관 관계가 없는 것으로 나타났다. 그러나, 대체로 NSE치가 증가된 군에서 임파선 병기가 좀 더 진행된 경향을 보였다.

결론적으로, NSE치가 높은 비 소세포성 폐암은 치료에 대한 반응과 생존율을 포함한 임상 경과에 있어서 NSE치가 정상인 비 소세포성 폐암과는 다름을 알 수 있었다. 따라서, NSE치가 증가된 비 소세포성 폐암에서는 약물치료 및 방사선 치료의 병합요법이 요구되며, NSE치는 예후인자의 하나로서 가치가 있다고 보여진다.