The Clinical Value of Lymphatic Micrometastases in Patients with Non-small Cell Lung Cancer

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Introduction: In early stage non-small cell lung cancer (NSCLC), presence of lymphatic micrometastases and isolated tumor cells, primarily detected by immunohistochemistry, is suggested to be a prognostic factor. However, there is no consensus whether immunohistochemistry should be used routinely in lymph node assessment.

The goal of our study was to determine whether recurrent disease is associated with the presence of lymphatic micrometastases and/or isolated tumor cells, at the time of the lung resection.

Methods: We retrospectively analyzed the prevalence of lymphatic micrometastases and/or isolated tumor cells in two groups of patients, who underwent a curative resection for early stage NSCLC. Group I had a follow-up of 5 years without recurrent disease. Group II consisted of a matched group of patients with recurrent disease. Patients were originally classified as having negative mediastinal lymph nodes.

All lymph nodes obtained by mediastinoscopy and thoracotomy were re-examined by serial sectioning and immunohistochemistry.

Results: Micrometastases and/or isolated tumor cells were found in one of 16 patients in group I, which was significantly different from six of 16 patients in group II. (Fisher exact test, 4.6; p, 0.04; risk ratio, 2.4).

Serial sectioning and immunohistochemistry did not change N-stage for the single patient in group I, in contrast to all six patients in group II.

Conclusion: Presence of lymphatic micrometastases and/or isolated tumor cells is associated with distant recurrence in patients with early stage NSCLC. We recommend the routine use of serial sectioning and immunohistochemistry in lymph node assessment to improve the accuracy of staging.

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In patients with non-small cell lung cancer (NSCLC), regional lymph node involvement is a major prognostic factor, and in the absence of distant metastases, assessment is essential to determine the appropriate therapy. In clinical practice, nodal assessment is based on computed tomography scan and fluorodeoxyglucose-positron emission tomography (FDG-PET), to provide anatomic and metabolic information, respectively. Histologic proof of lymph node involvement is preoperatively obtained by endoscopic needle biopsy and/or mediastinoscopy. Moreover, in case of a lung resection, both intrapulmonary and mediastinal lymph nodes are recommended to be resected, enabling classification in a pathologic stage of the disease.[1]

However, despite an intended curative treatment, 25 to 50% of patients with early stage lung cancer develop a recurrence during follow-up,[2] thus, suggesting occult disease and inaccurate staging. This occult disease may be represented by lymphatic spread of isolated tumor cells and micrometastases, defined as small clusters of tumor cells of 0.2 mm or less and clusters of 0.2 mm to 2 mm in diameter, respectively.[3]

Several reports[4–6] have addressed the prognostic significance of lymphatic isolated tumor cells and micrometastases, but controversy remains about their clinical impact.[7–11] If clinically important, detection of isolated tumor cells and micrometastases not only affects staging and therapy, but may also act on the position of invasive diagnostics in the workup of patients with lung cancer, because there is a different yield of lymphatic tissue obtained by different diagnostic tools.

The goal of this study was to determine whether recurrent disease was associated with the presence of lymphatic isolated tumor cells and/or micrometastases at the time of the lung resection.

PATIENTS AND METHODS

Design

To determine the clinical value of lymphatic isolated tumor cells and micrometastases, we analyzed their preva-

lence in two groups of patients who had undergone a complete resection for early stage NSCLC in a retrospective case control study.

All patients were originally classified as having negative mediastinal lymph nodes, both after clinical workup and based on the final pathology report after surgery.

Group I consisted of patients without any sign of recurrence during a follow-up of at least 5 years after the operation. This group was matched with a second group of patients, who did develop recurrent disease during follow-up, despite their complete resection (group II).

Patients were matched for age, sex, performance status, weight loss, histology, type of resection, and pathologic tumor, node, metastasis stage.[2] Patient characteristics are presented in Table 1.

Patients were included in this study retrospectively from a surgical data base. Follow-up information was collected from the patient's medical files of the referring pulmonologists.

Surgery

Surgical treatment consisted of (at least) a lobectomy with en bloc resection of the lobar lymph nodes. Dissection of interlobar and hilar lymph nodes was routinely performed, as well as a lobe specific mediastinal lymph node dissection.[1] On average, 4.5 mediastinal lymph node stations (range 3–6) per patient were dissected. Surgery was supposed to be

complete if the bronchial, vascular, and pleural resection margins were free, and there was no involvement of the mediastinal lymph nodes.

None of the patients in this study received induction- nor adjuvant chemo- or radiotherapy.

Pathology

Of the patients included in this study, first a re-examination of the original hematoxylin and eosin stained slides of both the primary tumor, bronchial resection margin, and lymph nodes obtained by mediastinoscopy and thoracotomy was carried out to confirm histology and pathologic stage.

Next, all formalin-fixed and paraffin-embedded lymph nodes were once more sectioned at two levels of 50- and 85-μm deep. At each level, a 5-μm tissue section was taken and hematoxylin-eosin stained for pathologic assessment. Second, two 5-μm tissue sections at levels of 60- and 65-μm deep were taken and immunohistochemically stained with the keratin monoclonal antibodies Cytokeratin pan AE1/ AE3 (1:200; Neomarkers, Fremont, CA) and Cytokeratin 8/Cam5.2 (1:20; Becton & Dickinson, Franklin Lakes, NJ) using the standard avidin-biotin-complex technique.[12] After the first incubation with the keratin antibodies, a second incubation was performed with horse-antimouse biotinylated antibody (Vector laboratories, Burlingame, CA). Subsequently, all attached antibodies were developed using the avidin-biotin-peroxidase method Vectastain, ABC-kit Elite standard (1:100, Vector Laboratories). Finally, all tissue sections were shortly counterstained with hematoxylin. Appropriate negative and positive controls were runned parallel with the staining procedures.

Assessment of these additional slides after immunohistochemistry and reclassification of nodal status was done by a pathologist unaware of a recurrence during follow-up.

Statistics

Based on previous reports,[4–9,11] we hypothesized a prevalence of isolated tumor cells and/or micrometastases in our patients of 25% and intended to demonstrate a different prevalence between the group with and without recurrent disease of at least 35%. The α (error probability) was set to 0.05. A power analysis (with a z-test proportion method for differences between two proportions) revealed that the study population had to consist of two groups of 16 patients each. A Pearson χ^2 test (Fisher exact test) was used to demonstrate the difference between both groups. A one-tailed significance level of 0.05 was considered significant. Statistical analyses were performed using SPSS software, version17.0 (SPSS, Inc., Chicago, IL).

RESULTS

Pathologic Assessment

In addition to the lobar and interlobar lymph nodes of each patient, 145 mediastinal lymph node stations, with an average of 2.4 lymph nodes per station (range 1–7), in 32 patients were available for re-examination.

Reassessment of the original hematoxylin and eosin stained slides, to confirm the histologic diagnosis and stage,

TABLE 1. Clinical and Pathologic Characteristics of Patients With and Without Recurrence After 5-yr Follow-Up (*n* = 32)

	No Recurrence, Group I (*n* = 16)	Recurrence, Group II (*n* = 16)
Age		
Mean (range)	64 (54–77)	62 (38–70)
Sex		
Male	15	13
Female	1	3
ECOG performance status		
0	1	1
1	15	15
Weight loss[a]		
Yes	3	4
No	13	12
Histology		
Squamous cell carcinoma	13	12
Adenocarcinoma	1	4
Large cell carcinoma	2	—
Type of resection		
Lobectomy	7	6
Pneumonectomy	9	10
Stage (pTNM)		
T1N0	2	1
T2N0	9	9
T2N1	5	6

[a] More than 5% in 6 mo before initial diagnosis.
ECOG, Eastern Cooperative Oncology Group; pTNM, pathologic tumor, node, metastasis.

revealed an overt hilar lymph node metastasis in one patient that was missed at first examination, but no mediastinal lymph node metastases were detected.

After subsequent immunohistochemical analysis, additional lymph node metastases were identified in seven of 32 patiens (22%).

In group I, consisting of patients without recurrent disease, micrometastases were detected in hilar lymph nodes in only one patient (6%). This patient had already been staged pT2N1, based on direct tumor invasion of an adjacent lymph node.

In group II, patients with recurrent disease, isolated tumor cells and/or micrometastases were identified in six patients (38%), which was significantly different from the single patient in group I (Fisher exact test, 4.6; *p*, 0.04; risk ratio, 2.4).

Influence on Stage

For the single patient in group I, the pathologic stage remained unchanged. In group II, initial staging was pT1N0 in 1 patient, pT2N0 in nine patients, and pT2N1 in six patients. In three of these nine patients staged pT2N0, isolated tumor cells and/or micrometastases were identified (33%), in two patients in both N1 and N2 lymph nodes, and in one patient in hilar lymph nodes only.

In three of the six patients, initially staged pT2N1 positive mediastinal lymph nodes were identified (50%).

Because of the serial sectioning and immunohistochemistry, five of six patients from group II were upstaged: from stage Ib to stage IIIa in two patients, from stage Ib to IIb in one patient, and from stage IIb to IIIa in two patients. In the remaining patient from group II, initially staged IIb, the N-stage changed from N1 to N1 (i+), because only isolated tumor cells were found in mediastinal lymph nodes, but no micrometastases were found.

The yield of pathologic re-examination and stage migration observed by serial sectioning and immunohistochemistry are shown in Table 2.

Time to Recurrence

For the patients in group II, the time interval from the operation until the first sign of a metastasis ranged from three to 48 months, with a mean interval of 12.8 months. The interval tended to be shorter for patients with micrometastases (mean 8.6 months, range 5–16) than for patients without micrometastases (mean 15.4 months, range 3–48), but there was no significant difference.

In all patients with micrometastases, the first clinical recurrence was at a distant site.

DISCUSSION

Our study shows that the prevalence of lymphatic micrometastases and isolated tumor cells is significantly increased in patients who underwent a complete resection for NSCLC and developed distant recurrent disease during follow-up, compared with a matched group of patients without recurrent disease. This demonstrates that minimal lymph node involvement represents tumor dissemination with a significant clinical impact, that is, prediction of tumor recurrence.

Although micrometastases can be detected by using hematoxylin and eosin staining only, detection may be improved by serial sectioning, immunohistochemistry, or molecular techniques as (reverse transcription-) polymerase chain reaction. Moreover, these methods are essential to detect isolated tumor cells, but thus far none of these methods is routinely used in lymph node assessment in NSCLC. We retrospectively demonstrated the presence of lymphatic micrometastases and isolated tumor cells by pathologic re-examination with serial sectioning and immunohistochemistry at two levels.

The overall prevalence of micrometastases and isolated tumor cells in our patients of 22% is within the range of expected and comparable to other studies, with a prevalence ranging from 16 to 74%.[4–9,11] Obviously, the prevalence may be influenced by the study design. By definition half of our study population had a favorable outcome, with an expected limited prevalence of occult disease.

Because of the serial sectioning and immunohistochemistry, five of 16 patients with recurrent disease seemed to have mediastinal dissemination. Whether their prognosis is the same as for patients with overt N2 disease cannot be answered from this study. Minimal N2 disease may represent a favorable subgroup in patients with mediastinal lymph node involvement,[13] on the other hand, in a recent study by Riquet

TABLE 2. Stage Migration of Seven Patients with Lymph Node Involvement Detected by Serial Sectioning and Immunohistochemistry

Patient (Study nr.)	Histology	Original p Stage	Yield of Re-Examination	p Stage After IHC
Group I: no recurrence				
26	Squamous cell	T2N1	Micrometastases N1	Unchanged
Group II: with recurrence				
6	Squamous cell	T2N0	Micrometastases N1 + N2	T2N2
9	Adeno	T2N1	Micrometastases + ITCs N2	T2N2
10	Adeno	T2N1	ITCs N2	T2N1 (i+)
14	Squamous cell	T2N0	ITCs N1/micrometastases N2	T2N2
19	Squamous cell	T2N1	Micrometastases N2	T2N2
27	Adeno	T2N0	Micrometastases N1	T2N1

IHC, immunohistochemistry; ITCs, isolated tumor cells.

et al.,[14] patients with micrometastatic N2 disease seemed to have the same poor outcome as patients with bulky N2 disease, although immunohistochemistry was not used to detect these micrometastases. The authors suggest micrometastases to be the consequence of a more aggressive biologic behavior, resulting in a worse outcome. Moreover, a relationship of micrometastases with micropapillary adenocarcinoma has been suggested previously.[15]

Rather than being a separate entity, micrometastases and isolated tumor cells may also just represent an early phase in the development of lymph node metastases and thus being the result of a more accurate pathologic assessment. This would be in line with a high prevalence of mediastinal micrometastases in patients with overt N1 disease, as found in our study.

The occurrence of any lymphatic dissemination may well be the result of specific biologic features of a carcinoma that can be different from one tumor to another. Detterbeck et al.[16] proposed four distinguished patterns of biologic behavior of lung cancer, with a separate propensity for local invasion, lymphatic and hematogenous dissemination, and multifocal spread in lung parenchyma. Patients with micrometastatic lymph node involvement without metastases at a distant site seem to have at least a propensity for lymphatic dissemination, detected in an early phase. Nevertheless, because all patients with lymphatic micrometastases in our study had a first recurrence at a distant site, the propensity for lymphatic dissemination seems to predict the propensity for hematogenous dissemination as well, as has been found by others.[17]

In our study in 10 patients with recurrent disease, no lymphatic micrometastases were detected. Although some lymphatic micrometastases may be missed, because we performed immunohistochemistry on two additional slides of each lymph node only, the propensity for hematogenous dissemination was probably independent from lymphatic dissemination in these patients. Moreover, the time interval until their first recurrence for these patients was not significantly different from patients with lymphatic micrometastases, but both groups are small and our study was not designed to find a difference between these patient subsets.

If lymphatic micrometastases represent clinically important tumor dissemination, the question raises about the optimal staging and treatment algorithm. When immunohistochemistry is routinely applied in case of a cervical mediastinoscopy, the diagnostic yield is expected to increase,[18] resulting in a new subset of patients with minimal mediastinal involvement. Results of surgery as a first line treatment in patients with preoperatively detected N2 involvement are poor,[19] so probably induction or even definitive chemoradiotherapy will be offered to these patients.

On the other hand, mediastinal staging based on computed tomography, FDG-PET, and eventually esophageal ultrasound or endobronchial ultrasound-fine needle aspiration is gaining interest with an expected lower yield of lymphatic tissue,[20] underestimating the incidence of micrometastases and isolated tumor cells. After a subsequent resection, including mediastinal lymph node dissection, the use of serial

sectioning and immunohistochemistry will then increase the incidence of unexpected N2 involvement and hereby the indication for adjuvant therapy. Which of these treatment sequences is most optimal for this subset of patients with microscopic N2 disease is unclear and has to be determined.

Routine use of serial sectioning and immunohistochemistry in lymph node assessment will add extra cost and time to the pathology department, what may hamper general application. However, the price of serial sectioning and immunohistochemical staining at two levels in our study was only $22.50 per lymph node and assessment took approximately 5 minutes extra. Because we examined an average of 4.5 mediastinal lymph node stations per patient, containing 2.4 lymph nodes, the extra cost with regard to the mediastinal lymph nodes was $245.00 and approximately 1 hour of time. In comparison, the price of FDG-PET in our institution is approximately $1700.00, and assessment also takes half an hour.

Despite our results, confirmation in a large prospective trial is desirable. Preferably, only patients without any lymph node involvement at all after staining with hematoxylin and eosin are included, so that micrometastases are the only kind of dissemination.

In conclusion, the prevalence of lymphatic micrometastases detected by serial sectioning and immunohistochemistry is higher in patients with NSCLC with recurrent disease after a complete resection then in patients without recurrent disease, thus, representing a kind of tumor dissemination with a clear impact on prognosis. We recommend the routine use of serial sectioning and immunohistochemistry in lymph node assessment of both lymphatic tissue obtained by mediastinoscopy and after thoracotomy, to improve the accuracy of staging. The optimal treatment for patients with minimal mediastinal lymph node involvement still has to be determined.

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