Five-Year Follow-up Study of Independent Clinical and Flow Cytometric Prognostic Factors for the Survival of Patients with Non-Small Cell Lung Carcinoma

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ABSTRACT

Fresh surgical specimens of tumors of 187 patients with previously untreated non-small cell lung carcinomas were investigated by means of flow cytometry. The aim of the study was to look for cellular prognostic indicators for survival times of these patients in addition to the well-known clinical prognostic factors. All patients had a minimum of 5 years follow-up. Patients with aneuploid tumors had significantly shorter survival times than those with diploid tumors ($P < 0.001$). Identical results are obtained when the analysis is restricted to just those patients with $T_4$ tumors or to patients with metastatic tumors at time of surgery or who were classified as Stage III ($P < 0.01$). These data indicate that DNA ploidy is a strong and independent prognostic factor in patients with non-small-cell lung carcinoma. Patients having tumors with a high proliferative activity died significantly ($P < 0.05$) earlier than patients having tumors with lower proliferative activity. As with tumor ploidy, survival time in patients with high or low proliferative tumor activities was independent of whether the patients had $T_4$-tumors, metastases, or were in Stage III. Univariate and multivariate analyses of the data in this study demonstrate two groups of independent prognostic factors for the survival of patients with non-small-cell lung carcinoma: a group of clinical factors and a group of flow cytometric factors.

INTRODUCTION

Although many histological types of lung cancer exist, the vast majority of cases fall into one of the two groups that are treated very differently: small and non-small-cell lung carcinomas. Histological features, clinical course, and response to treatment indicate that small cell lung carcinomas are a separate entity indeed, whereas NSCLC$^2$ represent a mixed group of tumors with overlapping histologies, clinical courses, and responses to treatment. Thus, an accurate prediction of tumor progression and patient survival remains a major problem for these tumors. The aim of this study, therefore, was to look for cellular prognostic indicators in NSCLC in addition to the well known clinical prognostic factors.

There is increasing evidence that in a variety of tumors DNA content may be of considerable prognostic value with respect to survival (1–14). Some studies on lung carcinomas have also shown a possible relationship between DNA ploidy and biological behavior of these tumors (15–18), while others provide no such evidence (19).

In a preliminary investigation we found that patients with NSCLC whose tumors were aneuploid had a shorter survival time than those with diploid tumors (18). Subsequently, we were able to demonstrate that patients whose tumors had a high proliferative activity also had a worse prognosis (20). In this early study the follow-up was only 1.5 years and even while there may be a difference in the survival 2 years after surgery, by 3 years the differences can disappear (21). In this investiga-

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gaussian fittings according to the method of Fleischer and Pelka (25). A computerized subtraction of exponentially decreasing corrections beginning with the peak of cellular debris was included in the evaluation program (26). Cell cycle analyses with interspersed cell populations (e.g., Fig. 1, right) were deleted from the study.

Documentation and Analysis of Data. The data were stored by the central data processing system of the German Cancer Research Center (VW/CMS operating system, program package SURVIVAL). The method for analysis of survival was the statistical failure time model with censored data according to the method of Kaplan and Meier (27). The multivariate relationship was analyzed according to Cox regression (28, 29). Both statistical methods were integrated in a program package by Edler et al. (30). The cutoff point for low or high fraction of cell cycle phases was determined by the CRITLEVEL procedure (31). This is an efficient graphic tool for determining those levels of quantitative prognostic factors at which the most pronounced deterioration of prognosis takes place.

RESULTS

The prognosis of patients with NSCLC is largely determined by the stage of disease and this is confirmed by the present study (Fig. 2). Extent and location of primary tumor (T), nodal involvement (N), and distant metastases (M) are well-known prognostic factors and their significance is also clearly established in our study. Histology, on the other hand, was not of significant prognostic value.

The results of DNA content analysis and length of survival are summarized in Fig. 3. Analysis of all patients show that those with aneuploid tumors had significantly shorter survival times ($P < 0.001$) than did those with diploid tumors (Fig. 3, top left). While the overall number of patients with diploid tumors is only 27, representing about 15% of the total number of patients studied, it is noteworthy that 16 or almost 60% of these patients survived 5 years or more. Although we have not been able to determine whether all of these 16 patients had no evidence of disease at 5 years the finding that no deaths occurred after the 120th week would tend to substantiate this.

To exclude the influence or possible introduction of a bias into the analysis we further analyzed these data according to patients with $T_3$ tumors, NM+, and in Stage III. The analysis of these homogeneous groups of patients may be found in Fig. 3, top right and bottom. Patients classified as Stage III or with $T_3$ tumors or metastasis at time of surgery and having diploid tumors lived longer ($P < 0.05$) than did those with aneuploid tumors. This finding can also be extended to other homogeneous groups of patients who were treated for instance with surgery alone. Similarly patients with diploid tumor receiving surgery alone survived significantly longer ($P < 0.002$) than did those with aneuploid tumors. This means that DNA ploidy is a significant prognostic factor for the survival of patients with NSCLC.

The results of the analyses in 122 cases of the proportion of tumor cells in different stages of the cell cycle are demonstrated in Fig. 4 and Table 1. Patients who had tumors with a low proliferative activity as demonstrated by more than 78% of the cells in Go-G1, or less than 22% of the cells in S-G2-M lived longer ($P < 0.05$) than patients with more active tumors (Fig. 4). The same trends can be demonstrated when the S-phase and G2-M cells are equal to or less than 8 and 14%, respectively. The same results are obtained when the analysis is restricted to just those patients with $T_3$ tumors, or with metastases at time of surgery, or who were classified Stage III (Table 1). This finding means that proliferation is a strong prognostic factor for the survival time of patients with NSCLC as well.

The correlation coefficient according to Pearson was tested
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HISTOLOGY

LOG-RANK
n.s.

○ EPIDERMOID CA (n=100)
△ LARGE CELL CA (n=26)
□ ADENO CA (n=49)

T

LOG-RANK
p<0.01

T1,2 (n=57)
T3 (n=118)

NM

LOG-RANK
p<0.001

NM− (n=70)
NM+ (n=103)

STAGE

LOG-RANK
p<0.01

STAGE I,II (n=48)
STAGE III (n=127)

Fig. 2. Survival curves of patients with NSCLC (Kaplan-Meier-estimates) subdivided according to histology (top left) T (top right), lymph node involvement and metastases (NM, bottom left), and stage (bottom right). NM−, without lymph node involvement and without metastases; NM+, with lymph node involvement or with metastases.

for significance by Fisher's Exact Test. No significant correlation was found between any clinical and any cytometric factors, clearly indicating independence between clinical and cytometric variables. Univariate and multivariate (28) analyses of the data in this study demonstrate two groups of independent prognostic factors for the survival of patients with non-small cell lung carcinoma: a group of clinical and a group of flow cytometric factors.

DISCUSSION

With most diseases and in particular with cancer it is important to have a complete work-up before treatment. This affords the physician some evaluation of the prognosis and the treatment to use. In lung cancer correct staging is important because the prognosis for survival depends on size and location of the neoplasm and whether it has metastasized.

In addition to histology which in our study has no prognostic value flow cytometry of the primary tumors allows further characterization of the tumors. Flow cytometry can provide two pieces of information about the tumor: assessment of ploidy which seems to be correlated with malignant aggressiveness and measurement of the fraction of the different phases of the cell cycle, which is an index of proliferative activity.

The present report shows that cytometric analysis clearly has prognostic importance in patients with NSCLC. Regardless of stage, or whether the patients had a T1 tumor or were metastatic, patients with DNA diploid tumors lived longer than patients having DNA aneuploid tumors. This difference in survival was still significant in patients who had a minimum of 5 years' follow-up. Therefore, we conclude that DNA ploidy is an independent prognostic factor from clinical characteristics in patients with NSCLC. In addition, we found that the survival curves of patients with diploid tumors plateaued at 2 years, whereas patients with aneuploid tumors continued to die. Heddley et al. (10) working with breast cancer patients published similar results.

Several authors (10, 32, 33) measured the DNA content in nuclear suspensions prepared from archival paraffin-embedded tumor size. The advantage of their procedure is that survival and disease progression data are already available. A disadvantage of this technique is that the overall quality of DNA histograms obtained from paraffin-embedded tissue is generally inferior to that obtained with fresh tissue (32). Using this method Zimmerman et al. (33) substantiated our results by recently publishing a report that patients with aneuploid lung tumors had significantly shorter survival than those with diploid tumors. In our study only fresh tumor specimens were processed and we could therefore also examine cell cycle parameters.

A disadvantage with flow cytometry is that cell cycle analysis cannot be done in all cases. Whereas in this study we were able to obtain DNA indices of all 187 tumors, cell cycle analyses
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Fig. 3. Survival curves of patients with NSCLC (Kaplan-Meier-estimates) subdivided according to ploidy in all patients (top left) and according to T3 (top right), NM+ (bottom left), and Stage III (bottom right).

Fig. 4. Survival curves of all patients with NSCLC (Kaplan-Meier-estimates) subdivided according to cell cycle phases (G0-G1; S phase; G2-M; S-G1-M).
were possible in only 122 cases (65%). One-third of the patients (n = 65) could not be included in cell cycle analysis because of the overlapping of DNA steniles. The results of the analyses show that patients whose tumors had a high proliferative activity, (G₀-G₁ proportion <78%; S-phase proportion >8%; G₂-M proportion >14%; S-G₂-M proportion >22%) died significantly earlier than patients with lower proliferative activity. As with tumor ploidy, survival time in patients with high or low proliferative tumor activities was independent of whether the patients had T₃ tumors, metastases (NM+), or were in different stages.

Each method of cell cycle analysis has its own specific limitations with both cell preparation and the computer evaluation. In our analysis the original distribution of S-phase cells is separated from background debris by computerized subtraction. Nevertheless an overestimation of the S-phase fraction cannot be excluded. Indeed, a significant correlation between the labeling index determined by autoradiographic means and the S-phase fraction determined by flow cytometry is demonstrable only by omission of S-phase values above 16% (20). In the present study we divided the groups according to S-phase < 8% without restrictions. This may be the reason why the S-phase fraction was not as significant a prognostic factor as the other cell cycle parameters. Based on our data we believe that classification of the patients according to S-G₂-M fraction is more useful than S-phase fraction as a prognostic factor for patients with NSCLC.

In conclusion, the results of this present study demonstrate clinical and flow cytometric prognostic factors for the survival of patients with NSCLC. The group of clinical and the group of flow cytometric factors are independent.

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REFERENCES


Table 1 Median survival times of patients with NSCLC subdivided according to cell cycle distribution

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<tr>
<th>Clinical Characteristics</th>
<th>S phase ≤ 8%</th>
<th>S phase &gt; 8%</th>
<th>S-G₂-M&lt; 22%</th>
<th>S-G₂-M&gt; 22%</th>
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<tr>
<td>Clinical Characteristics</td>
<td>Total no. of patients (dead)</td>
<td>MST* (wk)</td>
<td>Total no. of patients (dead)</td>
<td>MST* (wk)</td>
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<td>All patients</td>
<td>45 (27) 117 69 (53) 62</td>
<td>0.041 60 (37) 119 54 (43) 56</td>
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<td>T₂</td>
<td>30 (20) 84</td>
<td>49 (43) 45</td>
<td>0.020 41 (29) 79 38 (34) 39</td>
<td>0.065</td>
</tr>
<tr>
<td>NM+</td>
<td>24 (16) 88</td>
<td>43 (34) 43</td>
<td>0.103 37 (24) 88 33 (20) 38</td>
<td>0.019</td>
</tr>
<tr>
<td>Stage III</td>
<td>32 (21) 84</td>
<td>51 (44) 85</td>
<td>0.025 44 (30) 79 39 (35) 41</td>
<td>0.032</td>
</tr>
<tr>
<td>Surgery alone</td>
<td>33 (17) 144</td>
<td>45 (32) 89</td>
<td>0.074 41 (21) 144 37 (28) 87</td>
<td>0.041</td>
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* Median survival time.
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