Detection of Occult Micrometastases in Patients With Clinical Stage I Non–Small-Cell Lung Cancer: A Prospective Analysis of Mature Results of CALGB 9761 (Alliance)

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ABSTRACT

Purpose

Outcomes after resection of stage I non–small-cell lung cancer (NSCLC) are variable, potentially due to undetected occult micrometastases (OM). Cancer and Leukemia Group B 9761 was a prospectively designed study aimed at determining the prognostic significance of OM.

Materials and Methods

Between 1997 and 2002, 502 patients with suspected clinical stage I (T1-2N0M0) NSCLC were prospectively enrolled at 11 institutions. Primary tumor and lymph nodes (LNs) were collected and sent to a central site for molecular analysis. Both were assayed for OM using immunohistochemistry (IHC) for cytokeratin (AE1/AE3) and real-time reverse transcriptase polymerase chain reaction (RT-PCR) for carcinoembryonic antigen.

Results

Four hundred eighty-nine of the 502 enrolled patients underwent complete surgical staging. Three hundred four patients (61%) had pathologic stage I NSCLC (T1, 58%; T2, 42%) and were included in the final analysis. Fifty-six percent had adenocarcinomas, 34% had squamous cell carcinomas, and 10% had another histology. LNs from 298 patients were analyzed by IHC; 41 (14%) were IHC-positive (42% in N1 position, 58% in N2 position). Neither overall survival (OS) nor disease-free survival was associated with IHC positivity; however, patients who had IHC-positive N2 LNs had statistically significantly worse survival rates (hazard ratio, 2.04, $P = .017$). LNs from 256 patients were analyzed by RT-PCR; 176 (69%) were PCR-positive (52% in N1 position, 48% in N2 position). Neither OS nor disease-free survival was associated with PCR positivity.

Conclusion

NSCLC tumor markers can be detected in histologically negative LNs by AE1/AE3 IHC and carcinoembryonic antigen RT-PCR. In this prospective, multi-institutional trial, the presence of OM by IHC staining in N2 LNs of patients with NSCLC correlated with decreased OS. The clinical significance of this warrants further investigation.

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INTRODUCTION

Unlike many other malignancies, complete surgical resection of early-stage non–small-cell lung cancer (NSCLC) yields unpredictable patient outcomes. Stage I NSCLC 5-year survival rates range from 40% to 90%.¹⁻³ Locoregional and distant recurrences develop in 15% to 20% of patients with stage I disease within 5 years of resection.^{4,5} One possible explanation for this wide variation is occult metastases (OM) in regional nodes. Current surgical pathology

techniques may misclassify patients with stage II or III disease as having stage I; more detailed lymph node (LN) assessment theoretically may better categorize patients undergoing resection. Improved staging accuracy should result in better prognostication and allow for identification of patients whose cancer is likely to recur. Such high-risk patients may then be offered adjuvant interventions to decrease the likelihood of relapse.

Standard practice for hilar, mediastinal, and lobar nodes removed during pulmonary resection is to cut the nodes serially into 3- to 4-mm thick

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slices. These slices are then embedded in paraffin, sectioned by microtome, stained with hematoxylin and eosin (H&E), and examined for malignant cells with light microscopy. With this technique, there remains a possibility of missing a small focus of metastatic cancer because of sampling error or camouflage by normal cells. Whether these microscopic OM are clinically relevant is controversial.

Since the 1980s, investigators have used cytokeratin staining to enhance detection of epithelial cells to detect OM. In breast cancer, immunohistochemistry (IHC)-detected regional node involvement predicts recurrence with high reliability.⁶ In NSCLC, the American College of Surgeons Oncology Group Z0040 trial evaluated IHC for Cam5.2 and AE1 in bone marrow (BM), pleural lavage, and LNs and found a significant difference in disease-free survival (DFS) and overall survival (OS) with OM in LNs.⁷

Reverse transcriptase polymerase chain reaction (RT-PCR) for tumor-specific mRNA has the potential to be highly sensitive for just a few tumor cells within a histologically negative node. Quantitative RT-PCR can estimate the tumor burden in tissue, as opposed to qualitative RT-PCR, which gives a simple yes or no answer. Carcinoembryonic antigen (CEA) is a glycoprotein involved in cell-to-cell adhesion that has been expressed in breast and GI malignancies^{8,9} and has also been detected in peripheral blood of patients with NSCLC.^{10,11} This marker was chosen for quantitative RT-PCR in LNs and BM in the Cancer and Leukemia Group B (CALGB) 9761 trial as an exploratory analysis to see if it is useful in identifying and quantifying metastases in patients with NSCLC.

Herein we present the results of CALGB 9761 as follow-up to the preliminary reports from the trial.^{12,13} (CALGB is now part of the Alliance for Clinical Trials in Oncology.) The study was designed to test the hypothesis that the presence of OM in stage I NSCLC would negatively affect both time to recurrence and survival after surgical resection. If this hypothesis proves correct, it might change the standard of care for pathologic evaluation of nodes in patients with lung cancer, and would affect treatment algorithms for early-stage NSCLC.

MATERIALS AND METHODS

Objectives

The stated primary objective of CALGB 9761 was to determine if detection of OM by IHC or RT-PCR in histologically negative LNs or BM was associated with poorer survival among patients with stage I NSCLC. Secondary objectives were as follows: to evaluate the incidence of OM in LNs and BM; to assess the sensitivity of IHC compared with RT-PCR; to look at the relationships among tumor size, stage, and OM by IHC or RT-PCR; to evaluate the relationship between OM and DFS; and to see how the site of OM affects the incidence and site of recurrence as well as survival.

Study Design

The study schema is shown in Figure 1. The inclusion criteria were clinical stage I known or suspected NSCLC, T1 (0 to 3 cm) or T2 (> 3 cm, endobronchial, or with visceral pleural invasion) according to the American Joint Commission on Cancer (AJCC) 6th edition staging manual. All patients were evaluated by history and physical examination, performance status (PS), chest x-ray, and chest computed tomography (CT) before enrollment. Patients were required to be clinically N0, ie, nodes < 1 cm on chest CT scan, or negative by mediastinoscopy. Positron emission tomography (PET)/CT was not used in this study. Patients were required to be surgical candidates for lobectomy or pneumonectomy by thoracotomy or thoracoscopic technique, older than 18 years, with no prior history of lung cancer or other malignancy with the exception of nonmelanoma skin cancer, cervical carcinoma in situ, or cancers other than lung if disease free for ≥ 5 years. Neoadjuvant therapy was not used in any patient. This protocol was approved by all individual participating site institutional review boards and all patients gave written informed consent. Patients underwent surgical resection, by lobectomy in nearly every case, and mediastinal lymphadenectomy (details of tissue acquisition are outlined in section Tissue Collection and Processing). Follow-up occurred every 6 months until disease recurrence or death by any cause for 5 years, and consisted of history and physical examination; routine follow-up imaging was not required or tracked for this protocol and was performed at the discretion of the surgeon.

Fig 1. Study schema for Cancer and Leukemia Group B (CALGB) 9761. Note requirement for sampling at least five nodal stations on the right side, six on the left side. CEA, carcinoembryonic antigen; IHC, immunohistochemistry; NSCLC, non–small-cell lung cancer; RT-PCR, reverse transcriptase polymerase chain reaction.

Tissue Collection and Processing

Eleven centers participated in the protocol on the basis of surgical volumes and CALGB membership status. BM aspiration was performed at the beginning of the operation, by harvesting 5 to 10 mL from the iliac crest into a sterile syringe, placing half in an EDTA tube, and snap-freezing the other half. All samples were shipped to a central laboratory (R.A.K. and M.A.M.). LN sampling was performed before lung resection: levels 4, 7, 9, 10, and 11 on the right side, and levels 5, 6, 7, 9, 10, and 11 on the left side. Nodes were processed as outlined in Figure 1. Nodes were bisected, with one half sent for routine histology and the other half retrieved and snap-frozen in liquid nitrogen and analyzed at a central laboratory (R.A.K. laboratory at University of Minnesota, for PCR analysis; IHC was performed in a Clinical Laboratory Improvement Amendments–certified laboratory at University of Minnesota). To avoid nodal contamination by the primary tumor, nodes were harvested before lung mobilization or dissection occurred, and separate instruments were used for each node to avoid cross-contamination. After processing of the lung resection specimen by the institutional pathologist, additional samples of station 11 LNs were harvested and 1.0 cm³ from the primary tumor was removed by the pathologist; both were snap-frozen. Tissue from patients who proved to have benign disease was kept as a negative control for both IHC and RT-PCR.

RT-PCR and IHC Techniques

IHC was performed with a polyclonal anticytokeratin antibody cocktail on all LNs.¹⁴ Primary tumors were examined to confirm the diagnosis of NSCLC and to assay for AE1/AE3 expression (100% were positive). Each LN sample was handled in a uniform fashion so that H&E stains were matched to IHC slides. Specifically, three sections were cut from each block of tissue: the first for routine H&E staining; the second for cytokeratin IHC staining; and the third as a control for IHC. For IHC, we used Dako prediluted AE1/AE3 product N1590 (Dako, Carpinteria, CA), a 20-minute pretreatment with protease 760-2018 (Ventana Medical Systems, Tucson, AZ), and 3,3'-diaminobenzidine detection kit 760-001 (Ventana Medical Systems). Slides were reviewed by two independent pathologists (R.T.V. and N.A.), according to the methods described previously.¹⁴

Fig 2. CONSORT diagram: Patient enrollment and exclusion. NSCLC, non-small-cell lung cancer.

Fig 3. (A) Hematoxylin and eosin stain of a lymph node found positive by immunohistochemistry (IHC) stain for cytokeratin. No tumor is apparent. Original magnification, $\times 100$. (B) IHC stain for cytokeratin of the same node, different section. A small focus of tumor stained brown (arrow). Original magnification, $\times 100$. (C) Higher magnification of tumor focus shown in Fig 3B. Original magnification, $\times 200$. An IHC-positive focus of tumor in this node (arrow) had a maximum diameter of approximately 40 microns. Courtesy of Robin T. Vollmer, MD.

Table 1. Demographic and Clinical Characteristics for Patients by Immunohistochemistry Status of Lymph Nodes

Characteristic	IHC-Positive (n = 41)	IHC-Negative (n = 257)	Total (N = 298)	χ^2 P
Age in years, median (range)	67 (33-79)	68 (37-86)	68 (33-86)	.43
Sex				
Male	22 (54)	138 (54)	160 (54)	1.0
Female	19 (46)	119 (46)	138 (46)	
Race				
White	39 (95)	232 (90)	271 (91)	.32 (white v nonwhite)
Black	1 (2)	23 (9)	24 (8)	
Other	1 (2)	2 (1)	3 (1)	
PS				
0	27 (66)	184 (72)	211 (71)	.68 (PS = 0 v PS = 1, 2)
1	12 (29)	65 (25)	77 (26)	
2	0 (0)	5 (2)	5 (2)	
Data missing	2 (5)	3 (1)	5 (2)	
Weight loss in previous 6 months				
≤ 5% or no loss	39 (95)	224 (87)	263 (88)	.29 (≤ 5% v > 5%)
> 5%	2 (5)	25 (10)	27 (9)	
Data missing	0 (0)	8 (3)	8 (3)	
Histology				
Adenocarcinoma	18 (44)	150 (58)	168 (56)	.22 (adenocarcinoma v squamous)
Squamous	16 (39)	85 (33)	101 (34)	
Others	7 (17)	22 (9)	29 (10)	
T category				
T1	26 (63)	146 (57)	172 (58)	.43
T2	15 (37)	111 (43)	126 (42)	
IHC-detected N category				
N1	17 (42)	NA	NA	
N2	24 (58)	NA	NA	
Adjuvant CT or RT				
Yes	9 (22)	48 (19)	57 (19)	.62
No	32 (78)	209 (81)	241 (81)	

NOTE. Data are presented as No. (%) unless otherwise indicated.

Abbreviations: CT, chemotherapy; IHC, immunohistochemistry; NA, not applicable; PS, performance status; RT, radiotherapy.

RT-PCR for the presence of CEA mRNA was performed as previously described.^{13,15} The cut point chosen for positive versus negative was threshold PCR cycle ≤ 45 defined as positive and > 45 as negative. Results were verified at intervals of approximately every 100 samples by random sampling of 10% of the samples for repeat RT-PCR analysis. Primary tumors were also tested for CEA expression, which was noted in approximately 90%.

Data Quality Control and Statistical Analysis

We estimated that IHC or RT-PCR markers would be expressed in LNs of approximately half the patients. The study was designed to have 80% power to detect a reduction in 3-year survival from 70% for patients without the IHC marker to 50% for patients with the marker (hazard ratio [HR], 1.94), assuming an equal percentage of patients with IHC-positive and IHC-negative results and using a log-rank test conducted at the two-sided significance level of .01. Including a Bonferroni correction for type I error calculation, accrual of 500 patients was planned, anticipating exclusion of 40% to 50% due to benign disease, small-cell cancer, or more advanced disease. The final target was 225 patients.

The results for OM based on cytokeratin stains and RT-PCR were compared by Fisher's exact test.¹⁶ Logistic regression was used to evaluate the independent variable tumor size (diameter) and presence of OM in LNs as the dependent variable.¹⁷ Spline functions were used to explore a nonlinear relationship between tumor size and OM incidence (data not shown).¹⁸ Kaplan-Meier curves were used to display OS (from surgical resection to death) and DFS (from surgery to recurrence or death from any cause, whichever came first).¹⁹

The Cox proportional hazards model²⁰ was used to examine the relationship between OM and OS, adjusting for age (continuous variable),

PS (PS = 0 v PS = 1, 2), sex (male v female), race (white v nonwhite), weight loss (≤ 5% loss in the previous 6 months or no loss v > 5% loss), histology type (adenocarcinoma v squamous cell carcinoma), tumor size, and previous chemotherapy or radiation received (yes v no). The explanatory variables in the final models were chosen using stepwise algorithm with entry significance level of .2 and stay significance level of .05. The OM variable was forced into the model. The proportional hazards assumption for the final model was checked for each variable using log-log and scaled Schoenfeld residual plots.²¹ The variable-specific score test and the overall score test for proportional hazards assumption were also performed. There was no significant evidence that these models violated the proportionality assumption. All P values reported are two sided.

The Alliance Statistics and Data Center conducted data collection and statistical analyses. Data quality was ensured by review of data by the same group, and by the study chairperson following Alliance Statistics and Data Center policies. Analyses were based on the study database, which was frozen on September 16, 2009.

RESULTS

This study was activated in April 1997, closed in January 2002 after accruing 501 patients, and terminated in June 2010. Among the accrued patients, 304 patients had stage IA or IB NSCLC; exclusions are delineated in Figure 2. R0 resections were achieved in 261 patients by standard lobectomy, in 21 by video-assisted thoracic surgery lobectomy, in two by wedge resection, and in 20 cases operative reports were not available. Compliance with submission of

Fig 4. (A) Overall survival by immunohistochemistry (IHC) status of lymph nodes in patients with stage I non-small-cell lung cancer. Log rank $P = .19$. (B) Disease-free survival by IHC status. Log rank $P = .51$.

all requested nodal stations was 39% (118 of 304). At least three nodal stations were sampled and submitted for central review in 81% of cases (246 of 304; Appendix Table A1, online only). Median follow-up was 8.4 years (0.97 to 11.4 years) and was complete in all but seven patients: one was lost to follow-up and six withdrew from

the study. By termination of follow-up, 156 patients had died. Patterns of recurrence were local only in 24 patients, local and distant in 18, and distant only in 27. Fifty-seven patients (19%) received adjuvant therapy. BM specimens were only available in a small number of patients. The quality of the samples was highly variable; many samples were paucicellular and, therefore, BM data were not evaluable in the final analysis.

IHC Data Analysis

IHC was performed on 298 of the 304 patients with stage IA or IB NSCLC. Forty-one (13.8%) were IHC-positive. An example of an H&E-negative, but IHC-positive, LN is depicted in Figures 3A-3C. Table 1 contains clinical and demographic data based on IHC status. There were no significant differences in baseline characteristics between IHC-positive or IHC-negative patients. Kaplan-Meier curves for OS and DFS by IHC status are shown in Figures 4A and 4B; no significant difference was noted. Table 2 gives the estimates of HR of the effect of IHC-positive with N1 or N2 node involvement relative to IHC-negative from Cox proportional hazards models. Known prognostic factors, increasing age, male sex, and PS 1 or 2 significantly correlated with shorter OS and DFS. OS was statistically significantly related to IHC status after adjusting for those covariates ($P = .01$). Specifically, patients who were IHC-positive within an N2 node had worse survival relative to IHC-negative patients (HR, 2.04, 95% CI: 1.14 to 3.66). Five-year survival was 50% (95% CI: 29.1% to 67.8%) for N2 IHC-positive compared with IHC-negative 5-year survival of 66.9% (95% CI: 60.9% to 72.2%), $P = .017$. Patients who were IHC-positive within an N1 node had no statistically significant difference in survival compared with IHC-negative patients.

PCR Data Analysis

RT-PCR data were available for 256 patients with stage IA and IB disease (Table 3). Overall, 68.8% of patients had OM by RT-PCR. Appendix Table A2 (online only) gives the estimates of the effect of PCR-positive N1 or N2 nodes relative to PCR-negative from Cox proportional hazards models. No statistically significant relationship between PCR status and OS or DFS was identified (Appendix Figs A1A and A1B, online only).

Table 2. Relationship Among Overall Survival, Disease-Free Survival, and Immunohistochemistry Status of Lymph Nodes From Cox Proportional Hazards Model

Variable	Overall Survival			Disease-Free Survival		
	HR	95% CI	<i>P</i>	HR	95% CI	<i>P</i>
Univariate						
IHC-positive N1 v IHC-negative	1.12	0.57 to 2.19	.75	1.03	0.52 to 2.01	.94
IHC-positive N2 v IHC-negative	1.61	0.96 to 2.71	.073	1.36	0.81 to 2.28	.25
Multivariate						
IHC-positive N1 v IHC-negative	1.26	0.61 to 2.59	.53	0.98	0.47 to 2.00	.94
IHC-positive N2 v IHC-negative	2.04	1.14 to 3.66	.017	1.61	0.90 to 2.86	.11
Age	1.05	1.03 to 1.07	< .001	1.04	1.02 to 1.06	< .001
PS = 0 v PS = 1, 2	0.52	0.36 to 0.74	< .001	0.59	0.42 to 0.83	.0022
Male v female	2.08	1.45 to 2.97	< .001	1.75	1.24 to 2.46	.0015
Adjuvant therapy (yes v no)				1.70	1.16 to 2.49	.0061

Abbreviations: HR, hazard ratio; IHC, immunohistochemistry; PS, performance status.

Table 3. Demographic and Clinical Characteristics by Polymerase Chain Reaction Status of Lymph Nodes

Characteristic	PCR-Positive (n = 176)	PCR-Negative (n = 80)	Total (N = 256)	χ^2 P
Age in years, median (range)	67 (38-86)	68 (43-80)	68 (38-86)	NS
Sex				
Male	91 (52)	52 (65)	143 (56)	.05
Female	85 (48)	28 (35)	113 (44)	
Race				
White	157 (89)	76 (95)	233 (91)	.13 (white v nonwhite)
Black	17 (10)	4 (5)	21 (8)	
Other	2 (1)	0 (0)	2 (1)	
PS				
0	128 (73)	55 (69)	183 (71)	.35 (PS = 0 v PS = 1, 2)
1	41 (23)	23 (29)	64 (25)	
2	3 (2)	2 (2)	5 (2)	
Data missing	4 (2)	0 (0)	4 (2)	
Weight loss in previous 6 months				
≤ 5% or no loss	156 (89)	70 (88)	226 (88)	.71 (≤ 5% v > 5%)
> 5%	15 (8)	8 (10)	23 (9)	
Data missing	5 (3)	2 (2)	7 (3)	
Histology				
Adenocarcinoma	109 (62)	35 (44)	144 (56)	.004 (adenocarcinoma v squamous)
Squamous	50 (28)	37 (46)	87 (34)	
Others	17 (10)	8 (10)	25 (10)	
T category				
T1	104 (59)	46 (57)	150 (59)	.81
T2	72 (41)	34 (43)	106 (41)	
PCR-detected N category				
N1	92 (52)	NA	NA	
N2	84 (48)	NA	NA	
Adjuvant CT or RT				
Yes	41 (23)	11 (14)	52 (20)	.08
No	135 (77)	69 (86)	204 (80)	

NOTE. Data shown as No. (%) unless otherwise indicated.

Abbreviations: CT, chemotherapy; NA, not applicable; NS, not significant; PCR, polymerase chain reaction; PS, performance status; RT, radiotherapy.

Staging System Effect

We performed secondary analysis on the relationship between T category and OM using the AJCC 7th edition lung cancer staging system. Just over 10% of patients were upstaged to stage II on the basis of tumor size. All end points were reanalyzed, controlling for T category using the AJCC 7th edition staging schema. There were no changes in the impact of IHC- or RT-PCR-identified OM on OS or DFS taking into account the change in T category.

DISCUSSION

Patients designated as having stage I disease by conventional histopathology were in fact harboring metastatic tumor in N1 or N2 nodes demonstrable by IHC for a pan-cytokeratin antibody cocktail in 13.8% of cases and by RT-PCR for CEA in nearly 69%. IHC-positive nodes were in the N2 stations in 58% of patients; PCR-positive nodes were N2 in 48%. Despite this high frequency of micrometastatic disease, only occult N2 disease by IHC affected survival significantly (HR, 2.04), with a decrease in 5-year survival of nearly 17%. A secondary objective of CALGB 9761 was to ascertain sensitivity of the two techniques for identifying OM. Clearly RT-PCR is more sensitive, but perhaps CEA is not as specific for NSCLC and thus the clinical impact is insignificant. We did not identify a relationship between tumor size or T status and the frequency of OM by either technique, even when reanalyzing

our data using the AJCC 7th edition staging schema. No significant impact on DFS was identified using IHC or RT-PCR to detect OM.

We know that patients with stage I NSCLC do experience a high rate of recurrence, and 5-year survival rates are quite low compared with many other stage I malignancies. Many theories for the heterogeneity within stage I NSCLC have been proposed: Do some lung cancers express tumor suppressors that disappear after resection? Is it due to varying tumor biology (eg, proliferative rate, expression of intercellular matrix-invading enzymes, tumor microenvironment, neovascularization, promoter methylation, or evasion of immune destruction)?^{22,23} Circulating tumor cells can now be detected by RT-PCR; it remains to be seen if they are clinically relevant.

Histopathologic characteristics have also been implicated as prognostic predictors, eg, visceral pleural invasion, degree of differentiation, and lymphovascular invasion.^{5,24} Perhaps better gross pathology processing would lead to more accurate stage identification. Ramirez et al²⁵ found that 90% of lung resection specimens have additional unidentified LNs after the pathologist is finished with them; additional N1 nodes are histologically positive in up to 11% of cases. The presence of OM is a logical explanation for tumors that are classified as stage I by conventional histopathology to demonstrate a worse prognosis. However, the current rigorously designed and executed prospective study only showed a significant difference in survival when N2 nodes demonstrated positivity by IHC, but not by RT-PCR.

One other major clinical trial looked at OM in stages I to III NSCLC.⁷ A larger sample size (580 patients who were N0 by H&E) may have allowed for detection of a relatively small difference not seen in our study; or perhaps the choice of cytokeratin markers resulted in differing sensitivities between the two studies. Most likely, there are a multitude of factors that explain the heterogeneity of outcomes in early-stage lung cancer. Eventually, pooling of multiple clinicopathologic prognostic indicators should allow for creation of a scoring system that can be used to identify patients at highest risk for recurrence who, if proven in future clinical trials, should receive some form of adjuvant therapy.

There are several potential limitations of this trial. Sample size may not have been large enough to detect a significant survival impact—perhaps the effect size of OM on survival and recurrence was overestimated. This study occurred before the routine use of PET/CT scans, introducing the possibility of undetected advanced cancer. RT-PCR was positive in only 90% of the primary tumors sampled; thus the nodes in 10% of patients are guaranteed to be negative by this technique. CEA may not be the optimal marker to indicate presence or virulence of OM. Logistical difficulties with retrieval, cellularity, and quality of BM samples prevented the investigators from addressing the impact of occult BM involvement. When we compared the IHC data with the RT-PCR data, we found poor concordance. This implies epigenetic regulation of CEA expression in NSCLC OM, a finding worthy of future studies, but not possible with the current samples. It may also be due to evaluation of different markers in the nodes for the presence of OM. In addition, the lack of correlation of RT-PCR with outcome underscores the relative lack of utility in clinical practice for RT-PCR in NSCLC. Finally, some patients did receive adjuvant therapy, and this may have reduced the impact of OM. The lack of positive findings in N1 nodes by IHC and PCR-positive N1/N2 LNs refutes previously derived conclusions from numerous retrospective studies in early-stage NSCLC. Nonetheless, the strength of this trial lies in the rigorous and standardized approach from a surgical and tissue-processing

standpoint to generate data regarding the clinical significance of OM in stage I NSCLC.

In summary, the presence of micrometastases by IHC staining in N2 LNs of patients with NSCLC, otherwise designated stage I or N0 by standard pathologic techniques, correlated with decreased OS. We did not demonstrate a difference in OS or DFS if N1 nodes were deemed positive by IHC, or in either N1 or N2 disease detected by RT-PCR. Our findings suggest that consideration may be given to routinely staining for cytokeratin markers in histologically negative LNs to look for OM. Treatment decisions on the basis of these findings remain a complex issue. Our data suggest that further investigations are needed into other factors, such as tumor biology, to fully understand the behavior of early-stage NSCLC.

AUTHORS' DISCLOSURES OF POTENTIAL CONFLICTS OF INTEREST

Disclosures provided by the authors are available with this article at www.jco.org.

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Appendix

Fig A1. (A) Overall survival by polymerase chain reaction (PCR) status of lymph nodes in patients with stage I non-small-cell lung cancer. Log rank $P = .15$. (B) Disease-free survival by PCR status. Log rank $P = .24$.

Table A1. Nodal Station Sampling by Lobe Location

Lobe	Total No. of Cases	No. of Missing Stations							
		Any	0	1	2	3	4	5	6
Right upper	118	64	54	40	15	9	0	0	0
Right middle	12	6	6	2	1	2	1	0	0
Right lower	44	23	21	14	4	3	0	2	0
Left upper	90	61	29	31	14	12	2	1	1
Left lower	40	30	10	18	7	5	0	0	0
Total	304	184	120	105	41	31	3	3	1
%	100	61	39	34.5	13	10	1	1	0.5

NOTE: If accessible, five stations were requested on the right side, six stations on the left side.

Table A2. Relationship Among Overall Survival, Disease-Free Survival, and Polymerase Chain Reaction Status of Lymph Nodes From Cox Proportional Hazards Model

Variable	Overall Survival			Disease-Free Survival		
	HR	95% CI	<i>P</i>	HR	95% CI	<i>P</i>
Univariate						
PCR-positive N1 v PCR-negative	0.66	0.44 to 1.01	.06	0.72	0.48 to 1.07	.10
PCR-positive N2 v PCR-negative	0.79	0.52 to 1.19	.26	0.79	0.53 to 1.19	.26
Multivariate						
PCR-positive N1 v PCR-negative	0.83	0.54 to 1.29	.41	0.83	0.54 to 1.26	.38
PCR-positive N2 v PCR-negative	0.78	0.49 to 1.23	.29	0.76	0.49 to 1.19	.23
Age	1.04	1.02 to 1.07	.001	1.04	1.02 to 1.06	.001
PS = 0 v PS = 1, 2	0.58	0.39 to 0.85	.005	0.62	0.42 to 0.90	.012
Male v female	1.82	1.23 to 2.69	.003	1.81	1.25 to 2.62	.002

Abbreviations: HR, hazard ratio; PCR, polymerase chain reaction; PS, performance status.