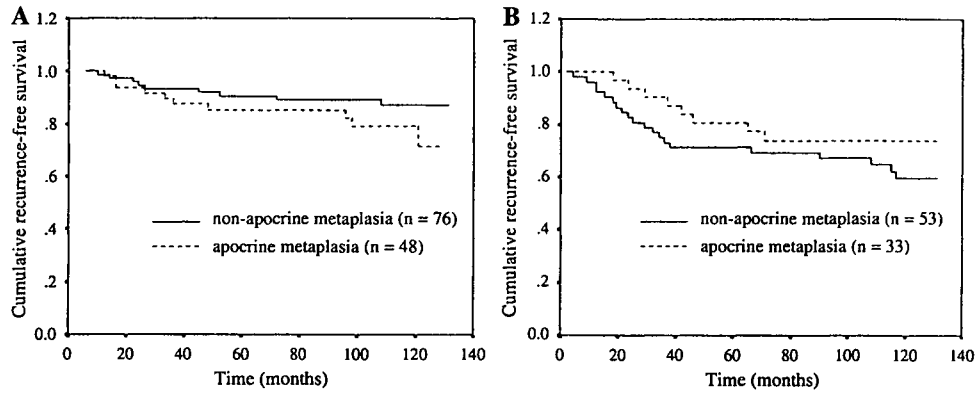

Fig. 4 Recurrence-free survival in patients with non-apocrine metaplasia (*full line*) and apocrine metaplasia (*short dotted line*). **a** Without chemotherapy ($p = 0.14$). **b** With chemotherapy ($p = 0.21$)

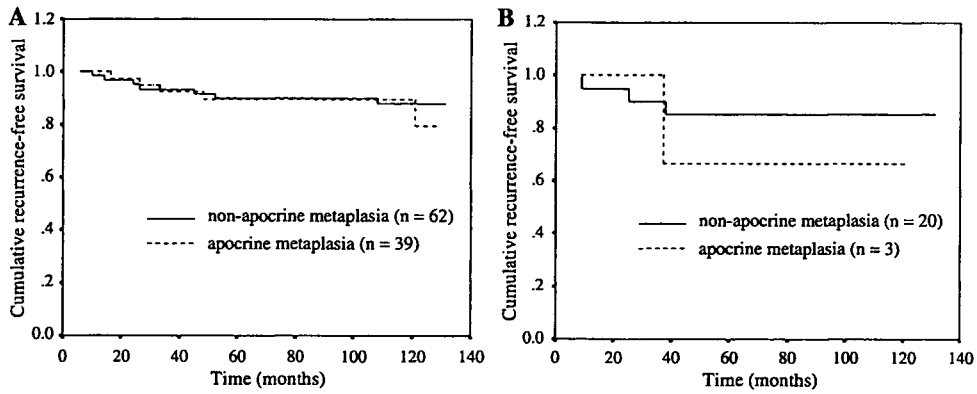

Fig. 5 Recurrence-free survival of node-negative patients with non-apocrine metaplasia group (*full line*) and apocrine metaplasia group (*short dotted line*). **a** Without chemotherapy ($p = 0.72$). **b** With chemotherapy ($p = 0.46$)

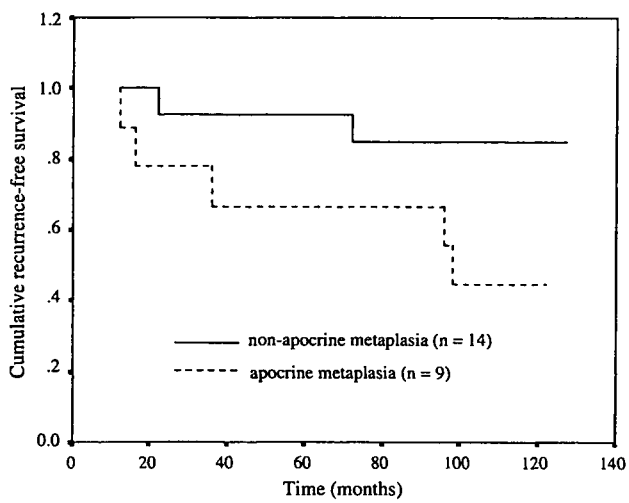

Fig. 6 Recurrence-free survival of node-positive patients without chemotherapy for non-apocrine metaplasia (*full line*) and apocrine metaplasia (*short dotted line*) ($p < 0.05$)

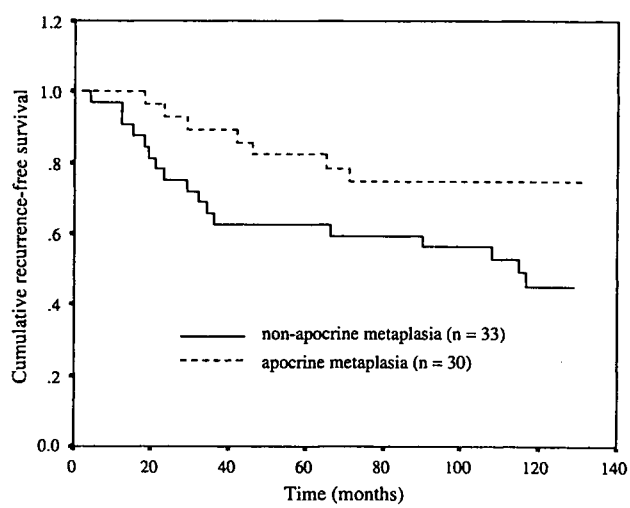

Fig. 7 Recurrence-free survival of node-positive patients with chemotherapy for non-apocrine metaplasia (*full line*) and apocrine metaplasia (*short dotted line*) ($p < 0.05$)

between non-AM and AM groups whether with presence or absence of chemotherapy. Using Cox multiple-regression analysis including tumor size, age, lymph node metastasis, hormonal status, HER2 status and AM, the persistence of lymph node metastasis was the only predictor of outcome. No associations between AM and prognosis were observed.

Discussion

The present study set up the hypothesis based on our institutional experience of neoadjuvant therapy that AM is useful as a predictive factor for therapeutic efficacy of chemotherapy. We then analyzed our institutional data retrospectively. We selected cases with invasive tumors 1.1–3.0 cm in diameter to ensure that all tumors had a measurable invasive size and to investigate lesions of comparable size. As the morphologic diagnostic criteria of AM remain unclear [2, 3, 6, 26], we proceeded with this study based on a definition of AM using our own scoring system with the size of cytoplasmic granules and the abundance of cytoplasm each classified into three categories. As 40% of tumors showing score 2 for abundance of cytoplasm did not exhibit the full characteristics of AM, a total score of three points was classified as incomplete AM. In all tumors including incomplete or complete AM, more than half of the area was occupied by AM, so we did not evaluate the percentage area occupied by AM. Cases of incomplete and complete AM were combined to form an AM group, because only one of six cases of complete AM received chemotherapy, so statistical analyses could not be performed among complete AM, incomplete AM and non-AM receiving chemotherapy. As this study focused on AM rather than apocrine cancer, incomplete and complete AMs were combined.

Some immunohistochemical analyses have reported apocrine carcinomas as ER-negative, PgR-negative, AR-positive, bcl-2-negative and GCDFP-15-positive [1, 7]. We performed these immunohistochemical analyses to improve the accuracy of diagnosis for AM, but no factors other than GCDFP-15 appeared as apocrine characteristics. GCDFP-15 positivity was high in the AM group, so we also analyzed prognoses between GCDFP-15-positive and GCDFP-15-negative tumors, but no significant differences were identified.

Among patients who had undergone chemotherapy, AM tended to be associated with better prognosis than non-AM, but no significant difference was identified. We also examined RFS in patients stratified according to lymph nodal status. In the lymph node-positive group, significant differences were seen in prognosis between AM and non-AM with or without chemotherapy. Recently, all patients identified as lymph node-positive have received chemotherapy as adjuvant therapy, according to the guidelines [27, 28]. However, in 1996, no guidelines regarding adjuvant therapy had been devised, so not all lymph node-positive patients received chemotherapy. We found that among lymph node-positive patients without chemotherapy, AM was associated with significantly worse prognosis than non-AM. Conversely, among lymph node-positive patients who received chemotherapy, patients with AM showed significantly better prognosis than those with non-AM. This suggests that AM responds well to chemotherapy, improving the prognosis of patients with AM.

Among lymph node-positive patients who received chemotherapy, the rate of metastasis to >10 lymph nodes, which is associated with very poor prognosis, was 10% for the AM group and 33% for the non-AM group. No significant difference in the distribution of the number of lymph node metastases was seen between AM and non-AM groups, but the possibility remains that the prognosis for patients with metastasis to >10 lymph nodes is so poor that the prognosis for non-AM group RFS is markedly skewed. We therefore excluded cases with metastasis to >10 lymph nodes and compared prognosis between AM and non-AM groups. No significant difference was observed between these groups if chemotherapy had been administered. However, in the absence of chemotherapy, the AM group showed clearly worse prognosis than the non-AM group. The AM group might thus have achieved comparable prognosis to the non-AM group largely due to the markedly good response to chemotherapy. In multivariate analysis, AM did not remain as an independent prognosticator. However, in lymph node-positive cases, AM without chemotherapy showed worse prognosis than non-AM, while AM with chemotherapy showed the same or better prognosis than non-AM. This suggests AM as a factor influencing therapeutic effect.

The two factors of structural and morphological features are important when considering histological classification of breast cancer. Recent histological classifications have mixed the histologic names based on structural features and morphologic features. IDC-NST, which comprises a majority of breast cancers, is diagnosed based on the structural features. Conversely, apocrine carcinoma is diagnosed based on the cytologic features of AM. The association of focal or incomplete AM is ignored and is not reflected in the diagnosis. Thus, the ultimate type of breast cancer with AM is diagnosed as apocrine cancer. With the increasing importance of pharmacotherapies, histological classifications that include predictors of response to therapy are needed. The present results indicate that AM could represent a useful predictive factor. We therefore suggest a reconstruction of the histological classification system based on structural classifications with the addition of cytological appearances, such as "scirrhous carcinoma

with complete AM," "solid-tubular carcinoma without AM" and so on. This classification extinguishes the existence of apocrine carcinoma, which is named based only on morphologic features. These new histological classifications of breast cancer could make pathological diagnosis more clinically useful and meet the demands of the times.

References

1. O'Malley FP, Bane A. An update on apocrine lesions of the breast. Histopathology. 2008;52:3–10.
2. Takeuchi H, Tsuji K, Ueo H, Kano T, Maehara Y. Clinicopathological feature and long-term prognosis of apocrine carcinoma of the breast in Japanese women. Breast Cancer Res Treat. 2004;88:49–54.
3. Durham JR, Fechner RE. The histologic spectrum of apocrine lesions of the breast. Am J Clin Pathol. 2000;113:S3–18.
4. Tavassoli FA, Devilee P, World Health Organization Classification of Tumours. Pathology and genetics of tumours of the breast and female genital organs. Lyon: IARC Press;2003.
5. Rosen PP. Apocrine carcinoma. In: Weinberg RW, Donnellan K, Palumbo R, editors. Rosen's breast pathology. 2nd ed. Philadelphia: Lippincott Williams & Wilkins;2001. p. 483–95.
6. Azzoparidi JG. Problems in breast pathology. Major problems in pathology. vol. 11. London: W.B. Saunders;1979.
7. Eusebi V, Millis RR, Cattani MG, Bussolati G, Azzopardi JG. Apocrine carcinoma of the breast. A morphologic and immunocytochemical study. Am J Pathol. 1986;123:532–41.
8. Mossler JA, Barton TK, Brinkhous AD, McCarty KS, Moylan JA, McCarty KS Jr. Apocrine differentiation in human mammary carcinoma. Cancer. 1980;46:2463–71.
9. Haagensen CD. Diseases of the breast. 3rd ed. Philadelphia: W.B. Saunders;1986.
10. Miller WR, Telford J, Dixon JM, Shivas AA. Androgen metabolism and apocrine differentiation in human breast cancer. Breast Cancer Res Treat. 1985;5:67–73.
11. Faratian D, Bartlett J. Predictive markers in breast cancer—the future. Histopathology. 2008;52:91–8.
12. Faneyte IF, Schrama JG, Peterse JL, Remijnse PL, Rodenhuis S, van de Vijver MJ. Breast cancer response to neoadjuvant chemotherapy: predictive markers and relation with outcome. Br J Cancer. 2003;88:406–12.
13. Fiegl H, Jones A, Hauser-Kronberger C, Hutarew G, Reitsamer R, Jones RL, et al. Methylated NEUROD1 promoter is a marker for chemosensitivity in breast cancer. Clin Cancer Res. 2008;14:3494–502.
14. Sandri MT, Johansson HA, Zorzino L, Salvatici M, Passerini R, Maisonneuve P, et al. Serum EGFR and serum HER-2/neu are useful predictive and prognostic markers in metastatic breast cancer patients treated with metronomic chemotherapy. Cancer. 2007;110:509–17.
15. Di Leo A, Tanner M, Desmedt C, Paesmans M, Cardoso F, Durbecq V, et al. p-53 Gene mutations as a predictive marker in a population of advanced breast cancer patients randomly treated with doxorubicin or docetaxel in the context of a phase III clinical trial. Ann Oncol. 2007;18:997–1003.
16. Horii R, Akiyama F, Ito Y, Matsuura M, Miki Y, Iwase T. Histological features of breast cancer, highly sensitive to chemotherapy. Breast Cancer. 2007;14:393–400.
17. Ichikawa W, Takahashi T, Suto K, Shirota Y, Nihei Z, Shimizu M, et al. Simple combinations of 5-FU pathway genes predict the outcome of metastatic gastric cancer patients treated by S-1. Int J Cancer. 2006;119:1927–33.
18. Aschele C, Lonardi S, Monfardini S. Thymidylate synthase expression as a predictor of clinical response to fluoropyrimidine-based chemotherapy in advanced colorectal cancer. Cancer Treat Rev. 2002;28:27–47.
19. Moliterni A, Ménard S, Valagussa P, Biganzoli E, Boracchi P, Balsari A, et al. HER2 overexpression and doxorubicin in adjuvant chemotherapy for resectable breast cancer. J Clin Oncol. 2003;21:458–62.
20. Di Leo A, Gancberg D, Larsimont D, Tanner M, Jarvinen T, Rouas G, et al. HER-2 amplification and topoisomerase IIalpha gene aberrations as predictive markers in node-positive breast cancer patients randomly treated either with an anthracycline-based therapy or with cyclophosphamide, methotrexate, and 5-fluorouracil. Clin Cancer Res. 2002;8:1107–16.
21. Akiyama F, Horii R. Therapeutic strategies for breast cancer based on histological type. Breast Cancer. 2009;16(3):168–72.
22. Farmer P, Bonnefoi H, Becette V, Tubiana-Hulin M, Fumoleau P, Larsimont D, et al. Identification of molecular apocrine breast tumours by microarray analysis. Oncogene. 2005;24:4660–71.
23. Sorlie T, Tibshirani R, Parker J, Hastie T, Marron JS, Nobel A, et al. Repeated observation of breast tumor subtypes in independent gene expression data sets. Proc Natl Acad Sci USA. 2003;100:8418–23.
24. Carey LA, Dees EC, Sawyer L, Gatti L, Moore DT, Collichio F, et al. The triple negative paradox: primary tumor chemosensitivity of breast cancer subtypes. Clin Cancer Res. 2007;13:2329–34.
25. Perou CM, Sørlie T, Eisen MB, van de Rijn M, Jeffrey SS, Rees CA, et al. Molecular portraits of human breast tumours. Nature. 2000;406:747–52.
26. Haagensen CD, Bodian C, Haagensen DE Jr. Breast carcinoma: risk and detection. Philadelphia: W.B. Saunders;1981.
27. Goldhirsch A, Wood WC, Gelber RD, Coates AS, Thürlimann B, Senn HJ, et al. Progress and promise: highlights of the international expert consensus on the primary therapy of early breast cancer 2007. Ann Oncol. 2007;18:1133–44.
28. National Comprehensive Cancer Network. NCCN Clinical Practice Guidelines in Oncology™ Breast Cancer V.I.2008.

Breast Cancer
DOI 10.1007/s12282-009-0185-x

ORIGINAL ARTICLE

Malignant transformation of breast fibroadenoma to malignant phyllodes tumor: long-term outcome of 36 malignant phyllodes tumors

Makoto Abe · Satoshi Miyata · Seiichiro Nishimura · Kotaro Iijima · Masujiro Makita · Futoshi Akiyama · Takuji Iwase

Received: 11 June 2009 / Accepted: 26 October 2009

Abstract
Background Malignant phyllodes tumor of the breast is a rare neoplasm for which clinical findings remain insufficient for determination of optimal management. We examined the clinical behavior of these lesions in an attempt to determine appropriate management. We evaluated long-term outcome and clinical characteristics of malignant phyllodes tumors arising from fibroadenomas of the breast.
Methods A total of 173 patients were given a diagnosis of phyllodes tumor and underwent surgery at the Cancer Institute Hospital in Japan between January 1980 and December 1999. Of these patients, 39 (22.5%) were given a diagnosis of malignant phyllodes tumor; in three of these cases, detailed medical records were lost. Malignant phyllodes tumors were classified into two groups based on history of malignant transformation. Of the 36 malignant cases, 11 (30.6%) were primary and were given a diagnosis of fibroadenoma, experienced recurrence during the follow-up period, and were diagnosed with malignant phyllodes tumor (cases with a history of fibroadenoma). The other group was defined as cases without history of fibroadenoma and in whom lesions initially occurred as malignant phyllodes tumors. Based on differences between the two groups, overall survival curves were plotted using the Kaplan–Meier method, and statistical comparisons were performed using the log-rank test and Peto and Peto's test.
Results The outcome of cases with history of fibroadenoma was significantly better than that of cases without history of fibroadenoma.
Conclusions Patients with malignant phyllodes tumors but without prior history of malignant transformation who exhibit rapid growth within 6 months require aggressive treatment.

Keywords Malignant phyllodes tumor · Fibroadenoma · Malignant transformation · Breast tumor · Cohort study

Introduction

Phyllodes tumor of the breast is an uncommon fibroepithelial breast neoplasm that accounts for 0.3–1.0% of cases of female breast carcinoma [1]. On the other hand, fibroadenomas are the most frequent benign tumors of the breast after fibrocystic disease. The histogeneses of fibroadenoma and phyllodes tumor of the breast appear to be closely related. Because of the similarity of the epithelial cells in phyllodes tumors to cells in fibroadenomas, many believe phyllodes tumors to arise from a preexisting fibroadenoma [2, 3]. Whether all phyllodes tumors originate as fibroadenomas or whether they can arise de novo without a preexisting fibroadenoma is a matter of ongoing debate. In a study in 1995, Noguchi et al. [3] reported three

M. Abe · S. Nishimura · K. Iijima · M. Makita · T. Iwase
Department of Breast Oncology, Cancer Institute Hospital, 3-10-6 Ariake, Koto-ku, Tokyo 135-8550, Japan

S. Miyata
Department of Genome Center, Cancer Institute Hospital, 3-10-6 Ariake, Koto-ku, Tokyo 135-8550, Japan

F. Akiyama
Department of Pathology, Cancer Institute Hospital, 3-10-6 Ariake, Koto-ku, Tokyo 135-8550, Japan

M. Abe (✉)
Department of Breast Surgery, Murakami Memorial Hospital, Asahi University, 3-23 Hashimoto-cho, Gifu 500-8523, Japan
e-mail: makoto-abe@murakami.asahi-u.ac.jp

Published online: 26 December 2009

cases of fibroadenoma that were diagnosed by excisional biopsy and recurred as benign phyllodes. Clonal analysis showed that all three fibroadenomas were monoclonal in origin. It was speculated that phyllodes tumors begin as fibroadenomas, and that subsequently a single stromal cell undergoes mutation and develops into a phyllodes tumor composed mainly of monoclonal stromal cells but partially of monoclonal epithelial cells. Kuijper et al. [4] studied clonal progression in fibroadenomas and phyllodes tumors and concluded that fibroadenomas can progress in an epithelial direction to carcinoma in situ or in a stromal direction to phyllodes tumors. The incidence of monoclonal fibroadenoma is quite low, and this tumor can subsequently progress to phyllodes tumor. Valdes et al. [5] presented a case of malignant transformation of a fibroadenoma to cystosarcoma phyllodes after 5 years of radiologic stability, and demonstrated that monoclonal fibroadenomas can progress to phyllodes tumors by clonal analysis performed on fine-needle aspiration (FNA) sample. However, performing clonal analysis on all fibroadenomas is time consuming and not cost effective. Malignant phyllodes tumors are very uncommon. Successful management of them will require determination of their clinical characteristics. In this study, we specifically, evaluated the outcomes of and prognostic factors for the malignant transformation from fibroadenomas to malignant phyllodes tumors.

Patients and methods

Patient population

We treated 173 patients with the diagnosis of phyllodes tumors between 1980 and 1999 at the Department of Breast Surgery, Cancer Institute Hospital in Japan and classified the tumors as benign, borderline or malignant using the histological classification (Table 1) proposed by the Japanese Breast Cancer Society, which is similar to that proposed by Pietruszka and Barnes [6]. In total, 39 patients were diagnosed with malignant phyllodes tumors, though three of these patients were excluded because their medical records had been lost. The clinical features of 36 patients with malignant phyllodes tumors were retrospectively reviewed and collated.

Classification of cases

"Malignant transformation" was considered to have occurred when a fibroadenoma became a benign phyllodes tumor, or when a benign phyllodes tumor became a malignant phyllodes tumor. Eleven patients (30.6%) had been diagnosed as having fibroadenoma previously. Ten patients underwent excisional biopsy, exhibited recurrence in the region near the scar, and were diagnosed with malignant phyllodes tumors, while in one patient the tumor was demonstrated histopathologically (Fig. 1). These 11 cases were classified as cases with history of fibroadenoma. Another 25 patients (69.4%) were initially diagnosed with malignant phyllodes tumors. We compared findings for these two groups of patients.

Table 1 Histologic features used in classification of phyllodes tumors subtypes [1, 6]

Histologic features	Benign	Borderline	Malignant
Stromal cellular atypia	Mild	Marked	Marked
Mitotic activity	<4/10 HPF	4–9/10 HPF	≧10/10 HPF
Stromal overgrowth	Absent	Absent	Present
Tumor margins	Circumscribed	Circumscribed or infiltrative	Infiltrative

HPF high-power field

Statistical analysis

For this study, the two groups were compared with respect to age, tumor size, surgical treatment, surgical margins, duration of signs and symptoms, local recurrence, metastases, and survival. Overall survival curves were plotted using the Kaplan–Meier method, and statistical comparisons were performed using the log-rank test and Peto and Peto's test [7]. Comparisons of clinical background factors were made between those two groups using Welch's *t*-test and Fisher's exact test.

Result

Patient demographics

There were 36 patients diagnosed with malignant phyllodes tumors between the years 1980 and 1999. All were female, with a median age of 43.6 (range 16–88) years. The median size was 91.4 (range 15–320) mm. The median duration of follow-up was 68.5 (range 2–287) months. Of 36 patients, nine (25%) had local recurrence and 14 (39%) had hematogenous metastases.

Outcomes of groups with or without history of fibroadenoma

In the group with history of fibroadenoma, four of 11 (36.4%) patients developed generalized hematogenous

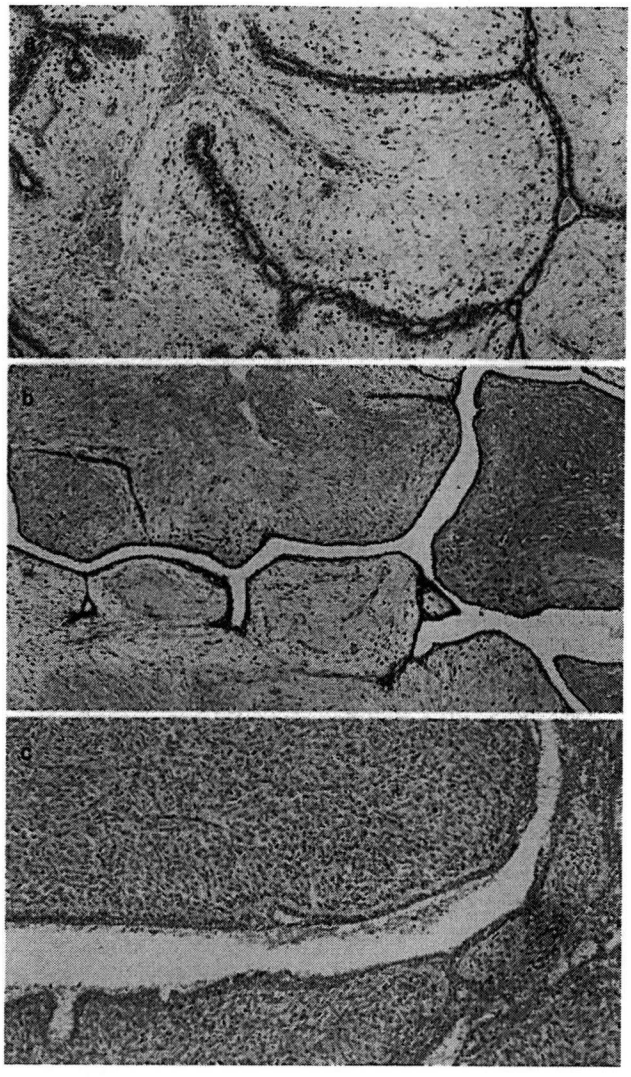

Fig. 1 Histologic features of typical tumor of fibroadenoma; benign and malignant phyllodes tumors are demonstrated. **a** Fibroadenoma. **b** Benign phyllodes tumor. **c** Malignant phyllodes tumor. Hematoxylin and eosin (H&E, ×400)

metastases. Three of the four died, and the remaining patient underwent three partial resections of lung metastases and remains alive 137 months after mastectomy.

However, in the group without history of fibroadenoma, 13 of 25 (52%) patients died. Ten patients developed generalized hematogenous metastases; nine of these 10 patients died early (at 2–27 months after diagnosis), and only one patient died later, at 134 months after final mastectomy, with pleural effusion and ascites. The other three patients died of other diseases. Moreover, some of the patients without history of fibroadenoma exhibited aggressive tumor growth, and died despite surgical resection. However, these were no early deaths among patients without history of fibroadenoma. The overall 20-year survivals of the two groups are shown in Fig. 2. The outcome

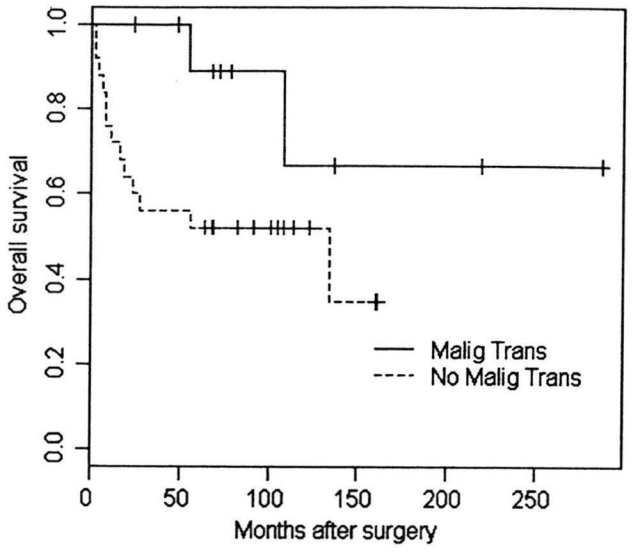

Fig. 2 Twenty-year survival of patients treated for primary malignant phyllodes tumors. *Malig trans* malignant transformation group (cases with history of fibroadenoma); *no malig trans* group without malignant transformation (cases without history of fibroadenoma)

of cases with history of fibroadenoma was significantly better than that of cases without history of fibroadenoma (log-rank test $p = 0.0551$, Peto and Peto's test [7] $p = 0.0409$). Multivariate analysis using the Cox model was used for stepwise regression to select the best subset of predictors from candidate covariates, yielding the following statistically significant variables as effective predictors of survival: tumor size, tumor growth rate in duration of symptoms (SIZE/DOS), surgical treatment, and malignancy (Table 2).

Table 3 summarizes tumor-related characteristics according to malignant transformation. The difference between the two groups in DOS was significant (Welch's t-test $p = 0.01437$). The cases without history of fibroadenoma had shorter DOS than the cases with history of fibroadenoma. There were no differences in age, tumor size, surgical treatment, surgical margins, local recurrence, metastasis or survival outcome between the two groups.

Discussion

Phyllodes tumor of the breast is a rare fibroepithelial lesion that accounts for less than 1% of all primary breast neoplasms [1]. The majority of phyllodes tumors have been described as benign (35–64%), with the remainder divided between borderline and malignant subtypes. The malignant subtype is found in approximately 25–30% of resected phyllodes tumors [1, 2]. Malignant phyllodes tumor sometimes metastasizes to the lungs. The median rate of metastasis reported after surgery for malignant phyllodes

Table 2 Multivariate analysis of Cox proportional-hazards model

Variable	Coefficient	Hazard ratio (95% CI)	*p* Value
Malignant transformation	1.545	4.69 (0.998–22.036)	0.05
Tumor size	0.00504	1.01 (0.999–1.011)	0.093
SIZE/DOS	0.0749	1.08 (1.023–1.136)	0.005
Surgical treatment	2.546	12.76 (1.054–154.426)	0.045

SIZE/DOS tumor growth rate in duration of symptoms, *SIZE* tumor size, *DOS* duration of symptoms

Table 3 Patients and tumor-related characteristics by malignant transformation

	Malig trans	No malig trans	*p*-Value
Age (mean, years)	42.5	44.1	0.721
Tumor size (cm)	8.2	9.5	0.5372
<5	4	7	
5–10	2	11	0.3226
>10	5	7	
Treatment			
Mastectomy	11	19	0.1479
Lumpectomy	0	6	
Surgical margins (cm)			
<0.5	1	1	0.5238095
≧0.5	10	24	
DOS (months)	33.6	34.6	0.9471
0–6	1	12	
6.1–12	2	0	0.01437
>12	8	11	
Unknown	0	2	
Local recurrence			
Yes	3	6	≧0.999
No	8	19	
Metastasis			
Yes	4	10	≧0.999
No	7	15	
Follow-up			
Alive	8	12	0.2767
Dead	3	13	0.4559
Died of disease	3	10	

DOS duration of symptoms; *malig trans* malignant transformation group (cases with history of fibroadenoma); *no malig trans* group without malignant transformation (cases without history of fibroadenoma)

tumors is 25–35% [2]. A report [8] from the M.D. Anderson Cancer Center on a subset of 30 women with the malignant histological subtype estimated that 5- and 10-year overall survival rates were 79% and 42%, respectively. Recently, Macdonald et al. [9] reviewed data on primary nonmetastatic malignant phyllodes tumors ($n = 821$) obtained from the National Cancer Institute's (NCI) Surveillance, Epidemiology, and End Results program and reported that predicted cause-specific survival rates were 91%, 89%, and 89%, at 5, 10, and 15 years, respectively, with median follow-up of 5.7 years. Another recent report [10] noted that the relative cumulative survival of malignant phyllodes patients was 87.4% at 10 years. In our study, the overall survival rates were 65%, 60%, and 52%, at 5, 10, and 20 years.

Previously reports [9] in the literature have suggested that stromal overgrowth, tumor size, surgical margin status, and types of surgery were predictive of local or distant recurrence after primary surgery. Patients with stromal overgrowth, tumor size >5 cm [8], and positive margins of excision [11] were found to have a high rate of distant failure. In the present series, the outcome of cases with history of fibroadenoma was significantly better than that of those without history of fibroadenoma. Multivariate analysis using the Cox model was used for stepwise regression technique to select the best subset of predictors, and revealed that size, SIZE/DOS, surgical treatment, and malignancy were effective predictors of survival. Nonmalignant transformation, large size, rapid growth, and mastectomy were significantly correlated with poor survival.

The course from fibroadenoma to phyllodes tumor was slow, but these tumors became histologically more malignant with every local recurrence. Recurrence was perhaps due to residual tumor secondary to inadequate excision of initial fibroadenoma, which can progress to phyllodes tumor. Chen et al. [12] reported that 22 of 172 phyllodes tumors patients had previously undergone fibroadenoma excisions, but that none of them had metastases. All 19 of these 22 patients had a first local recurrence of benign phyllodes tumor. According to his study of recurrent phyllodes tumors, the majority of recurrent tumors were histologically similar to the initial tumors; however, seven patients (19%) developed a malignant recurrence from an initially benign or borderline tumor [13]. Moreover, preoperative diagnosis of phyllodes tumors is difficult. Rapid growth and/or large size of apparent fibroadenomas may be the only imaging finding suggestive of phyllodes tumor. Whole-breast ultrasound showed that nearly one-third of women with phyllodes tumors had concurrent fibroadenoma [14]. It is important to examine most fibroadenomas with ultrasound, and to assess their rate of growth, if any. Rapid tumor growth or sudden increase in size is the most important clinical characteristic for prediction of progression. It is, however, difficult to assess the reliability of this observation, since no objective measurements of tumor growth rate were performed [1, 9]. Some reports notes that phyllodes tumors begin as fibroadenoma, and that subsequently a single stromal cell undergoes mutation and

develops into a phyllodes tumor composed mainly of monoclonal stromal cells and partially of monoclonal epithelial cells. The results of monoclonal analysis of our excisional biopsy samples would thus be of great interest.

To our knowledge, this is the first report on the frequency and prognosis of malignant transformation from fibroadenoma to malignant phyllodes tumor. About 20–30% of cases of malignant phyllodes tumors begin as fibroadenomas, and these have better prognosis than those that do not.

Conclusions

The prognosis of malignant phyllodes tumor arising from a preexisting fibroadenoma is relatively good. Patients with malignant phyllodes tumors but without prior history of malignant transformation who exhibit rapid growth within 6 months require aggressive treatment.

Acknowledgments We are grateful for the secretarial assistance provided by Ms. Rie Gokan.

References

1. Anderson BO, Lawton TJ, Lehman CD, Moe RE. Phyllodes tumors. In: Harris JR, Lippman ME, Morrow M, Osborne CK, editors. Disease of the breast. 3rd ed. Philadelphia: Lippincott Williams & Wilkins publishers; 2004. p. 991–1006.
2. McDivitt RW, Urban JA, Farrow JH. Cystosarcoma phyllodes. Johns Hopkins Med J. 1967;120:33–45.
3. Noguchi S, Yokouchi H, Aihara T, Motomura K, Inaji H, Imaoka S, et al. Progression of fibroadenoma to phyllodes tumor demonstrated by clonal analysis. Cancer. 1995;10:1779–85.
4. Kuijper A, Buerger H, Simon R, Schaefer KL, Croonen A, Boecker W, et al. Analysis of the progression of fibroepithelial tumors of the breast by PCR-based clonality assay. J Pathol. 2002;197:575–81.
5. Valdes EK, Boolbol SK, Cohen J-M, Feldman SM. Malignant transformation of a breast fibroadenoma to cystsarcoma phyllodes: case report and review of the literature. Am Surg. 2005;71:348–53.
6. Pietruszka M, Barnes L. Cystsarcoma phyllodes: clinicopathologic analysis of 42 cases. Cancer. 1978;41:1974–83.
7. Peto R, Peto J. Asymptotically efficient rank invariant test procedures (with discussion). J R Stat Soc Ser A Stat Soc. 1972;135:185–206.
8. Chaney AW, Pollack A, Mcneese MD, Zagars GK, Pister PWT, Pollock RE, et al. Primary treatment of cystsarcoma phyllodes of the breast. Cancer. 2000;89:1502–11.
9. Macdonald OK, Lee CM, Tward JD, Chappel CD, Gaffney DK. Malignant phyllodes tumors of the female breast. Association of primary therapy with cause-specific survival from the surveillance, epidemiology, and end results (SEER) program. Cancer. 2006;107:2127–33.
10. Asoglu O, Ugurlu MM, Blanchard K, Grant CS, Reynolds C, Cha SS, et al. Risk factors for recurrence and death after primary surgical treatment of malignant phyllodes tumors. Ann Surg Oncol. 2004;11:1011–7.
11. Fou A, Schnabel FR, Hamele-Bena D, Wei XJ, Cheng B, Tamer ME, et al. Long-term outcomes of malignant phyllodes tumors patients: an institutional experience. Am J Surg. 2006;192:492–5.
12. Chen W-H, Cheng S-P, Tzen C-Y, Yang T-L, Jeng K-S, Liu C-L, et al. Surgical treatment of phyllodes tumors of the breast: retrospective review of 172 cases. J Surg Oncol. 2005;91:185–94.
13. Tan EY, Hoon TP, Yong WS, Wong HB, Hui HG, Yeo AWY, et al. Recurrent phyllodes tumors of the breast: pathological features and clinical implications. ANZ J Surg. 2006;76:476–80.
14. Foxcroft LM, Evans EB, Porter AJ. Difficulties in the pre-operative diagnosis of phyllodes tumors of the breast: a study of 84 cases. Breast. 2006;27:27–37.

The Prognostic Impact of Main Bronchial Lymph Node Involvement in Non-Small Cell Lung Carcinoma: Suggestions for a Modification of the Staging System

Yoshihisa Shimada, MD, Masahiro Tsuboi, MD, PhD, Hisashi Saji, MD, PhD, Kuniharu Miyajima, MD, PhD, Jitsuo Usuda, MD, PhD, Osamu Uchida, MD, PhD, Naohiro Kajiwara, MD, PhD, Tatsuo Ohira, MD, PhD, Takashi Hirano, MD, PhD, Harubumi Kato, MD, PhD, and Norihiko Ikeda, MD, PhD

Department of Thoracic Surgery and Oncology, Tokyo Medical University, Tokyo, Japan

Background. The therapeutic strategies for non-small cell lung carcinoma (NSCLC) with N1 and N2 disease differ remarkably. Debate exists about the definition of the borderline between N1 and N2 stations. This study evaluated the prognostic effect of N1 disease, especially focused on the significance of the main bronchial node (No. 10) vs N2 disease.

Methods. The records of 1601 patients who underwent complete pulmonary resection for NSCLC were reviewed to examine the clinical features of lymph nodal involvement.

Results. There were 1086 patients (67.8%) with pN0 disease, 202 (12.6%) with pN1, and 274 (17.1%) with pN2 disease; overall 5-year survival rates were 74.7%, 56.1% and 28.9%, respectively ($p < 0.001$). Overall 5-year survival rates were 60.2% in hilar N1 and 49.6% in intralobar N1. Overall 5-year survival rates were 58.6% in N1 without node 10 and 35.1% in N1 with node 10. A significant difference was observed between N0 and N1 without node 10 ($p < 0.001$), and N1 without node 10 and N1 with node 10 ($p = 0.033$); however, the difference between N1 with node 10 and N2 was not significant. The status of node 10 involvement was an independent prognostic factor of pN1 patients, as well as age and gender.

Conclusions. Patients with node 10-positive N1 disease have an unfavorable prognosis, and the disease behaves like N2 disease. The definition of clear borderline between N1 and N2 is mandatory to achieve a uniform classification map. This study offers further information for clinical and therapeutic purposes.

(Ann Thorac Surg 2009;88:1583–8)

Staging of lung cancer based on the T N M classification is the method internationally accepted for the clinical setting of the disease to evaluate the prognosis, decide appropriate management, and analyze the results of treatment. The current T N M classification was initially proposed by Mountain in 1986 [1] and revised in 1997 [2].

Although this staging classification has been accepted, the anatomic definition of lymph node location—especially the boundary between N1 and N2 stations—has not been completely accorded. Currently, some variations of the lymph node map can be found, and thus considerable discordance exists regarding the designation of sites among investigators in the United States, Europe, and Japan [3]. The American Joint Committee on Cancer (AJCC) [4], Naruke and colleagues [5], and The American Thoracic Society (ATS) [6] introduced the concept of lymph node maps in 1973, 1978, and 1983, respectively.

The Mountain and Dresler modification of the ATS (MD-ATS) map was promulgated in 1997 [2]. Mountain and coworkers defined the boundary between N1 and N2 at the pleural reflection. The Naruke-Japanese map did not mention pleural reflection, however; they defined the lymph node station in relation to the bronchial tree and mediastinal structures [5, 7]. The main discrepancy between these two maps is that the Narke-Japanese map considers lymph nodes around the main bronchus and in the subcarinal space among the inferior border of the main bronchus to be station 10 (N1), whereas most of those are labeled as station 4 or 7 (N2) in the MD-ATS map [8].

A rational approach to the management of lung cancer requires accurate staging to plan the most appropriate treatment and to estimate the prognosis. Patients with pathologically proven N2 are no longer indicated for initial resection. Chemotherapy, chemoradiotherapy, or induction therapy, followed by resection, is the standard treatment of choice [9, 10]. Because the therapeutic strategies for patients with N1 and N2 disease differ greatly, the boundary between N1 and N2 stations where metastasis is confirmed pathologically is most essential. More-

Accepted for publication April 16, 2009.

Address correspondence to Dr Tsuboi, Department of Thoracic Surgery and Oncology, Tokyo Medical University, 6-7-1, Nishishinjuku, Shinjuku-ku, Tokyo, 160-0023, Japan; e-mail: mtsuboi@za2.so-net.ne.jp.

Published by Elsevier Inc

0003-4975/09/$36.00
doi:10.1016/j.athoracsur.2009.04.065

over, these discordant classifications may lead to a nonunivocal staging, rendering the comparison of different clinical studies difficult. Therefore, we need to define the boundary of lymph node location more precisely and reach consensus on the basis of the most recent evidence.

We reviewed the records of patients with completely resected non-small cell lung cancer (NSCLC) to examine the clinical features of lymph nodal involvement. The purpose of our study was to evaluate the prognostic impact of N1 disease, with a special focus on the significance of involvement of the main bronchial node (No. 10) compared with N2 disease.

Patients and Methods

Of 1601 patients who underwent complete pulmonary resection for NSCLC from 1990 to 2004 at Tokyo Medical University, 202 pN1 patients (12.6 %) without distant metastasis were the focus. Data collection and analyses were approved, and the need for obtaining informed consent from each patient was waived by the Institutional Review Board.

All of those patients underwent lobectomy or pneumonectomy with systemic lymph nodal dissection of the hilum and mediastinum. The histologic tumor type was determined according to the World Health Organization classification. Staging was determined according to the international T N M staging system [2]. All dissected lymph nodes were pathologically examined and classified according to anatomic location by the numbering system of Naruke and colleagues [5].

The station of N1 lymph nodes were classified main bronchial lymph node as No. 10, interlobar as No. 11, lobar bronchial as No. 12, segment bronchial as No. 13, and subsegmental as No. 14. N1 lymph nodes were generally classified into two groups as follows, hilar lymph nodes as No. 10 and 11, and intralobar lymph nodes as No. 12, 13, and 14. We further classified N1 lymph nodes involvement into two groups: pN1 disease who were No. 10-positive as the No. 10+ N1 group, and pN1 disease who were No. 10-negative as the No. 10− N1 group. Single-station metastasis was defined as involvement of only one station, whereas multiple-station metastasis was defined as involvement of more than one station.

For staging, all patients underwent a physical examination, chest roentgenogram, computed tomography (CT) imaging of the thorax, brain, and upper abdomen; bone scintigraphy, and bronchoscopy. The tumor marker, carcinoembryonic antigen (CEA) was also examined preoperatively. Serum CEA levels were measured using Latex photometric immunoassay (Mitsubishi Chemical Medience, Tokyo, Japan), and the upper limit of normal serum CEA levels was 3.0 ng/mL according to the manufacturers.

After resection, the patients were examined at 3-month intervals for 3 years, at 6-month intervals for the next 2 years, and thereafter at 1-year intervals in general. The evaluations included physical examination, chest roentgenogram, CT of the chest, and tumor marker measurement. Abdominal and brain CT as well as bone scintigraphy were done each year.

Table 1. Clinicopathologic Profiles of Patients With pN1 and pN2 Non-Small Cell Lung Cancer, 1990 to 2004

Variable	pN1 (n = 202)	pN2 (n = 274)
Age, median (range), y	64 (31–82)	65 (25–87)
Gender, No.		
Male	158	193
Female	44	81
pT status, No.		
T1	72	63
T2	99	153
T3	19	31
T4	12	27
Location, No.		
Right	117	176
Left	85	98
Histology, No.		
Adenocarcinoma	94	178
Squamous	84	68
Large	14	20
Adenosquamous	3	3
Other	7	5
Operation, No.		
Pneumonectomy	25	36
Lobectomy	177	238
Serum CEA, median (range) ng/mL	3.0 (1.0–213.5)	2.0 (1.0–140.0)

CEA = carcinoembryonic antigen.

Patients with cancer recurrences were carefully divided into two groups according to the site of initial relapse: locoregional or distant. Locoregional recurrence was defined as any recurrent site within the ipsilateral hemithorax, mediastinum, or supraclavicular lymph nodes. All other sites of recurrence were considered distant metastases.

Survival was calculated by the Kaplan-Meier method, and differences in survival were determined by log-rank analysis in which the initial day of treatment was the day of operation. The cause of death was recorded as cancer-related, due to other diseases, or unknown. Deaths that were not because of cancer were censored. Multivariate analysis of clinicopathologic factors was performed using the Cox proportional hazard regression model. A value of $p < 0.05$ was considered statistically significant. Hazard ratios (HR) and 95% confidence intervals (CI) are presented.

Results

Demographics

Of 1601 patients who underwent complete pulmonary resection for NSCLC from 1990 to 2004 at Tokyo Medical University, lymph node involvement was recognized in 1086 (67.8 %) as pN0, 202 (12.6 %) as pN1,

Table 2. Survival at 5 Years in Patients with pN1 Non-Small Cell Lung Cancer According to Prognostic Factors, 1994 to 2002

Variables	Patients, No.	5-Year Survival, %	*p* Value
Age, years			
<65	105	63.4	0.006
≥65	97	48.5	
Gender			
Male	158	50.7	0.007
Female	44	78.0	
pT status			
T1	72	58.7	0.319
T2/T3/T4	130	54.8	
Location			
Right	117	56.3	0.858
Left	85	56.0	
Histology			
Adenocarcinoma	94	55.7	0.830
Not adenocarcinoma	108	56.4	
Operation			
Pneumonectomy	25	46.4	0.303
Lobectomy	177	57.5	
Serum CEA, ng/mL			
<3.0	115	54.7	0.662
≥3.0	45	56.7	
pN1 status			
Intralobar[a]	124	60.2	0.146
Hilar[b]	78	49.6	
No. 10−	181	58.6	0.033
No. 10+	21	35.1	

[a] No. 12, 13, 14 lymph node metastasis. [b] No. 10, 11 lymph node metastasis.

CEA = carcinoembryonic antigen.

and 274 (17.1 %) as pN2 disease. The 202 pN1 patients (158 men, 44 women) were a median age of 64 years (range, 31 to 82 years). Operative procedures included 177 lobectomies and 25 pneumonectomies. The histologic classification was adenocarcinoma in 94 patients, squamous cell carcinoma in 84, large cell carcinoma in 14, adenosquamous in 3, and others in 7. The median value of preoperative serum CEA was 3.0 ng/mL (range, 1.0 to 213.5 ng/mL) (Table 1).

The distribution of pathologic T status was 72 pT1, 99 pT2, 19 pT3, and 12 pT4. The mean value of preoperative serum CEA was 3.0 ng/mL (range, 1.0 to 213.5 ng/mL). Among 202 patients with p-N1 disease, 124 had metastasis of intralobar nodes but not hilar nodes. Patients with hilar N1 nodes metastases were further categorized as 21 with No. 10+ N1 disease and 181 with No. 10− N1 disease (Table 2).

Prognosis

The median follow-up for survivors was 55 months (range, 1 to 200 months). The survival curves for the 1086 pN0, 202 pN1, and 274 pN2 patients are shown in Figure

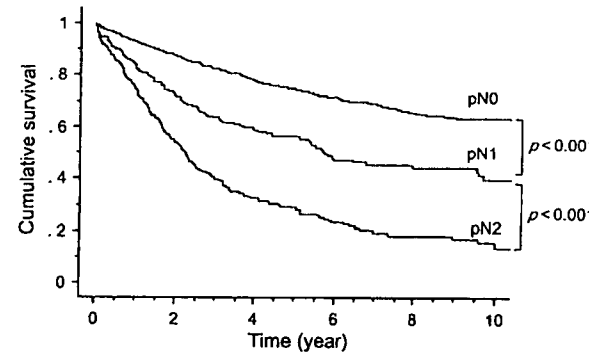

Fig 1. Kaplan-Meier curves show cumulative survival of patients undergoing complete resection for non-small cell lung carcinoma according to pathologic nodal status.

1. The overall 5-year survival rates were 74.7%, 56.1%, and 28.9%, respectively, and this difference was statistically significant ($p < 0.001$).

The association of various prognostic factors was examined by univariate analysis in 202 pN1 patients. Age and gender had a significant effect on survival ($p = 0.006$ and $p = 0.007$, respectively). The 21 No. 10+ N1 patients had significantly worse outcome than the 181 No.10− patients ($p = 0.033$). The overall 5-year survival of No. 10+ N1 patients was 35.1%, which was similar to that of pN2 patients (28.9%; Fig 2; Table 2). However, there was no significant difference in survival when pN1 patients were divided into hilar N1 (No. 10 and No. 11; n = 78) and intralobar N1 (No. 12, 13, and 14; n = 124; $p = 0.146$; Fig 3). There were also no significant differences on survival between the 156 patients with pN1 disease who had single-station metastasis and the 46 with multiple-station metastasis ($p = 0.742$; data not shown). This result implied that lymph node involvement of No. 10 is a poor

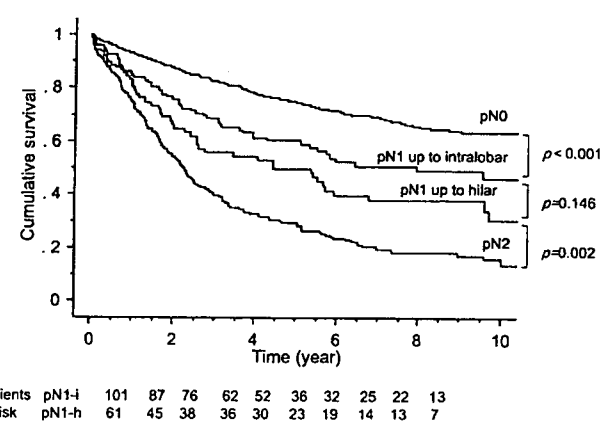

Fig 2. Kaplan-Meier curves show cumulative survival of patients undergoing complete resection for pN1 non-small cell lung carcinoma according to pathologic nodal status. Disease was classified as pN0, intralobar pN1, hilar pN1, and pN2. (Intralobar = No. 12, 13, 14 regional lymph nodes; hilar = No. 11, 10 regional lymph nodes.)

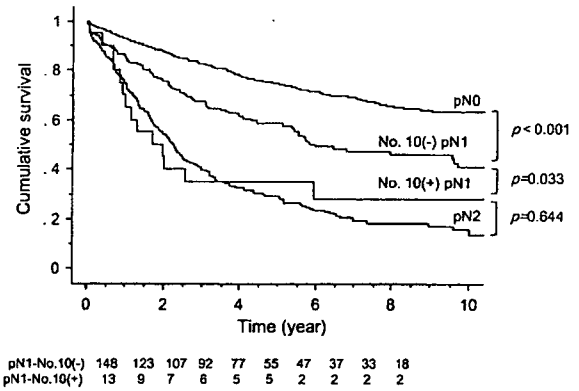

Fig 3. Kaplan-Meier curves show cumulative survival of patients undergoing complete resection for pN1 non-small cell lung carcinoma according to pathologic nodal status. Disease was classified as pN0, pN1 without No. 10 lymph nodes metastasis, pN1 with No. 10 lymph nodes metastasis, and pN.

prognostic marker in pN1 patients compared with No. 10 and No. 11 hilar lymph nodes. In multivariate analysis, the status of No. 10 lymph node involvement was an independent prognostic factor of pN1 patients as well as age and gender (HR, 1.933; 95% CI, 1.089 to 3.430; p = 0.024; Table 3).

We evaluated postoperative cancer recurrence in 100 pN1 patients for limited period, from 1996 to 2002, and 43 (43.0%) had cancer recurrence. Detailed data on cancer recurrence were not available for 6 patients. The initial relapse site was analyzed in the remaining 37 patients. The recurrences were locoregional in 10 (27.0%) and distant in 27 (73.0%). No statistical difference was observed in the distribution of the site of recurrence, locoregional or distant, between No. 10+ N1 and No. 10− N1 patients. Locoregional relapse occurred in 2 No. 10+ N1 patients (33.0%) and distant metastasis occurred 4 patients (67.0%). For those without No. 10 lymph node involvement, locoregional relapse occurred in 8 patients (25.8%) and distant metastasis in 23 (74.2%). The lung was the most common site for distant metastasis in both groups (data not shown).

Comment

During the past 30 years, different types of lymph node maps have been proposed. The distinction of lymph node stations is a most crucial topic that is still not entirely resolved by many lung cancer surgeons. One of the most significant problems concerning lymph node involvement under debate among thoracic oncologists is the definition of the borderline between N1 and N2 stations, which must be clarified, because this discordance could distort therapeutic strategies and stages reported in different studies.

In Naruke's map, lymph nodes in the subcarinal space along the inferior border of the mainstem bronchus are station No. 10 [5], whereas in MD-ATS map, these are labeled as level 7, hence N2 nodes [8]. The borderline between the N1 and N2 station is not clearly defined in Naruke's map. The No. 10 station is defined simply as "nodes around the main bronchus," and adjacent No.4 and No.7 were defined as "nodes at the tracheobronchial angle" and "nodes below tracheal carina," respectively [7].

In the MD-ATS map, the pleural reflection was set as a clear borderline for N1-N2 stations [8], with N1 nodes as those located distal to the mediastinal pleural reflection and within visceral pleura. This definition involves the proximal part of the hilar lymph nodes being classified within the N2 category because the proximal part of the mainstem bronchus lies within the mediastinal pleural envelope. However, concerning the borderline between N1 and N2 station, Asamura and colleagues [11] reported that the pleural reflection is recognized as a plane rather than as a line, and the reflecting line can be easily moved by retracting the lung anteriorly or posteriorly.

Some patients considered to have T1 2N1 M0 stage II disease in Japan would be considered to have T1 2N2 M0 stage IIIA disease in all other countries. This difference in nodal diagnosis might be a cause of staging migration.

In this study, we used Naruke's lymph node map to review the records of 1601 consecutive patients who had undergone complete resection for NSCLC. We also examined the spread pattern of lymph node metastases and investigated the outcome according to the level of the involved nodes.

Some investigators reported hilar lymph node metastasis is a significant unfavorable prognostic factor in p-N1 disease [12–18]. First, we divided N1 lymph nodes into two stations as follows, hilar lymph nodes (No. 10 and 11) and intralober lymph nodes (No. 12, 13, and 14). However, the difference in survival between hilar N1 and intralobar N1 was not significant.

Second, we further categorized patients with hilar N1 node metastases as those with and those without main bronchus node (No. 10) involvement. Among the 202 p-N1 patients, the 21(10.4%) identified with No. 10-positive N1 disease had a significantly worse prognosis than those with No. 10− N1 disease (p = 0.031). Moreover, the overall 5-year survival of patients with No. 10+

Table 3. Factors Influencing Survival in Patients With pN1 Non-Small Cell Lung Cancer by Multivariate Analysis

Variables	Favorable	Unfavorable	OR (95% CI)	p Value
Gender	Female	Male	2.109 (1.152–3.862)	0.016
Age, y	<65	≥65	1.771 (1.188–2.639)	0.005
No. 10 LN involved	Negative	Positive	1.933 (1.089–3.430)	0.024

CI = confidence interval, LN = lymph node; OR = odds ratio.

N1 disease was 35.1%, which was similar to the 28.9% survival in N2 disease. Multivariate analysis demonstrated that No. 10 lymph node involvement was one of the independent prognostic factors of pN1 patients as well as age and gender. Although the number of patients who were No. 10+ in this study is relatively small, we found that pN1 with No. 10+ disease behaves like a more advanced stage. Matsuoka and colleagues [19] reported the same results, including multivariate analysis, as ours concerning the survival benefit for the N1 disease with or without No. 10 involvement.

Several authors reported that the mode of metastasis in interlobar N1 tended to resemble that of N0, whereas that of hilar N1 behaved like N2 disease [12, 13, 15, 17, 18]; however, the modality of recurrence in our study for the limited period was not affected by the level of pN1 involvement. Our result that distant metastasis was predominant in the recurrent pattern over locoregional recurrence in p-N1 patients implies that nodal involvement might be a surrogate marker for distant metastasis, even if the site of metastasis is the interlobar lymph nodes.

Previous studies suggest that multiple levels of N1 stations are associated with a worse outcome than single-level disease [14, 20–23]. We were unable to identify the differences. Concerning the prognostic effect of the number of involved N1 stations, which may be one of the strong predictable factors for poor survival, it is possible that these analysis did not include enough patients to lead to a valid conclusion.

When taken together, the discrepancy between the Naruke map and the MD-ATS map might contribute to borderline cases between N1 and N2, such as multiple-station N1 disease or hilar N1 disease. The staging committee of the International Association for the Study of Lung Cancer (IASLC) is proposing a new international lymph node map that provides very precise definitions of the anatomic boundaries of each lymph node station and reconciles the differences between the Naruke map and the MD-ATS map [22].

The nodes around the junction of the hilum and mediastinum are key points at issue. Indeed, one of the most important problems is to decide whether the main bronchus nodes belong to the N1 or N2 station in relation to prognosis as well as anatomy. In this study, we found a difference in survival among patients with nodal metastasis up to either station 11 or station 10, whereas survival did not differ among patients with nodal metastases up to either station 10 or N2 station. This result suggested that nodes could be designated as intermediate between N1 and N2 and that there might be a borderline between N1 and N2 nodes around the main bronchus in accordance with the Naruke map. Moreover, our study demonstrated that the involvement of main bronchial nodes has a prognostic significance similar to that of single-station N2 and could be considered as an early N2 disease.

We fervently hope to have a single, accurate map of lymph node stations that can be used universally. Otherwise, it will be difficult to make progress in therapeutic strategies for lung cancer.

In conclusion, survival in patients with pN1 disease differs according to the type of lymph node involvement. Patients with No. 10 involvement have an unfavorable prognosis, and the disease behaves like N2 disease. The definition of a clear borderline between N1 and N2 is mandatory to achieve a uniform classification map. Further clinical studies may give more accurate information about the real prognostic value of No. 10 involvement to improve the clinical assessment and therapeutic strategies.

We are indebted to Professor J. Patrick Barron of the International Medical Communication Center of Tokyo Medical University for his review of this manuscript.

References

1. Mountain CF. A new international staging system for lung cancer. Chest 1986;89:225–33S.
2. Mountain CF. Revisions in the international system for staging lung cancer. Chest 1997;111:1710–7.
3. Watanabe S, Ladas G, Goldstraw P. Inter-observer variability in systematic nodal dissection: comparison of European and Japanese nodal designation. Ann Thorac Surg 2002;73: 245–248; discussion 248–9.
4. Cancer AJCC. Clinical staging system for carcinoma of the lung. Chicago: American Joint Committee for Cancer Staging and End Results Reporting; 1973.
5. Naruke T, Suemasu K, Ishikawa S. Lymph node mapping and curability at various levels of metastasis in resected lung cancer. J Thorac Cardiovasc Surg 1978;76:832–9.
6. American Thoracic Society. Medical section of the American Lung Association. Clinical staging of primary lung cancer. Am Rev Respir Dis 1983;127:659–64.
7. Kato H, Kwade N, Kobayashi K, et al. Classification of lung cancer. The Japan Lung Cancer Society. 1st English ed. Tokyo: Kanehara Co, Ltd; 2000:6–14.
8. Mountain CF, Dresler CM. Regional lymph node classification for lung cancer staging. Chest 1997;111:1718–23.
9. Thomas M, Rube C, Hoffknecht P, et al. Effect of preoperative chemoradiation in addition to preoperative chemotherapy: a randomised trial in stage III non-small-cell lung cancer. Lancet Oncol 2008;9:636–48.
10. van Meerbeeck JP, Kramer GW, Van Schil PE, et al. Randomized controlled trial of resection versus radiotherapy after induction chemotherapy in stage IIIA-N2 non-small-cell lung cancer. J Natl Cancer Inst 2007;99:442–50.
11. Asamura H, Suzuki K, Kondo H, Tsuchiya R. Where is the boundary between N1 and N2 stations in lung cancer? Ann Thorac Surg 2000;70:1839–45; discussion 1845–6.
12. Yano T, Yokoyama H, Inoue T, Asoh H, Tayama K, Ichinose Y. Surgical results and prognostic factors of pathologic N1 disease in non-small-cell carcinoma of the lung. Significance of N1 level: lobar or hilar nodes. J Thorac Cardiovasc Surg 1994;107:1398–402.
13. van Velzen E, Snijder RJ, Brutel de la Riviere A, Elbers HR, van den Bosch JM. Lymph node type as a prognostic factor for survival in T2 N1 M0 non-small cell lung carcinoma. Ann Thorac Surg 1997;63:1436–40.
14. Marra A, Hillejan L, Zaboura G, Fujimoto T, Greschuchna D, Stamatis G. Pathologic N1 non-small cell lung cancer: correlation between pattern of lymphatic spread and prognosis. J Thorac Cardiovasc Surg 2003;125:543–53.
15. Caldarella A, Crocetti E, Comin CE, Janni A, Pegna AL, Paci E. Prognostic variability among nonsmall cell lung cancer patients with pathologic N1 lymph node involvement. Epidemiological figures with strong clinical implications. Cancer 2006;107:793–8.

16. Okada M, Sakamoto T, Yuki T, et al. Border between N1 and N2 stations in lung carcinoma: lessons from lymph node metastatic patterns of lower lobe tumors. J Thorac Cardiovasc Surg 2005;129:825–30.
17. Riquet M, Manac'h D, Le Pimpec-Barthes F, Dujon A, Chehab A. Prognostic significance of surgical-pathologic N1 disease in non-small cell carcinoma of the lung. Ann Thorac Surg 1999;67:1572–6.
18. Ueda K, Kaneda Y, Saeki K, Fujita N, Zempo N, Esato K. Hilar lymph nodes in N2 disease: survival analysis of patients with non-small cell lung cancers and regional lymph node metastasis. Surg Today 2002;32:300–4.
19. Matsuoka K, Sumitomo S, Misaki N. Prognostic factors in patients with pathologic T1–2N1M0 disease in non-small cell carcinoma of the lung. J Thorac Oncol 2007;2:1098–102.
20. Martini N, Burt ME, Bains MS, McCormack PM, Rusch VW, Ginsberg RJ. Survival after resection of stage II non-small cell lung cancer. Ann Thorac Surg 1992;54:460–5; discussion 466.
21. Osaki T, Nagashima A, Yoshimatsu T, Tashima Y, Yasumoto K. Survival and characteristics of lymph node involvement in patients with N1 non-small cell lung cancer. Lung Cancer 2004;43:151–7.
22. Rusch VW, Crowley J, Giroux DJ, et al. The IASLC Lung Cancer Staging Project: proposals for the revision of the N descriptors in the forthcoming seventh edition of the TNM classification for lung cancer. J Thorac Oncol 2007;2:603–12.
23. Ueda K, Kaneda Y, Sakano H, et al. Independent predictive value of the overall number of metastatic N1 and N2 stations in lung cancer. Jpn J Thorac Cardiovasc Surg 2003;51:297–301.

The Society of Thoracic Surgeons Policy Action Center

The Society of Thoracic Surgeons (STS) is pleased to announce a new member benefit—the STS Policy Action Center, a website that allows STS members to participate in change in Washington, DC. This easy, interactive, hassle-free site allows members to:

- Personally contact legislators with one's input on key issues relevant to cardiothoracic surgery
- Write and send an editorial opinion to one's local media
- E-mail senators and representatives about upcoming medical liability reform legislation
- Track congressional campaigns in one's district—and become involved
- Research the proposed policies that help—or hurt—one's practice
- Take action on behalf of cardiothoracic surgery

This website is now available at www.sts.org/takeaction.

EUROPEAN JOURNAL OF CANCER 45 (2009) 1950–1958

available at www.sciencedirect.com

ScienceDirect

journal homepage: www.ejconline.com

Pretreatment neutrophil count as an independent prognostic factor in advanced non-small-cell lung cancer: An analysis of Japan Multinational Trial Organisation LC00-03

Satoshi Teramukai[a,*], *Toshiyuki Kitano*[b], *Yusuke Kishida*[a], *Masaaki Kawahara*[c], *Kaoru Kubota*[d], *Kiyoshi Komuta*[e], *Koichi Minato*[f], *Tadashi Mio*[g], *Yuka Fujita*[h], *Toshiro Yonei*[i], *Kikuo Nakano*[j], *Masahiro Tsuboi*[k], *Kazuhiko Shibata*[l], *Kiyoyuki Furuse*[m], *Masanori Fukushima*[a,b]

[a]*Department of Clinical Trial Design and Management, Translational Research Center, Kyoto University Hospital, 54 Shogoin Kawahara-cho, Sakyo-ku, Kyoto 606-8507, Japan*

[b]*Department of Translational Clinical Oncology, Kyoto University Graduate School of Medicine, 54 Shogoin Kawahara-cho, Sakyo-ku, Kyoto 606-8507, Japan*

[c]*National Hospital Organisation, Kinki-chuo Chest Medical Centre, 1180 Nagasone-cho, Kita-ku, Sakai 591-8555, Japan*

[d]*National Cancer Centre Hospital East, 6-5-1 Kashiwanoha, Kashiwa 277-8577, Japan*

[e]*Osaka Police Hospital, 10-31 Kitayama-cho, Tennoji-ku, Osaka 543-0035, Japan*

[f]*Gunma Prefectural Cancer Centre, 617-1 Takabayashi-Nishicho, Ohta 373-8550, Japan*

[g]*Department of Multidisciplinary Cancer Treatment, Kyoto University Graduate School of Medicine, 54 Shogoin Kawahara-cho, Sakyo-ku, Kyoto 606-8507, Japan*

[h]*Dohoku National Hospital, 7-4048 Hanasaki-cho, Asahikawa 070-8644, Japan*

[i]*National Hospital Organisation, Okayama Medical Centre, 1711-1 Tamasu, Okayama 701-1192, Japan*

[j]*National Kure Medical Centre, 3-1 Aoyama-cho, Kure 737-0023, Japan*

[k]*Kanagawa Cancer Centre, 1-1-2 Nakao, Asahi-ku, Yokohama 241-0815, Japan*

[l]*Koseiren Takaoka Hospital, 5-10 Eiraku-cho, Takaoka 933-8555, Japan*

[m]*The Japan Multinational Trial Organisation, 474 Uehonnojimae-cho, Teramachi-Oike agaru, Nakagyo-ku, Kyoto 604-0925, Japan*

ARTICLE INFO

Article history:
Received 3 December 2008
Received in revised form 14 January 2009
Accepted 16 January 2009
Available online 21 February 2009

Keywords:
Prognostic factors
Neutrophil count
Non-small-cell lung cancer

ABSTRACT

We examined the impact of pretreatment neutrophil count on survival in patients with advanced non-small-cell lung cancer (NSCLC). A total of 388 chemo-naïve patients with stage IIIB or IV NSCLC from a randomised controlled trial were evaluated. The effects of pretreatment peripheral blood neutrophil, lymphocyte and monocyte counts and neutrophil–lymphocyte ratio on survival were examined using the proportional hazards regression model to estimate hazard ratios after adjustment for covariates. The optimal cut-off value was determined by proportional hazards regression analysis with the minimum P-value approach and shrinkage procedure. After adjustment for prognostic factors, the pretreatment elevated neutrophil count was statistically significantly associated with short overall ($P = 0.0008$) and progression-free survival ($P = 0.024$), whereas no association was found between prognosis and lymphocyte or monocyte count. The cut-off value selected for neutrophil count was 4500 mm^{-3} (corrected hazard ratio, 1.67; 95% confidence interval (CI), 1.09–2.54). The median survival time was 19.3 months (95%CI, 16.5–21.4) for the low-neutrophil group (<4500 mm^{-3}, $n = 204$) and was 10.2 months (95%CI, 8.0–12.3) for the

* *Corresponding author:* Tel.: +81 75 751 4768; fax: +81 75 751 4732.
E-mail address: steramu@kuhp.kyoto-u.ac.jp (S. Teramukai).
0959-8049/$ - see front matter
doi:10.1016/j.ejca.2009.01.023

high-neutrophil group (≥4500 mm^{-3}, $n = 184$). We confirmed that pretreatment elevated neutrophil count is an independent prognostic factor in patients with advanced NSCLC receiving modern chemotherapy. Neutrophil count is easily measured at low cost, and it may be a useful indicator of patient prognosis.

1. Introduction

The prognosis for patients with advanced non-small-cell lung cancer (NSCLC) (TNM stage IIIB with a positive pleural effusion, or stage IV) has improved with recent advances in systemic chemotherapy, but still remains poor, with a median overall survival time between 4 and 15 months.[1] Prognostic factors identified in previous studies include tumour stage, performance status (PS), weight loss, sex, plasma lactate dehydrogenase (LDH) level and the presence of bone, liver or skin metastases.[2] Although novel immunological and histological biomarkers have been identified, these are often time-consuming to measure, and this is not part of the standard practice.

It is now evident that inflammatory cells in the tumour microenvironment have significant effects on tumour development.[3–6] Elevation in the pretreatment neutrophil count has been proposed as a prognostic factor for poor survival in patients with metastatic renal cell carcinoma,[7–9] and elevated neutrophil, monocyte or leucocyte count has been associated with poor survival in patients with metastatic melanoma.[10,11] A high-neutrophil–lymphocyte ratio may be related to poor prognosis in patients with colorectal cancer[12] and in those with advanced gastric cancer.[13] The European Lung Cancer Working Group found that the high-neutrophil count was an independent prognostic factor for poor survival in patients with unresectable advanced NSCLC[14] and in those with small-cell lung cancer.[15] A retrospective study found that neutrophil count was of prognostic value in patients with lung cancer.[16]

The aim of this study was to examine and confirm the impact of pretreatment peripheral blood neutrophil, monocyte and lymphocyte counts on overall and progression-free survival in a well-defined population of patients with advanced NSCLC being treated with regimens using newer chemotherapeutic agents in a randomised controlled clinical trial.

2. Patients and methods

2.1. Study population

A total of 401 chemo-naïve NSCLC patients with stage IIIB with pleural effusion or stage IV without brain metastasis, who had Eastern Cooperative Oncology Group (ECOG) PS of 0 or 1, were enrolled from 45 institutions in Japan between March 2001 and April 2005 into Japan Multinational Trial Organisation LC00-03[17] (registered with ClinicalTrials.gov identifier NCT00079287). Patients underwent one of two treatment regimens: intravenous vinorelbine (25 mg/m^2) plus gemcitabine (1000 mg/m^2) on days 1 and 8 every 21 d for three cycles, followed by intravenous docetaxel (60 mg/m^2) on day 1 every 21 d for three cycles [VGD arm, $n = 196$] versus intravenous paclitaxel (225 mg/m^2) and carboplatin (area under the curve = 6) for 3 h on day 1, every 21 d for six cycles [PC arm, $n = 197$]). As there were no significant differences between treatment groups in terms of either overall (hazard ratio: 0.996, $P = 0.974$) or progression-free survival (hazard ratio: 0.966, $P = 0.742$), the combined data from the two arms were analysed in this study. Of 393 eligible patients, information regarding pretreatment neutrophils in peripheral blood was not available for five patients. Thus, data from 388 patients were included in the present study.

2.2. Statistical analysis

Overall survival was defined as the time from randomisation until death from any cause, and progression-free survival was defined as the time from randomisation until objective tumour progression or death. Survival curves were estimated with the Kaplan–Meier method. Associations between the factors and the prognosis were examined with the log-rank test in univariate analyses. The prognostic impact of pretreatment peripheral blood neutrophil, lymphocyte and monocyte counts, and neutrophil–lymphocyte ratio were examined using the proportional hazards regression model to estimate hazard ratios after adjustment for covariates without variable selection. Optimal cut-off points for continuous variables were selected using the minimum P-value approach with correction of the P-value.[18] The corrected hazard ratio and its 95% confidence interval (CI) were estimated using a shrinkage procedure with bootstrap resampling.[19] All statistical analyses were done using SAS version 9.1 (SAS Institute, Cary, NC).

3. Results

3.1. Patients' characteristics

Of 388 patients, 276 patients had died, and the median follow-up time for the 112 surviving patients was 567 d (range: 70–1711 d). The characteristics of the 388 patients (276 men [71%], 112 women [29%], median age 65 years [range, 33–81 years]) included in the present study are shown in Table 1. Median pretreatment counts of neutrophils, lymphocytes and monocytes were 4304 mm^{-3}, 1386 mm^{-3} and 404.2 mm^{-3}, respectively. Spearman's rank correlations were 0.351 for neutrophils and monocytes, 0.034 for neutrophils and lymphocytes and 0.352 for monocytes and lymphocytes.

3.2. Relationship between pretreatment neutrophil, lymphocyte and monocytes counts and survival

In univariate analyses, pretreatment elevated counts of neutrophils were statistically significantly associated with short

Table 1 – Baseline patients characteristics (n = 388).

Characteristics	No.	%
Age, years, median (range)	65 (33–81)	
Sex		
Male	276	71
Female	112	29
Smoking history		
Non-smokers	96	25
Former smokers	107	28
Current smokers	168	43
Unknown	17	4
Stage		
IIIB	68	18
IV	320	82
Histologic type		
Squamous cell	76	20
Adenocarcinoma	274	70
Others	38	10
ECOG performance status		
0	154	40
1	234	60
Weight loss (from 6 months before enrolment)		
<5%	317	82
≥5%	71	18
LDH		
Normal (≤ULN)	279	72
High (>ULN)	109	28
Bone metastases		
No	280	72
Yes	108	28
Liver metastases		
No	357	92
Yes	31	8
Skin metastases		
No	379	98
Yes	9	2
Neutrophils, mm^{-3}, median (range)	4304 (205–17,100)	
Lymphocytes, mm^{-3}, median (range)	1386 (243–4200)	
Monocytes, mm^{-3}, median (range)[a]	404.2 (0–1620)	
Red blood cells, $\times 10^4$ mm^{-3}, median (range)	420 (286–579)	
Platelets, $\times 10^4$ mm^{-3}, median (range)[b]	26 (11–380)	

ULN: upper limit of normal.
a One missing value.
b Two missing values.

overall (Fig. 1A, $P < 0.0001$) and progression-free survival (Fig. 1B, $P = 0.0001$). Although lymphocyte count did not correlate with survival, there were significant relationships between high-neutrophil–lymphocyte ratio and short overall ($P < 0.0001$) and progression-free survival ($P = 0.005$). The elevated monocyte count was also significantly associated with short overall survival ($P = 0.004$), and was moderately related to short progression-free survival ($P = 0.052$). We selected sex, smoking history, stage, ECOG PS, weight loss, plasma LDH and the presence of bone, liver or skin metastases as the known pretreatment prognostic factors.[2,14] Adjusted hazard ratios for the relationship between pretreatment neutrophil, lymphocyte and monocyte counts and neutrophil–lymphocyte ratio and overall and progression-free survival after adjustment for the known prognostic factors are shown in Table 2. There was a statistically significant association between elevated neutrophil count and short overall ($P = 0.0008$) and progression-free survival ($P = 0.024$), and between high-neutrophil–lymphocyte ratio and short overall ($P = 0.011$) and progression-free survival ($P = 0.040$), whereas no association was found between lymphocyte or monocyte count and prognosis. The relationship between neutrophil count and both overall and progression-free survival was linear, whereas the relationship between neutrophil–lymphocyte ratio and overall survival was to some degree non-linear.

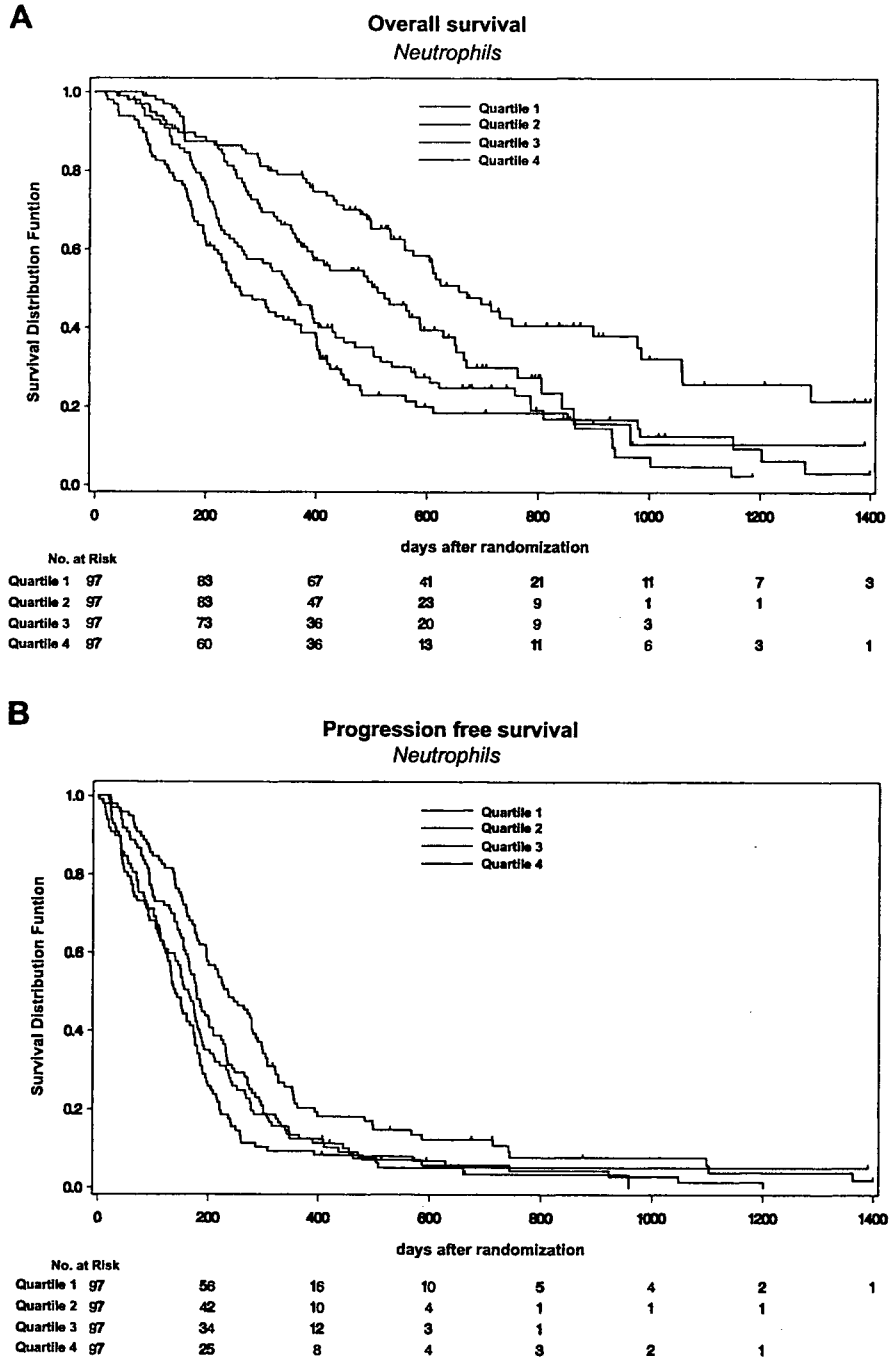

Fig. 1 – Kaplan–Meier estimates according to quartiles for the effect of pretreatment neutrophil count on (A) overall survival and (B) progression-free survival.

3.3. *Optimal cut-off value for pretreatment neutrophil count*

In selecting optimal cut-off values for the effect of neutrophil count on overall survival, the range between the 5th percentile (2205 mm^{-3}) and the 95th percentile (9657 mm^{-3}) for distribution of neutrophils was selected, and the possible cut-off points at intervals of 500 mm^{-3} from 2500 mm^{-3} to 9500 mm^{-3} were considered (giving 15 candidate cut-off points). Using the minimum P-value approach, the selected cut-off value for neutrophil count was 4500 mm^{-3} (corrected $P = 0.0009$) and the corrected shrunk hazard ratio was 1.67 (95%CI, 1.09–2.54, from 100 bootstrap samples; Table 3). The selected optimal cut-off value did not change even when we used the stratified proportional hazards model, stratified by the combination of all covariates. The median survival time was 19.3 months (95%CI, 16.5–21.4) for the low-neutrophil group (<4500 mm^{-3}, $n = 204$) and was 10.2 months (95%CI, 8.0–12.3) for the high-neutrophil group (≥4500 mm^{-3}, $n = 184$) (Fig. 2). The results of prognostic factor analysis for overall survival are shown in Table 4. In terms of the relative order of significance, neutrophil count was one of the most important

Table 2 – Multivariate Cox regression analysis for neutrophil, lymphocyte and monocyte counts.

Factors	Overall survival				Progression-free survival			
	Hazard ratio[a]	95%CI	P	P[b]	Hazard ratio[a]	95%CI	P	P[b]
Neutrophil count (mm^{-3})								
Quartile 1 (<3278)	1	–	–	0.0008	1	–	–	0.024
Quartile 2 (<4304)	1.25	0.86–1.82	0.251		1.19	0.88–1.61	0.258	
Quartile 3 (<5873)	1.76	1.22–2.53	0.002		1.32	0.97–1.78	0.076	
Quartile 4 (≥5873)	1.94	1.35–2.79	0.0003		1.61	1.18–2.19	0.003	
Lymphocyte count (mm^{-3})								
Quartile 1 (<1082.3)	1	–	–	0.251	1	–	–	0.545
Quartile 2 (<1386.1)	1.14	0.81–1.61	0.438		1.10	0.82–1.47	0.535	
Quartile 3 (<1821.8)	0.83	0.58–1.19	0.303		0.88	0.65–1.20	0.424	
Quartile 4 (≥1821.8)	1.13	0.80–1.59	0.495		0.95	0.70–1.28	0.732	
Neutrophil–lymphocyte ratio								
Quartile 1 (<2.093)	1	–	–	0.011	1	–	–	0.040
Quartile 2 (<2.914)	1.42	0.98–2.05	0.065		1.39	1.02–1.88	0.035	
Quartile 3 (<4.744)	1.83	1.27–2.62	0.001		1.50	1.09–2.06	0.012	
Quartile 4 (≥4.744)	1.56	1.09–2.24	0.015		1.48	1.09–2.02	0.013	
Monocyte count (mm^{-3})								
Quartile 1 (<289.9)	1	–	–	0.381	1	–	–	0.969
Quartile 2 (<402.3)	0.93	0.65–1.32	0.674		1.05	0.78–1.41	0.755	
Quartile 3 (<550.4)	1.07	0.75–1.52	0.712		0.99	0.72–1.35	0.924	
Quartile 4 (≥550.4)	1.26	0.89–1.78	0.203		1.04	0.76–1.42	0.792	

CI: confidence interval.
a Adjustment for sex, smoking, stage, ECOG PS, weight loss, LDH, bone metastases, liver metastases and skin metastases.
b P-values for global association.

Table 3 – Cutpoint analysis for neutrophil count and overall survival.

Neutrophil count (cut-off points, mm^{-3})	Uncorrected hazard ratio[a]	Uncorrected P-value
2500	1.95	0.016
3000	1.78	0.001
3500	1.40	0.021
4000	1.57	0.0007
4500	1.72[b]	<0.0001[c]
5000	1.49	0.002
5500	1.51	0.002
6000	1.46	0.008
6500	1.75	0.0004
7000	1.62	0.005
7500	1.59	0.015
8000	1.88	0.004
8500	1.86	0.007
9000	1.78	0.017
9500	1.89	0.009

a (Hazard of death in patients on or above the cut-off point) divided by (hazard of death in patients below the cut-off point), after adjustment for sex, smoking, stage, ECOG PS, weight loss, LDH, bone metastases, liver metastases and skin metastases.
b Corrected hazard ratio: 1.67 (95%CI, 1.09–2.54).
c Corrected P = 0.0009.

prognostic factors along with ECOG PS ($P < 0.0001$), LDH ($P = 0.001$) and smoking history ($P = 0.002$). The adjusted hazard ratios for the relationship between neutrophil count (<4500 mm^{-3} versus ≥4500 mm^{-3}) and survival according to the treatment groups were 1.62 (95%CI, 1.14–2.30) in the PC arm ($n = 195$) and 1.74 (95%CI, 1.22–2.48) in the VGD arm ($n = 193$). There was no interaction between the neutrophil count and the treatment arms (P for interaction = 0.437).

3.4. *Relationship between pretreatment neutrophil count and intensity of chemotherapy*

In order to evaluate the effect of neutrophil count on administration of chemotherapy and toxicity, we analysed the dose intensity of chemotherapeutic agents and the incidence of toxicity in each arm. In the VGD arm, there was no significant difference in the relative dose intensity of vinorelbine or

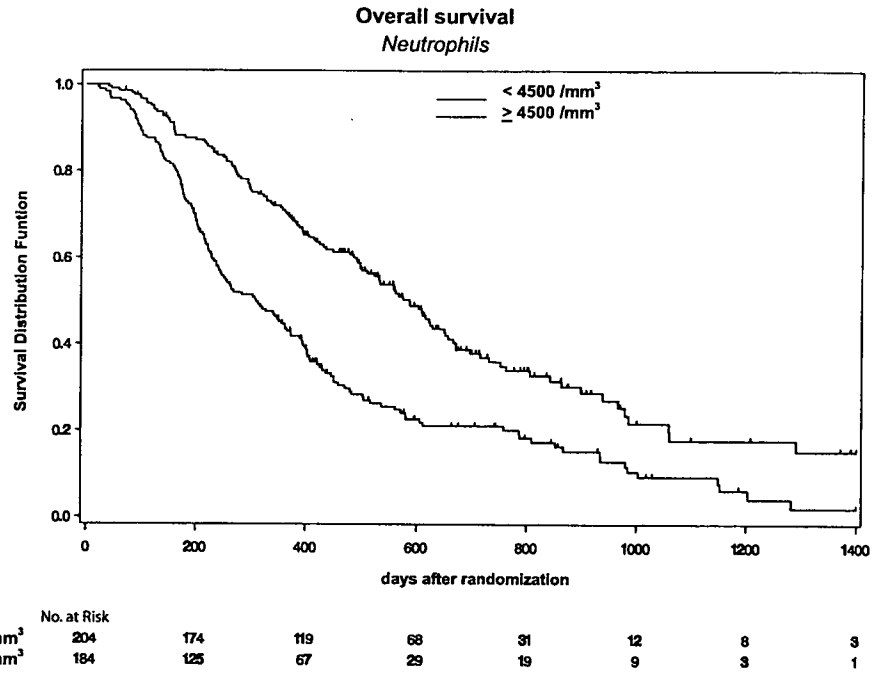

Fig. 2 – Kaplan–Meier estimates according to optimal cut-off point (4500 mm^{-3}) for the effect of pretreatment neutrophil count on overall survival.

gemcitabine between the low-neutrophil group (<4500 mm^{-3}) and the high-neutrophil group (≥4500 mm^{-3}). However, the relative dose intensity of docetaxel was significantly lower in the high-neutrophil group (median, 33%) than in the low-neutrophil group (median, 87%) ($P = 0.040$, Wilcoxon test).

The toxicity due to treatment was also analysed. In the VGD arm, the incidence of grade 3 or 4 non-haematological toxicity within the first three cycles of treatment was significantly higher in the high-neutrophil group than in the low-neutrophil group (26.5% versus 8.5%; $P = 0.002$, Fisher's exact test). Significantly fewer cycles were administered in the high-neutrophil group than in the low-neutrophil group (mean, 2.9 cycles versus 4.7 cycles; $P < 0.0001$, Wilcoxon test). None of the patients in the high-neutrophil group who experienced grade 3 or 4 non-haematological toxicity within the first three cycles completed the planned six cycles. The proportion of patients requiring reductions in the doses of vinorelbine or gemcitabine within the first two cycles of treatment was significantly higher in the low-neutrophil group (45.2%) than in the high-neutrophil group (26.4%) ($P = 0.007$, Fisher's exact test). No such differences in dose intensity or toxicity were seen in the PC arm.

4. Discussion

In multivariate analysis after adjustment for known prognostic factors, we found linear associations between pretreatment elevated neutrophil count and short overall and progression-free survival. As there was no such association for the lymphocyte count, the relationship between neutrophil-lymphocyte ratio and overall survival was also found, however, it was to some degree weak and non-linear. As a consequence, we consider that absolute neutrophil count may better serve as a prognostic factor. An optimal cut-off value for the relationship between neutrophil count and overall survival was identified as 4500 mm^{-3} (corrected hazard ratio, 1.67; 95%CI, 1.09–2.54). In the VGD arm, the low-neutrophil group (<4500 mm^{-3}) tended to have a lower incidence of severe non-haematological toxicity and tolerated longer administration of the chemotherapeutic agents compared with the high-neutrophil group. However, no such association was found in the PC arm, and pretreatment neutrophil count was equally predictive of prognosis in both treatment arms when analysed separately. We therefore do not consider it likely that the pretreatment neutrophil count serves as an indicator of intolerance to chemotherapy, rather than as an indicator of poor prognosis.

A number of studies in the last two decades have suggested an association between the neutrophil count or neutrophil–lymphocyte ratio and the prognosis of cancer patients,[7–16] although no acceptable explanations for the mechanisms underlying these observed associations have been proposed. Moreover, although neutrophilia often accompanies the diagnosis of cancer, the causes of neutrophilia in cancer patients are not fully understood, and are likely to be the result of a combination of factors. One obvious cause of neutrophilia is paraneoplastic production of myeloid growth factors by cancer cells themselves. Granulocyte-colony stimulating factor (G-CSF) is a growth factor that acts selectively on bone marrow granulocytic lineage cells, and is considered to play a central role in granulopoiesis. Administration of G-CSF was reported to increase bone marrow neutrophil precursors and shorten bone marrow transit time in mice and humans,[20–22] resulting in marked increases in the production of neutrophils. Granulocyte macrophage-colony stimulating factor (GM-CSF) and macrophage-colony stimulating factor