Intraoperative pleural lavage cytology is an independent prognostic indicator for staging non–small cell lung cancer

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Objectives: For patients undergoing lung resection for cancer, macroscopic evidence of metastasis is clearly associated with adverse prognosis. However, less is known about the significance of tumor cells detected by using tests such as pleural lavage cytology. To ascertain the frequency and quantify the effect of this finding on survival, we performed a prospective study of intraoperative pleural lavage cytology.

Methods: Pleural lavage cytology consisted of cytologic analysis of 100 mL of saline irrigated over the lung surface immediately after thoracotomy. Patients were excluded if they had an existing effusion, extreme adhesions, or lateral chest wall invasion or if resection was not performed. Survival was calculated by means of Kaplan-Meier analysis and compared by using log-rank tests. Cox regression was used to ascertain independent predictors of prognosis.

Results: From 1995 through 2003, we performed pleural lavage cytology on 292 patients undergoing thoracotomy for lung cancer. The mean age was 64 (SD, 10) years, and 196 (67%) patients were men. Of 292 samples, 13 (4.5%) showed evidence of malignant cells. The median time to follow-up was 15 months (interquartile range, 1-40 months), with a median survival of 49 months for patients with negative pleural lavage cytology results and 13 months for patients with positive pleural lavage cytology results ($P = .002$). Univariate prognostic predictors were positive pleural lavage cytology status ($P = .03$), stage ($P = .03$), adenocarcinoma ($P = .06$), and parietal pleural involvement ($P = .01$). In the final multivariate model only positive pleural lavage cytology status ($P = .006$) and stage ($P = .03$) remained significant.

Conclusions: Intraoperative pleural lavage cytology is a simple addition to intrathoracic staging and an independent predictor of prognosis. Positive results potentially affect survival by upstaging patients to stage IIIB or greater.

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Accurate and reproducible staging is the fundamental basis for patient management, evaluation of research, and communication in the treatment of lung cancer.[1] Investigating for local, lymphatic, and hematogenous invasion is part of the routine evaluation before lung resection. Although these staging methods tend to concentrate on macroscopic evidence of disseminated disease, advances in cytochemistry, immunohistochemistry, and polymerase chain reaction techniques facilitate detection of micrometastatic disease.[2] In patients without evidence of metastasis, isolated tumor cells have been detected in the bone marrow and lymph nodes, even though conventional stains have failed to detect such disease.[3,4]

Because these findings are becoming increasingly clinically relevant, thoracic surgeons are now evaluating the significance of tumor cells detected on washings of the pleural cavity. The contribution to staging and the utility to influence subsequent patient management are not clearly defined.

In an attempt to ascertain the frequency of tumor cells on pleural washings and to characterize the effect on prognosis, we performed a prospective study of pleural lavage cytology (PLC) in patients who had thoracotomy and lung resection for non–small cell lung cancer (NSCLC) at our institution.

Methods

This study was conducted from 1995 through 2003, including all patients with known or suspected NSCLC who had a thoracotomy for intended surgical resection. Patients with a confirmed diagnosis of NSCLC formed the population for this study, and we did not include any patients with benign disease. We excluded patients who had an existing effusion at the time of the operation (defined as visible evidence of fluid in the pleural cavity), extreme adhesions, or chest wall invasion or in whom resection was not performed.

Preparation of the Sample

Immediately after thoracotomy but before any manipulation of the tumor, 100 mL of normal saline was instilled into the pleural space through a wide-bore catheter. The fluid was irrigated over the visceral and parietal pleura, but forceful irrigation over the tumor was avoided. The sample was then aspirated and placed in a sterile container with 1 mL of 20% sodium citrate. The sample was mixed by means of repeated inversion. Isolation of cells from erythrocytes was achieved by using a centrifugal sedimentation system. Two 16-mL centrifuged tubes were prepared by layering 12 mL of sample over 3 mL of Lymphoprep medium (Nycomed Pharma AS) and centrifuging at 2000 rpm for 20 minutes. Cells were harvested from the sample-medium interface with a Pasteur pipette and resuspended in 2 mL of supernatant. Four cytologic slides of this fraction were prepared by using a Cyto-Tek centrifuge (Bayer Diagnostics), subsequently fixed in formal acetic alcohol, and stained with the Papanicolaou technique. The slides were then screened by a pathologist (AGN) for the presence of malignant cells.

Data Acquisition

Individual patient data were collated from a prospective histopathology database. Survival status was determined from the date of last follow-up in a hospital outpatient or general practitioner's clinic. Mortality status was documented from patient records and the NHS strategic tracing service.

Statistical Analysis

Patients were grouped according to the results of intraoperative PLC. Categoric data are presented as frequency (in percentages) and continuous data as means with SDs or medians with interquartile ranges. Comparisons of categoric data between the 2 groups were made by using χ^2 or Fisher exact tests. Continuous data were compared by using 2-tailed t tests or Mann-Whitney tests as appropriate to the distribution of the data.

Actuarial survival was estimated by using the Kaplan-Meier method and compared with the log-rank test. Cox proportional hazards regression was used to ascertain the individual contribution of factors associated with survival and to compare the risk-adjusted survival between the 2 groups. The criterion for variable retention was a P value of less than .1.

Results

From January 1, 1995, to January 1, 2003, a total of 292 PLC samples were received from patients who fulfilled the inclusion criteria and underwent surgical resection for NSCLC.

The mean age of the cohort was 64 (SD, 10) years, and 196 (67%) of the patients were men. Of 292 samples, 13 (4.5%) had evidence of malignant cells. Patients with positive PLC results were well matched for age and sex. However, more underwent pneumonectomy and had a higher stage compared with patients who had negative PLC results. In our series all patients with stage IV disease had M1 status by virtue of a second tumor in a different lobe. The baseline characteristics and stage are summarized in Tables 1 and 2, respectively.

The median time to follow-up was 15 months (interquartile range, 1-40 months), with a median survival of 49 (SE, 2.81) months for patients with negative PLC results and 13 (SE, 8.9) months for patients with positive PLC results ($P = .002$, Figure 1).

The univariate prognostic predictors were positive PLC status ($P = .03$), stage ($P = .03$), adenocarcinoma ($P = .06$), and parietal pleural involvement ($P = .01$), as summarized in Table 3. During statistical modeling, PLC status ($P = .002$) as a prognostic predictor was independent of N stage ($P < .001$) and T stage ($P = .9$). In the final multivariate model, only positive PLC status ($P = .006$) and overall stage ($P = .03$) were retained as independent prognostic predictors (Table 4 and Figure 2).

A subanalysis of the 12 patients with positive PLC results with stage IB to IIIB disease (after excluding one patient with stage IV disease) compared with the 17 patients with stage IIIB disease in the group with negative PLC results suggested poorer survival when PLC results were positive ($P = .06$, Figure 3).

Discussion

How common is intracoelomic disease in patients who undergo lung resection without evidence of pleural effusion? In our series the proportion was relatively low (4.5%). However, reports in the literature range between 9% and 38%. The proportion of positive lavage results varies not only with the patient population but also with the technique of acquiring the sample. The volume of lavage fluid used to

TABLE 1. Baseline characteristics, operation extent, and cell type

	PLC negative	PLC positive	*P* value
No.	279	13	
Age, y (SD)	64 (11)	63 (7)	.84
Male, n (%)	188 (67)	8 (62)	.66
Operation			
Pneumonectomy, n (%)	57 (20)	5 (38)	
Lobectomy, n (%)	193 (69)	5 (38)	
Bilobectomy, n (%)	15 (5)	1 (8)	.03
Segmentectomy, n (%)	7 (2)	0 (0)	
Nonanatomic resection, n (%)	7 (3)	2 (15)	
Histology			
Squamous, n (%)	114 (41)	2 (15)	
Adenocarcinoma, n (%)	118 (42)	10 (77)	
Large cell, n (%)	23 (8)	0 (0)	.12
Mixed, n (%)	14 (5)	1 (8)	
Other, n (%)	10 (4)	0 (0)	
Visceral pleura involvement, n (%)	87 (31)	7 (54)	.08
Parietal pleura involvement, n (%)	6	1	.21

acquire a sample ranges from 50 to 1500 mL, and different carrier solutions, such as saline and Ringer's lactate have been used.[3,5-9] Generally, most samples have been acquired immediately after thoracotomy and before surgical intervention.[3,6,8] However, some have performed PLC after surgical intervention,[10] and others have performed PLC both before and after lung resection.[5,7] Despite the inconsistencies in volume, carrier solutions, timing of collection, and processing, one finding has been consistent: a uniformly poor prognosis in patients with positive results.

Role for PLC in Staging

Although some studies have identified positive PLC results as an independent predictor of prognosis,[6] not all studies that report a poorer survival were able to confirm whether this was independent of stage.[3] This problem stems from small numbers of patients with positive PLC results and the variable effect on survival in each series. Our study supports positive PLC results as a prognostic indicator that is independent of overall stage and, in particular, nodal status. Where and how PLC could be implemented into conventional lung cancer staging is still unclear. It is recommended that the cytologic results of pleural and peritoneal washings be considered separate to the classification of isolated tumor cells and micrometastasis. In addition, identification of patients with positive PLC results requires the suffix of (cy+) if this is to be the basis of classification of this subset to T4 disease (ie, T4 [cy+]).[11]

In our series patients with positive PLC results had equivalent or possibly poorer survival to that of patients with stage IIIB disease ($P = .06$). This is despite having 83% (10/12) of such patients with stage IB to IIIA disease. Clearly, positive PLC results have the effect of upstaging patients to at least T4 status, a finding with prognostic implications similar to those of a malignant effusion. Although we did not have a group of patients with stage IV disease to ascertain whether survival was comparable, the median survival of 13 months is similar to that of patients with stage IV disease reported by Naruke and colleagues.[12]

TABLE 2. Staging characteristics

	PLC negative	PLC positive	*P* value
No.	279	13	
Stage			
IA, n (%)	51 (18)	0 (0)	
IB, n (%)	108 (39)	3 (23)	
IIA, n (%)	12 (4)	1 (8)	
IIB, n (%)	53 (19)	1 (8)	.01
IIIA, n (%)	35 (13)	5 (38)	
IIIB, n (%)	17 (6)	2 (15)	
IV, n (%)	3 (1)	1 (8)	
T category			
T1, n (%)	74 (27)	3 (23)	
T2, n (%)	181 (65)	8 (62)	
T3, n (%)	8 (3)	0 (0)	.51
T4, n (%)	16 (6)	2 (15)	
N category			
N0, n (%)	197 (71)	8 (62)	
N1, n (%)	47 (17)	1 (8)	.15
N2, n (%)	35 (13)	4 (31)	

A Unique Problem

Currently, patients with stage IIIB and IV disease are generally (with few exceptions) considered to have inoperable disease,[13] and surgeons can justifiably choose not to proceed to lung resection when faced with unexpected stage IIIB or IV disease in the operating theater. However, patients with positive PLC results belong to a small subgroup

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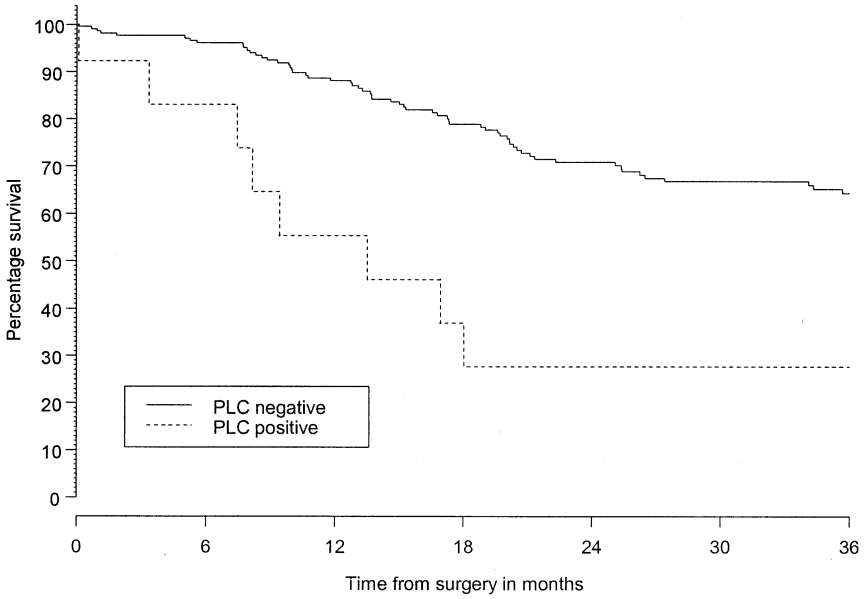

Figure 1. Overall survival by PLC status.

TABLE 3. Univariate predictors of survival

Variable	Odds ratio	*P* value
PLC	2.45	.03
Stage		(.03)*
I	1.00	
II	1.16	.29
III	1.23	.04
IV	1.01	.95
Age (per additional yr)	1.02	.12
Female sex	0.87	.28
Extent of resection		
Pneumonectomy	1.00	
Lobectomy	0.82	.17
Bilobectomy	0.91	.58
Segmentectomy	1.13	.63
Nonanatomic resection	0.86	.47
Histology		
Squamous	1.00	
Adenocarcinoma	1.31	.06
Large cell	1.18	.19
Mixed	0.99	.92
Other	0.35	.56
Maximum size (per additional mm)	0.99	.52
Visceral pleura involvement	0.88	.62
Parietal pleura involvement	10.33	.01

*The overall contribution of stage was assessed by using the likelihood method.

TABLE 4. Multivariate predictors of survival

Variable	Odds ratio	*P* value
Positive PLC results	2.52	.006
Stage		(.03)*
I	1.00	
II	1.17	.21
III	1.23	.01
IV	1.07	.80

*The overall contribution of stage was assessed by using the likelihood method.

with disease that is not detectable by means of conventional staging methods and who might have surgically resectable disease with a pathologic stage as early as IB (in our series). Because we are unlikely to develop an infrastructure that is quick enough to provide us the necessary information intraoperatively, the results of PLC are not available until after the operation, compounding the problem of the management of these patients. Even if we did use preoperative video-assisted thoracoscopy to obtain lavage fluid for PLC, the cost and potential morbidity for a detection rate of 4.5% would far outweigh the clinical utility of this investigation, especially when we do not as yet have a clear management plan for patients with positive results. With our present knowledge, it would not be appropriate to turn down patients with potentially resectable disease in the absence of further confirmatory studies.

Clinical Implications

Clearly the challenge is to define the optimal management strategy for this small but important subset of patients. A recent study of this subgroup by Ichinose and associates[14] suggested reduced carcinomatous pleuritis in patients with positive PLC results who had hypotonic cisplatin infused into the pleural space after lung resection. However, the study terminated prematurely before the effects on recur-

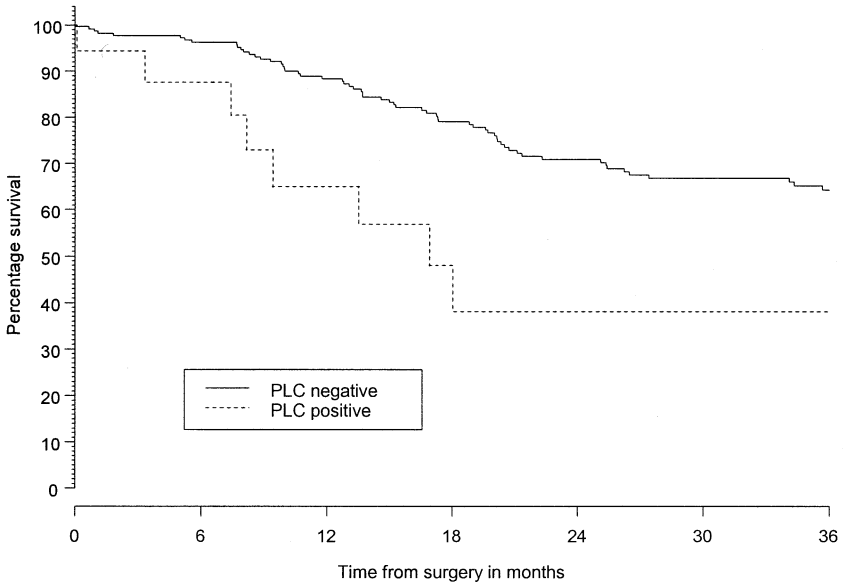

Figure 2. Survival by PLC status adjusted for stage.

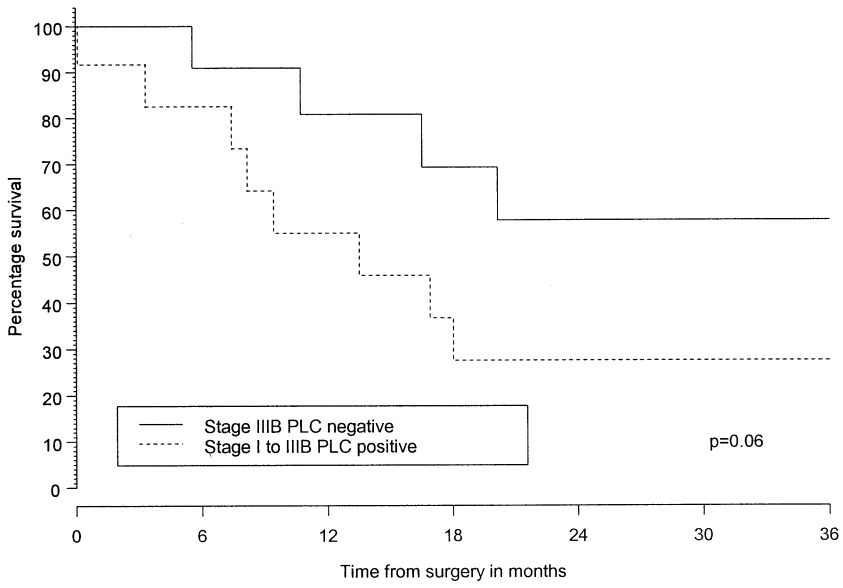

Figure 3. Survival comparing patients with stage IIIB disease with all patients with positive PLC results.

rence or mortality could be assessed. Realistically, until we can accurately and consistently identify patients in this subgroup, it would be difficult to evaluate the benefits of any adjuvant therapy. Moreover, this is based on the assumption that positive PLC results are an indicator of pleural extravasation of the primary tumor. It is equally conceivable that positive PLC results occur as a result of increasing metastatic potential and widespread microscopic disease detected on pleural washings.

Potential Limitations

Like many other studies on this topic, the numbers of patients with positive PLC results were small, limiting the ability to make robust conclusions. However, our data are clearly consistent with those of other reports as an adverse prognostic indicator. Because of sample size constraints, we were unable to ascertain with certainty whether visceral pleural involvement was associated with positive PLC results, and we did not have information on disease recurrence.

Future Direction

PLC is a relatively inexpensive (approximate cost is £40, US $65.45) and simple technique that identifies an important subset of surgical candidates with poor prognosis. However, standardization of sample acquisition and preparation is essential for widespread adoption and interpretation of future studies. Although numerous studies have been published on this topic, further studies or individual patient data meta-analyses of published trials are required to facilitate the accurate redefinition of a stage to this subgroup of patients.

Conclusions

Intraoperative PLC is a simple and inexpensive technique that identifies surgical patients with micrometastasis otherwise undetectable by means of conventional staging methods. The information provided by PLC conveys important additional prognostic information and potentially affects survival by upstaging patients to stage IIIB or greater. Standardization of sample acquisition and further studies are required to define the exact stage of patients with positive PLC results before it becomes widely adopted for staging. Further information on relapse patterns is needed before the prognostic information obtained can be used to assess the effect of adjuvant therapy.

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