Prognostic markers in resectable non-small cell lung cancer: a multivariate analysis

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Objective: To identify the prognostic significance of certain clinical, cellular and immunologic markers in resectable non-small cell lung cancer (NSCLC). **Design:** A cohort of patients with resectable NSCLC was prospectively followed up for 8 years (100% follow-up). **Setting:** A university hospital in a large Canadian city. **Patients:** One hundred and thirteen consecutive patients who underwent surgical resection of primary NSCLC. **Main outcome measures:** Presence of peritumoral B lymphocytes (identified with antibody to CD20) and T lymphocytes (antibody to CD43), along with tumour markers (carcinoembryonic antigen [CEA], keratin, cytokeratin, S-100 protein, vimentin, chromogranin) and other factors such as age, sex, cell type, American Joint Committee on Cancer (AJCC) stage, histologic grade, DNA ploidy and S-phase fraction were correlated with survival. **Results:** The mean age of patients in the study was 66.0 years; 60% were male. Histologic types of the tumours were: adenocarcinoma 57 (50.4%), squamous cell 47 (41.6%), adenosquamous 6 (5.3%) and large cell 3 (2.6%). AJCC stages were: I 66 (58.4%), II 20 (17.7%) and III 27 (23.9%). Histologic grades were: I (well differentiated) 31 (27.4%), II 50 (44.2%), III 29 (25.7%) and IV 3 (2.6%). Survival was 85% at 1 year (95% confidence interval [CI] 76%–90%), 44% at 5 years (95% CI 34%–53%) and 34% at 10 years (95% CI 22%–46%). Multivariate analyses using the Cox proportional hazards model for survival confirmed AJCC stage ($p < 0.001$) in all histologic subtypes to be the strongest factor of independent prognostic significance. It also revealed the presence of CD20-stained B lymphocytes ($p = 0.04$) in the peritumoral region of all tumours to be a positive prognostic factor. This relation was especially strong for nonsquamous cell carcinomas ($p < 0.001$). For squamous cell carcinomas, the immunohistochemical presence of CEA was of marginally negative prognostic value ($p = 0.04$). DNA ploidy and a high S-phase fraction showed no evidence of prognostic value for stage I tumours, but for stages II and III tumours there was strong evidence of prognostic value ($p < 0.001$ jointly). The evidence for DNA ploidy was especially strong in stages II and III squamous cell tumours ($p = 0.008$), and for a high S-phase fraction was strongest in stages II and III nonsquamous cell tumours ($p = 0.002$). **Conclusions:** AJCC stage remains the most important prognostic indicator from a variety of clinical variables and tumour markers in postoperative patients with resectable NSCLC. For nonsquamous cell lung carcinomas, the presence of peritumoral B lymphocytes was strongly associated with improved survival, suggesting an important role for humoral mediated immunity.

Objectif : Établir l'importance pronostique de certains marqueurs cliniques, cellulaires et immunologiques dans le cas du cancer bronchopulmonaire «non à petites cellules» résécable. **Conception :** Une cohorte de patients atteints d'un cancer bronchopulmonaire «non à petites cellules» résécable ont été suivis de façon prospective pendant huit ans (suivi à 100 %). **Contexte :** Un hôpital universitaire d'une grande ville canadienne. **Patients :** Cent treize patients consécutifs qui ont subi la résection chirurgicale d'un cancer bronchopulmonaire primitif «non à petites cellules». **Principales mesures de résultats :** La présence péritumorale de lymphocytes B (repérés à l'aide de l'anticorps du CD20) et de lymphocytes T (anticorps du CD43), ainsi que des marqueurs tumoraux (antigène carcino-embryonnaire [ACE], kératine, cytokératine, protéine S-100, vimentine, chromogranine) et d'autres facteurs tels

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l'âge, le sexe, le type de cellules, le stade selon l'American Joint Committee on Cancer (AJCC), le grade histologique, la ploïdie de l'ADN et la fraction de phase S ont été corrélés au taux de survie. **Résultats :** Les patients de l'étude avaient en moyenne 66,0 ans, et 60 % d'entre eux étaient de sexe masculin. La répartition des types histologiques des tumeurs était la suivante : 57 adénocarcinomes (50,4 %), 47 épidermoïdes (41,6 %), 6 épidermoïdes et glandulaires (5,3 %) et 3 à grandes cellules (2,6 %). La répartition des stades de l'AJCC était la suivante : I : 66 (58,4 %); II : 20 (17,7 %); III : 27 (23,9 %). La répartition des grades histologiques était la suivante : I (bonne différenciation) : 31 (27,4 %); II : 50 (44,2 %); III : 29 (25,7 %); IV : 3 (2,6 %). La survie s'établissait à 85 % après un an (intervalle de confiance [IC] à 95 % = 76 %–90 %), à 44 % après cinq ans (IC à 95 % = 34 %–53 %) et à 34 % après dix ans (IC à 95 % = 22 %–46 %). Les analyses multidimensionnelles de la survie effectuées à l'aide du modèle Cox de risque proportionnel ont confirmé que le stade de l'AJCC ($p < 0,001$) constituait le facteur le plus puissant sur le plan de l'importance prédictive indépendante pour tous les sous-types histologiques. Les analyses ont également révélé que la présence de lymphocytes B marqués par CD20 ($p = 0,04$) dans la région péritumorale de toutes les tumeurs constituait un facteur prédictif positif. Cette corrélation était tout particulièrement importante pour les carcinomes de type non épidermoïde ($p < 0,001$). Pour ce qui est des carcinomes de type épidermoïde, la présence immunohistochimique d'ACE avait une valeur prédictive légèrement négative ($p = 0,04$). Rien n'indiquait que la ploïdie de l'ADN et la fraction de phase S présentaient une valeur prédictive pour les tumeurs de stade I, bien qu'il y ait eu de solides preuves qu'elles avaient une valeur prédictive dans le cas des tumeurs de stades II et III ($p < 0,001$, ensemble). Les données probantes relatives à la ploïdie de l'ADN s'avéraient particulièrement convaincantes pour les tumeurs de type épidermoïde de stades II et III ($p = 0,008$), et celles relatives à la fraction de phase S avaient le plus d'importance lorsqu'il s'agissait de tumeurs de type non épidermoïde de stades II et III ($p = 0,002$). **Conclusions :** Chez les patients atteints d'un cancer bronchopulmonaire «non à petites cellules» résécable qui ont subi l'intervention chirurgicale, le stade de l'AJCC demeure l'indicateur pronostique le plus important d'une variété de variables cliniques et de marqueurs tumoraux. En ce qui concerne les carcinomes du poumon de type épidermoïde, un lien étroit a été établi entre la présence péritumorale de lymphocytes B et une amélioration de la survie, ce qui laisse croire que l'immunité à médiation humorale joue un rôle important.

Lung cancer continues to be the main cause of cancer death in Canada.[1] In the United States, more individuals die yearly from non-small cell lung cancer (NSCLC) than from colorectal and breast carcinomas combined.[2] The TNM staging in lung cancer has proven to be the most important prognostic factor for these patients.[3] However, reports of wide-ranging survival rates for similar tumours[4–11] have fuelled the search for better prognostic markers, ranging from clinical factors[9] to serum[5,12] and tumour molecular markers.[13] This discrepancy suggests a need for better prognostic factors to both improve the accuracy of survival predictions and guide therapeutic options after resection.

Numerous prognostic markers in resectable NSCLC have been evaluated, but conflicting reports and lack of clinical applicability have rendered many of these valueless.[13] Immunohistochemistry has enabled the assessment of various tumour markers, including those involved in a local immune response to neoplasms. Although immunodeficiency has been associated with poor prognosis in patients with cancer, including lung cancer, efforts at immunotherapy have yet to yield consistent, satisfactory results.[14,15] Also, little is known about the localized immune response to lung carcinomas at the site of the primary tumour, and its effect on prognosis. This report, undertaken at a single institution with 100% follow-up, aims to determine the prognostic value of such a localized immunologic response by identifying the lymphocytic subsets around a resectable tumour at the time of surgery and correlating their presence with long-term survival. Other suggested factors of prognosis and potential tumour markers are also assessed for their prognostic significance.

Patients and methods

Patient selection

One hundred and thirteen consecutive patients having primary NSCLC who underwent surgical resection and staging between January 1988 and December 1991 were studied prospectively. Data were entered into the institution's cardiothoracic surgery database. All patients were followed up by either the referring physician or surgeon (100% follow-up) until closure of the study (August 1998) or death.

Clinical and pathologic analysis

Data were prospectively gathered on the patient's age, gender, smoking history, family history of lung cancer, type of operative procedure, dose and timing of postoperative radiotherapy, and date of last follow-up or death. Lungs or lobes resected at surgery were inflated through the airways with 10% buffered formalin and fixed overnight. Segmental and wedge resections were injected with a syringe and needle containing formalin. They were sliced, and representative blocks from the tumour, lymph nodes and bronchial resection margins were processed for routine histologic examination and embedded in paraffin. Then, 5-μm thick sections were cut, stained with hematoxylin and eosin and, as required, periodic acid Schiff and mucicarmine stains. Histologic types of the tumours were determined according to

World Health Organization criteria.[16] Tumours were divided into 1 of 4 histologic grades: I (well differentiated), II (moderately differentiated), III (poorly differentiated), and IV (undifferentiated). Additional data included size of the tumour, status of regional lymph nodes, and sites of distant metastases. The American Joint Committee on Cancer (AJCC) stage of each tumour was determined using the TNM system.[3,17]

DNA flow cytometry

Formalin-fixed paraffin-embedded blocks from the primary tumours were selected for DNA flow cytometric (FCM) analysis. The method used for the preparation, staining and FCM analysis was similar to that described by Hedley and associates,[18] with slight modification. In brief, 50-μm sections were cut, deparaffinized and treated with pepsin (Sigma, St. Louis) and RNAse solutions (Boehringer Mannheim, Indianapolis). After incubation, the cells were centrifuged and stained in an appropriate volume of 50 mg/mL propidium iodide (Calbiochem, La Jolla, Calif.) to bring the final concentration of the nuclear suspension to 1 to 2×10^6 nuclei/mL. Nuclear DNA content was measured using an EPICS profile II flow cytometer (Beckman Coulter, Fullerton, Calif.) and the red fluorescence signals (>610 nm) were analyzed. A minimum of 20 000 events were measured in each analysis. Eight samples could not be included in this study because, despite repeated analysis, the quality of the DNA histograms was poor (coefficient of variance exceeding 7% or presence of excessive debris above 35% of the number of events).

By definition, the first peak observed in the histogram was classified as diploid and subsequent peaks aneuploid, since it was impossible to include external ploidy standards in paraffin-derived material.[19] Diploid tumour populations were defined as having a single G_0/G_1 peak. Tumours were considered aneuploid if there was evidence of a distinctly separate second G_0/G_1 peak. The cell cycle was analyzed with the use of multicycle software (Phoenix Flow Systems, San Diego). The mean (±2 standard deviations) of the diploid tumours was selected arbitrarily as a cutoff for a high S-phase fraction.

Immunohistochemistry

From selected blocks (1–3 per case), 5-mm thick paraffin sections were mounted on glass slides coated with a transparent white glue and dried overnight in a 37 °C oven. The sections were deparaffinized by 3 consecutive changes of xylol (10 minutes each) and brought to water through graded changes in ethanol concentration. After rinsing, sections were digested at 37 °C in 0.1% trypsin-calcium chloride, pH 7.8. All sections were then placed for 30 minutes in a 3.5% solution of hydrogen peroxide in methanol to block endogenous peroxidase and subsequently for 10 minutes in bovine serum albumin blocking solution. The sections were then incubated with the primary antibody, specifically MT1 antibody recognizing the CD43 epitope for T lymphocytes (Clonab, Denville, NJ) 1 in 50 dilution, and the L26 antibody recognizing the CD20 epitope on B lymphocytes (Dako, Carpenteria, Calif.) 1 in 200 dilution, cytokeratin (Becton Dickenson) 1 in 10 dilution, keratin (Dako) 1 in 400 dilution, vimentin (Dako) 1 in 50 dilution, chromogranin (Signet, Dedham, Mass.) 1 in 3 dilution, CEA (Dako) 1 in 300 dilution, and S100 protein (Dako). Incubation with the primary antibody was done in a moist heat chamber at 37 °C for 1 hour, after which the slides were rinsed with TRIS buffer, incubated with the secondary biotinylated antibody at 37 °C for 20 minutes, then with streptavidin horseradish peroxidase complex for 20 minutes and finally developed with diaminobenzidine. After counterstaining with Mayer's hematoxylin, the slides were dehydrated and mounted. The results of L26 and MT1 staining were interpreted as positive if more than 10% of the infiltrating lymphocytes were stained with the antibody. For the other antibodies, the results were interpreted as positive if more than 10% of the malignant cells stained with the antibody.

Statistical analysis

The Wilcoxon test for censored data, as computed by the SAS LIFETEST,[20] was used to test for significant survival difference among categories for categorical factors. This is the Gehan–Wilcoxon test when there are only 2 categories. For multivariate analysis, Cox regression[21] was done with EGRET (EGRET [1991] Statistics and Epidemiology Research Corporation, Seattle). For a binary factor, the log-rank test corresponds to the test of significance reported by Cox regression for the simple model relating survival to 1 factor. Kaplan–Meier survival estimates were computed and plotted with EGRET. The tics on the curves each represent the end-of-study time for a surviving patient (i.e., a censored time). Confidence intervals (CIs) are given as computed by EGRET, except at 0% or 100% survival, where these were replaced with Blyth–Still exact binomial intervals.[22] Residual analysis suggested a lack of fit of the Cox assumption of proportional hazards due to the survival difference between stage I and other stage data, so initial analyses were done separately for stage I data and stages II and III data. Best subset regression was performed for both groups for selection of best fitting Cox models. These included interaction terms involving stage (I v. II and III), cell type (squamous v. nonsquamous) and gender. The inclusion of interaction terms allowed good fits of Cox models to the entire data set. The 2 main effects corresponding to any interaction term were always included, as they should be.

For descriptive purposes, to avoid

having to interpret the meaning of interactions in Cox models, results are presented for subgroups of staging and cell type, and reported *p* values (2-sided) are from the SAS PROC LIFETEST Wilcoxon test applied only to the subgroup being discussed; *p* values greater than 0.01 are discounted to partly account for chance in covariate selection.

Results

Of the 113 patients in our study population, 68 (60.2%) were men and 45 (39.8%) were women. The mean age of the group at the time of surgery was 66.0 years, with a range (and standard deviation) from 43 to 84 (8.7) years. Current smokers numbered 104 (92.0%), and 12 (10.6%) had a positive family history for lung cancer. Surgical procedures included lobectomy in 83 patients (73.4%), pneumonectomy in 10 (8.8%), wedge excision in 10 (8.8%), segmentectomy in 6 (5.3%), and a combination of these in 4 (3.5%).

The histologic tumour types were as follows: 57 (50.4%) adenocarcinomas, 47 (41.6%) squamous cell carcinomas, 6 (5.3%) adenosquamous carcinomas, and 3 (2.6%) large cell carcinomas. Histologic grades were as follows: 31 (27.4%) grade I, 50 (44.2%) grade II, 29 (25.7%) grade III and 3 (2.6%) grade IV. The AJCC stages at time of resection were as follows: 66 (58.4%) stage I, 20 (17.7%) stage II and 27 (23.9%) stage III. There were no operative deaths.

Regarding ploidy status, 47 (41.6%) patients had diploid tumours, 58 (51.3%) had tumours with aneuploid populations, and in 8 (7.1%) the status was undetermined. The S-phase fraction (proliferative index) for all patients was recorded as low (69 [61.1%] patients), undetermined (23 [20.3%]) or high (21 [18.6%]).

Immunohistochemical marker analysis revealed the following: 60 (53.1%) patients had tumours that were positive for keratin, 101 (89.4%) were positive for cytokeratin, 95 (84.1%) were positive for CEA, 7 (6.2%) were positive for S-100 protein, 3 (2.6%) were positive for vimentin and 6 (5.3%) were positive for chromogranin. In the peritumoral tissue, 69 (61.1%) patientswere positive for CD43 (MT-1), and 65 (57.5%) were positive for CD20 (L26, Table 1). Ten (8.8%) specimens for CD43 and 11 (9.7%) specimens for CD20 could not be evaluated. CD43 served as a marker for T lymphocytes, and CD20 served as a marker for B lymphocytes.

Survival data

Survival was measured from the date of operation until closure of the study or death. Median follow-up of all patients was 40.2 months (range from 1.3 to 112.3 months). Survival for the study group at 1 year was 85% (95% CI 76%–90%), 57% (95% CI 46%–66%) at 3 years, 44% (95% CI 34%–53%) at 5 years and 34% (95% CI 22%–46%) at 10 years. Univariate and multivariate analyses demonstrated that survival was related significantly to AJCC stage ($p < 0.001$); survival decreased as stage increased (Fig. 1). However, survival was not influenced overall by either the histologic cell type ($p = 0.87$, Fig. 2) or the grade of the tumour ($p = 0.15$, Fig. 3). Univariate analysis of prognostic markers indicated the presence of B lymphocytes (CD20 staining) around the tumour margins to be the only independent prognostic factor ($p = 0.04$, Fig. 4). The presence of either T lymphocytes (p = NS) or other studied prognostic factors did not have any impact on survival (Table 1). The best fitting overall multivariate Cox regression included a strong L26 effect (hazard ratio 0.16, 95% CI [0.06–0.42]) and strong interactions between gender and L26 and between staging and DNA (DNA denotes the 3 categories shown in Fig. 5). However, owing to missing data for L26 and for DNA, this model was based on only 95 patients. More data were fit when Cox regressions were done separately for 47 patients with squamous cell carcinoma and for 60

Table 1

UnivariateAnalysis of Various Prognostic Factors for All 113 Patients

Clinical variables and tumour markers	Positive result, no. (and %)	*p* value
Sex, M/F	68/45	0.16
Mean (and SD) age, yr	66 (8.7)	0.96
Aneuploidy	58 (51.3)	0.15
L26 antibody	65 (57.5)	0.04
MT1 antibody	69 (61.1)	0.42
S100 protein	7 (6.2)	0.45
Keratin	60 (53.1)	0.69
CEA	95 (84.1)	0.28
Cytokeratin	101 (89.4)	0.71
Vimentin	3 (2.6)	0.90
Chromogranin	6 (5.3)	0.18

CEA = carcinoembryonic antigen.

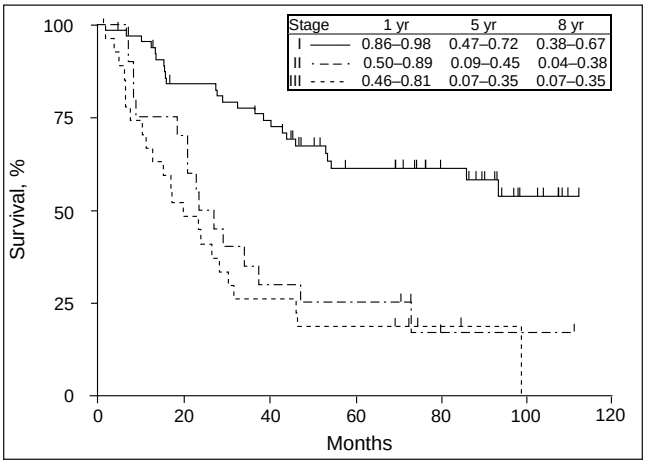

FIG. 1. Survival by American Joint Committee on Cancer stage for all 113 patients, $p < 0.001$. Figure legend indicates the 95% confidence intervals at 1, 5 and 8 years.

patients with nonsquamous cell carcinoma. The squamous cell model retained CEA and the staging and DNA interaction. The nonsquamous cell model retained L26 and the staging and DNA interaction. The gender and L26 interaction was not retained because of the relative weakness of the effect (p = 0.025). For these data, interactions were strong effects, so a regression tree approach, splitting by staging or cell type, yields a much clearer illustration of the results than presentation of the Cox regressions. Splitting by staging or cell type makes sense, since they are both statistically important for these data and, being well-established markers, are biologically important. The results that follow, and the figures, accurately reflect conclusions from the best fitting Cox models with interactions.

Stage I tumours

For the 66 patients with stage I tumours, complete data for all prognostic factors were available in 62. The Cox proportional hazards regression model did not identify any individual prognostic factors, and patients with squamous cell carcinoma had a similar survival to those with nonsquamous cell carcinoma (p = 0.18). However, in the 41 patients with stage I nonsquamous cell carcinomas, survival was significantly better in those with B-lymphocyte (CD20) infiltrates (p = 0.002); this was not the case for the 21 patients having stage I squamous cell carcinomas (p = NS).

Stage II and stage III tumours

For the 47 patients with stages II and III carcinomas, complete data for all prognostic factors were available in 43. No differences in survival were observed between squamous and nonsquamous cell carcinomas (p = NS). For the 19 patients with stage II or III nonsquamous cell carcinomas, survival was significantly better in patients with B-lymphocyte (CD20) infiltrates (p = 0.006). Also, a high S-phase fraction was an individually negative prognostic factor in this subgroup of patients (p = 0.002). In the subgroup of 26 patients having stage II or III squamous cell tumours, diploidy was of positive prognostic significance (p = 0.008). These effects are seen jointly

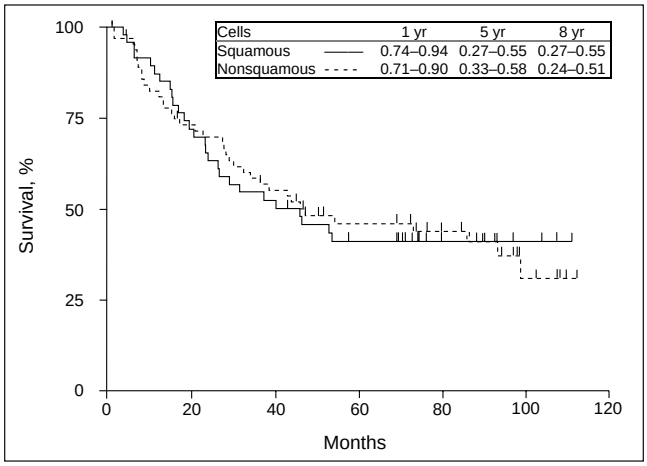

FIG. 2. Patient survival according to the histologic type of the tumour for all 113 patients, p = 0.87. Figure legend indicates the 95% confidence intervals at 1, 5 and 8 years.

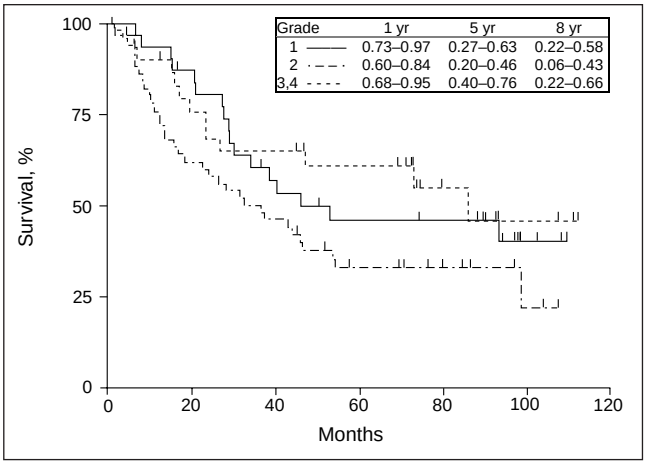

FIG. 3. Patient survival based on tumour grade for all 113 patients, p = 0.15. Figure legend indicates the 95% confidence intervals at 1, 5 and 8 years.

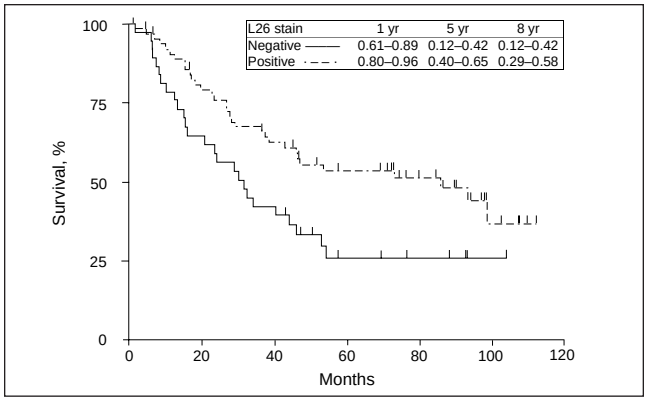

FIG. 4. Patient survival by L26 status (presence of peritumoral B lymphocytes) in 102 patients, p = 0.04. Figure legend indicates the 95% confidence intervals at 1, 5 and 8 years.

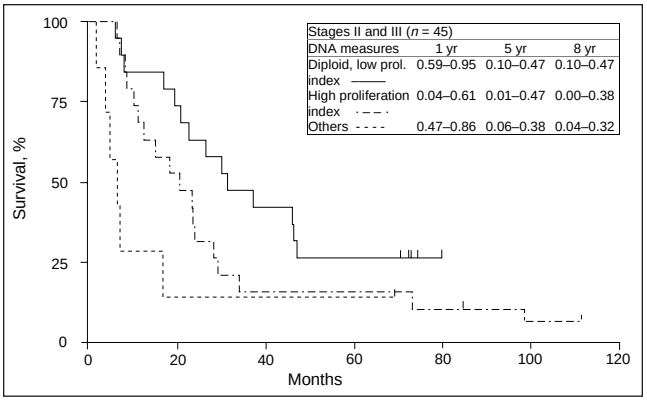

FIG. 5. Survival by S-phase fraction in 47 patients with stage II and stage III tumours, p = 0.001. Figure legend indicates the 95% confidence intervals at 1, 5 and 8 years.

in Fig. 5, based on all 47 patients.

Nonsquamous cell tumours

For all 66 nonsquamous cell carcinomas, a lower stage correlated with improved survival ($p = 0.003$). The only other independent factor of prognostic significance was the presence of B lymphocytes in tissue surrounding the tumour, identified by positive staining to CD20 (L26). Its presence was associated with a significant survival advantage ($p < 0.001$, Fig. 6).

Squamous cell tumours

For all 47 squamous cell carcinomas, lower stage was again associated with prolonged survival ($p < 0.001$). The only other important independent prognostic factor of statistical significance was CEA, where tumours testing positively for this antigen suggested a trend towardshorter survival ($p = 0.04$, Fig. 7). Contrary to the results of nonsquamous cell carcinomas, the presence of B lymphocytes (CD20) was not a significant prognostic factor in squamous carcinomas.

Discussion

The major value of prognostic markers in NSCLC should be to guide therapy after surgical resection. Resected tumours could be tested for specific prognostic indicators, leading to the selection of appropriate adjuvant therapy in selected patients. Unfortunately, there has been little success in consistently identifying such tumours, and adjuvant therapy in lung carcinomas has led to only modest survival advantages. It is possible that in prognosticating survival from resectable NSCLC, the patient's immune status at the time of resection may be important and measurable. Ongoing work suggests that immunotherapy may become a valuable adjuvant therapy. With this in mind, the present study aimed to determine the impact of lymphocytic markers, namely CD20 (L26) for B lymphocytes and CD43 (MT1) for T lymphocytes, along with a variety of tumour-related markers, for their prognostic significance.

Survival in our series was clearly linked to the stage of disease at the time of resection. Tumour stage is one of the few consistent prognostic factors.[13] However, our results suggesting no difference in survival based on histologic subtype or tumour differentiation simply adds more controversy to this issue. Unlike patients reported by the Lung Cancer Study Group,[5,23] our patients with squamous cell carcinoma did not have a superior outcome over patients with other histologic tumour types, supporting the results of other researchers.[6–9] Similarly, our results showing no change in survival based on tumour differentiation supported the findings of Harpole and associates[9] in contrast to those of other studies, which suggested an improvement in survival with well-differentiated lung tumours.[8,24,25] Finally, the results of our flow cytometry analysis revealed a 51.3% incidence of aneuploid tumours, well within the reported ranges of 45% to 76%.[26–28] As with the similar controversies surrounding histologic type and tumour grade, our results showing no significant benefit in overall survival with diploid tumours versus aneuploid tumours both supported[29–32] and disagreed[8,26,28,33] withprevious findings. However, our data did suggest that within specific groups, namely stages II and III tumours, a high S-phase fraction was associated with poor survival, and patients with diploid stage II or III squamous cell carcinomas had better survival. The association of a high S-phase fraction and poor survival has been both suggested[33] and refuted[31] in previous studies. For our analysis, it was useful to consider DNA ploidy

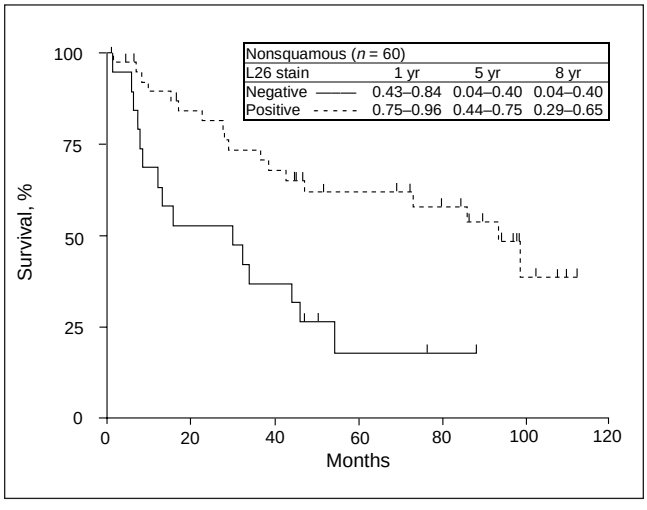

FIG. 6. Survival by L26 status (presence of peritumoral B lymphocytes) in 66 patients with nonsquamous cell carcinomas, $p < 0.001$. Figure legend indicates the 95% confidence intervals at 1, 5 and 8 years.

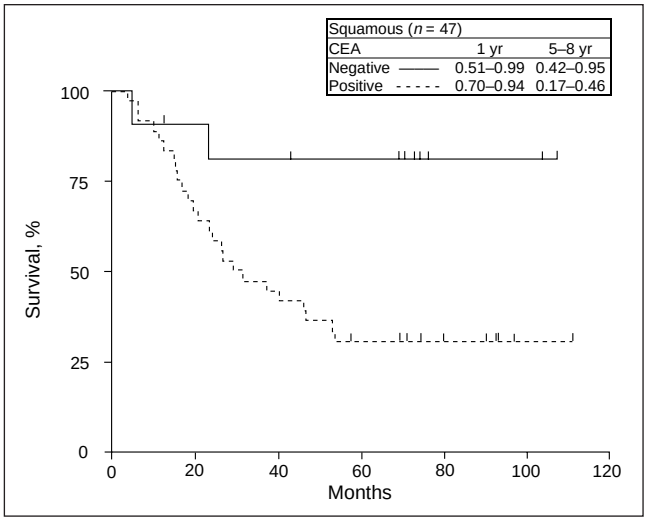

FIG. 7. Survival according to CEA status in 47 patients with squamous cell carcinomas, $p = 0.04$. Figure legend indicates the 95% confidence intervals at 1, 5 and 8 years.

and S-phase fraction jointly (i.e., the categories in Fig. 5). Still, it is unclear why this would have strong prognostic value for stages II and III tumours but not at all for stage I tumours. Accordingly, histologic type, tumour grade, DNA content and S-phase fraction cannot be considered reliable and independent prognostic indicators. With such conflicting literature, other factors of prognosis need to be assessed. To address this, we analyzed survival based on the patient's local immune response to lung cancer.

Long-term survival in our patients with NSCLC was significantly better when B lymphocytes were present in the peritumoral region at the time of resection. This observation suggests a specific link between immune function and survival. Although many factors may explain the variability in survival between patients with identically staged tumours, this link may be a reason for the host's ability to contain areas of micrometastasis, which are often present despite complete resection.[34] Also, the cell type and its virulence might be different at an unmeasurable level. The nodal status may be normal by histology, but actually contain occult nodal metastases when sensitive immunohistochemical techniques and specific monoclonal antibodies are utilized.[35,36] The presence of these previously undetectable nodal metastases was recently associated with shorter survival.[35,36] In fact, these reasons and potentially many more may explain the observed difference in survival among patients of the same stage. Despite tumour stage being the most important individual prognosticator of survival in our study, there exists such variability in the rate of recurrence that there are surely other factors involved. We postulate that patients' humoral immunity must play a determining role in their long-term survival. This notion is based on the observed improvement in survival seen in those patients who were found to have B lymphocytes in the tissue surrounding the tumour. Some researchers have previously quantitated lymphocyte subsets in patients with lung cancer,[37] whereas others have attempted to define the different immune functions of peripheral blood, regional lymph node, and tumour infiltrating lymphocytes.[38] However, the link between humoral immunity at the site of a lung tumour and its impact on survival remains unproven. Greater impact with nonsquamous cell neoplasms suggests that tumours of this histologic type may be more prone to meaningful immunogenicity by the host. In fact, our evidence for improved survival in tumours surrounded by B lymphocytes is strong for adenocarcinoma and adenosquamous carcinoma but negligible for squamous cell neoplasms.

There are many ideas to explain why the presence of peritumoral B cells is involved with prolonged survival. Possibly, this immune response helps to locally contain some tumours, thereby reducing the true incidence of occult micrometastases. If occult metastases are present, such immunity might prolong survival by limiting further tumour dissemination. Also, the presence of B cells may be a mirror of the host's overall immunity. Those able to mount an immune response may preselectively be in better overall condition. As is commonly seen in aging or multisystem disease, the overall immune response of the host may become weaker. Such poor immunity may actually contribute to tumorigenesis. In other words, our results cannot identify whether B cells were attracted by the tumour itself or if they were present as a reflection of the host's superior immune response. Efforts at modulating the host's immune response have been encouraging,[39,40] namely with adoptive and active immunotherapeutic agents such as transfer factor and *Nocardia rubra* cell wall skeleton. Other attempts at immunotherapy for stage I NSCLC have yielded controversial results. Efforts at using intrapleural bacille Calmette–Guérin[41] or *Corynebacterium parvum*[42] have not shown a survival advantage. Nevertheless, there have been some exciting developments, with undoubtedly many more on the horizon.[39]

Finally, the presence of CEA, as identified by immunohistochemistry, in our patients with squamous cell carcinoma was associated with a decrease in long-term survival. Measurement of preoperative serum CEA may be of value as a prognostic factor[43,44] but has not been shown to predict resectability. Previous studies also suggested that immunocytochemical CEA staining could neither predict survival nor be correlated with serum CEA.[45] Although CEA was not an individually prognostic factor in our whole group of patients, it was a weakly prognostic factor in the subgroup with squamous cell carcinoma. This association also remains to be proven. There is no clear explanation from published investigations[43] as to the exact mechanism of elevated CEA levels in lung cancer. Similarly, although the observed association between the squamous cell presence of CEA and survival is significant, its meaning is unclear but potentially important.

Two main limitations of this study must be addressed. First, because the histologic analysis for this study was performed between 1988 and 1991, several new and potentially more powerful tumour markers were not studied. Therefore no comment can be made on the prognostic value of p53, factor VIII, *erb-b2*, CD44, or retinoblastoma recessive oncogene in our patients.[46] Second, the relatively small sample of patients may decrease the power of the study. However, with proper statistical analysis, this potential drawback has been negated.

The explanations surrounding immunity and survival are admittedly speculative at this point, but they should not mask the observation that humoral (B-cell) immunity played a role as a prognostic marker in our series. Although no single serum or tu-

mour marker may ever adequately predict survival, the combination of stage and certain factors for specific tumours may provide clinicians with more accurate means of assessing prognosis. This work suggests that B-cell immunity for nonsquamous cell carcinomas and measurement of CEA for squamous carcinomas may be such factors.

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SESAP Questions
Questions SESAP

Category 6, Items 30 and 31

A 41-year-old man is brought to a community hospital for stabilization prior to transport after a motorcycle crash. He is comatose and hemodynamically labile and is intubated translaryngeally. Breath sounds are equal bilaterally. A pneumatic compression garment (MAST) was applied in the field because of an unstable pelvic fracture and transient episodes of hypotension and he was given three units of packed red blood cells.

After transport and with a fourth unit being infused the patient has a systolic pressure of 90 torr. He has a blown left pupil, widened mediastinum, unstable pelvic fracture and C3–4 subluxation.

30. Initial management should be

(A) immediate celiotomy
(B) computed tomographic scan of the head, abdomen, and pelvis
(C) angiographic embolization of a pelvic bleeding site
(D) diagnostic peritoneal tap / lavage
(E) a burr hole and emergency thoracotomy to repair traumatic aortic disruption

31. The LEAST likely possible injury contributing to this patient's hemodynamic lability would be a(n)

(A) unstable pelvic fracture
(B) cervical spine fracture
(C) traumatic aortic disruption
(D) solid intra-abdominal organ injury
(E) closed head injury

For the 2 incomplete statements above, select the answer that is best out of the 5 given for each item.

For the critique of Items 30 and 31, see page 209.