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Pleural Lavage Cytology Immediately after Thoracotomy as a Prognostic Factor for Patients with Lung Cancer

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Pleural lavage cytology was examined in 230 lung cancer patients just after opening the chest. There were 16 cases (7.0%) of positive pleural lavage cytology, and the results of pleural lavage cytology were related to the presence of pleural involvement by cancer, microscopical pleural dissemination and lymphatic permeation of the cancer cells. If the cancer involves the pleura or lymphatics of the submesothelial layer, being covered with visceral mesothelium, positive cytology may still be obtained. Pleural lavage cytology at opening of the chest seems to be available as a premonitory indicator for exfoliation and dissemination into the pleural cavity or subpleural lymphatic extension of cancer cells, and it was suggested that positive pleural lavage cytology has an influence on postoperative survival.

Key words: Lung cancer — Pleural lavage — Cytology — Prognosis

It has been reported that peritoneal lavage cytology carried out during operation is an important prognostic factor in patients with abdominal malignancy.[1-4] As regards lung cancer, there has been only one report describing pleural lavage cytology during surgery.[5] Eagan *et al.*[5] performed lavage cytology just prior to closing the chest cavity when the pulmonary resection was completed. The incidence of positive cytological results was correlated with lymph node status, cancer cell type (adenocarcinoma > other non-small cell lung cancer), stage and visceral pleural status. However, their results might reflect the exfoliation of cancer cells during surgical procedures.[6] The prognostic significance of pleural lavage cytology has not been established yet. Furthermore, the significance of pleural lavage cytology just after opening the chest, that is, without any surgical manipulation such as division of the pulmonary parenchyma and dissection of lymph nodes, should also be examined. We intended to clarify the usefulness of pleural lavage cytology, and this is a preliminary report of our study.

MATERIALS AND METHODS

Pleural lavage cytology has been examined in 230 patients with lung cancer during the period from August 1985 to January 1988. Immediately after opening the chest, the pleural cavity was washed with 50 ml of physiological saline solution. The solution was collected, put into a test tube containing 10 mg of EDTA, and centrifuged for 2.5 min at 2,500 rpm. Five milliliters of 0.85% ammonium chloride solution was added to the sediment, and after 10 min the mixture was centrifuged again for 2.5 min with 2,500 rpm. The obtaind sediment was stained with Giemsa and Papanicolaou's stains and with Alcian blue. If massive or moderate pleural effusion was found at thoracotomy, immediate cytological examination of the effusion was performed and pleural lavage was abandoned in cases of positive effusion cytology.

Pathological staging was according to the TNM and Staging System of UICC.[7] Histological type of the tumor was determined by applying the WHO classification.[8] The general rules for reporting of lung cancer proposed by the Japanese Lung Cancer Society[9] were employed for describing the nodal, pleural and d (pleural dissemination) status. Vascular involvement and lymphatic permeation of the cancer cells were examined pathologically.

The chi-square test was used to evaluate the significance of the relationship between positivity of the pleural lavage cytology and each of the clinico-pathological factors. Actual survival curves were plotted using the method of Kaplan and Meier, and the prognostic significance was evaluated by means of the generalized Wilcoxon's test. Subsequently the proportional hazards general linear model procedure in the SAS program package was performed.

RESULTS

The characteristics of the 230 patients examined are shown in Table I. There were 16 patients (7.0%) who showed positive lavage cytology out of the 230. As for pleural involvement, there was no case of positive cytology in the p0 group (visceral pleura not involved by tumor). Positive results of lavage cytology were seen at

Table I. Histologic Types and Pathological Status of the 230 Patients with Pleural Lavage Immediately after Opening of the Chest

Histologic type	Pathological stage					
	I	II	IIIA	IIIB	IV	Total
Adenocarcinoma	67	10	30	5	17	129
Squamous cell carcinoma	28	10	22	1	5	66
Large cell carcinoma	5	1	8	0	2	16
Adenosquamous carcinoma	1	0	1	0	2	4
Small cell carcinoma	1	0	3	1	1	6
Others	5	1	2	1	0	9
Total	107	22	66	8	27	230

Table II. Relationship between Pleural Involvement and Results of Pleural Lavage Cytology

Cytology	Pleural involvement				
	p0[a]	p1	p2	p3	Total
Positive	0[b]	8	4	4	16
Negative	117	37	13	46	213
Total	117	45	17	50	229[c]

a) See text for p0, p1, p2 and p3.
b) There is a significant difference with $P<0.01$ between the p0 group and the sum of p1+p2+p3 groups (chi-square test).
c) Pleural involvement was not identified in one case.

the rates of 17.7% (8/45), 23.5% (4/17) and 8.0% (4/50) in p1 (cancer had reached but not invaded through the visceral pleura), p2 (cancer had invaded the visceral pleura but not involved the parietal pleura) and p3 (cancer had invaded the pleura and the chest wall) groups. Though there was no statistically significant difference between each group, a significant difference with $P<0.01$ was seen between the p0 group and the total of p1+p2+p3 (Table II).

Histologic type, pathological stage and lymph nodal status had no correlation with positivity of pleural lavage cytology (Tables III and IV).

As for d-factor (pleural dissemination), there were 8 cases with pathologically confirmed pleural dissemination and all of them had little or only a physiological amount of pleural effusion at opening of the chest wall. In 2 cases cytological examination of the small amount of effusion was performed and was negative for cancer cells. In the other 6 cases with dissemination whose pleural effusion could not be obtained, 2 cases gave positive lavage cytology. That is, positive lavage cytological results were seen in 2 out of 8 (25%) disseminated cases with no evidence of "clinically" malignant pleural effusion. This correlation was statistically significant ($P<0.05$).

Vascular involvement (v-factor) and lymphatic permeation (ly-factor) of the tumor were examined pathologically in 222 cases. In 1 patient with positive lavage cytology, v- and ly-factors could not be evaluated. Two

Table III. Relationship between Histologic Type, Stage and the Result of Pleural Lavage Cytology

Stage	Histologic type						
	Ad	Sq	Ad/Sq	Sm	La	Others	Total
I	4/67[a]	0/28	1/1	0/1	0/5	0/5	5/107
II	1/10	0/10	0/0	0/0	0/1	0/1	1/22
IIIA	2/30	2/22	0/1	1/3	0/8	0/2	5/66
IIIB	1/5	1/1	0/0	0/1	0/0	0/1	2/8
IV	1/17	1/5	1/2	0/1	0/2	0/0	3/27
Total	9/129	4/66	2/4	1/6	0/16	0/9	16/230

Abbreviations: Ad, adenocarcinoma; Sq, squamous cell carcinoma; Ad/Sq, adenosquamous carcinoma; Sm, small cell carcinoma; La, large cell carcinoma.
a) Number of positive lavage cytology cases/total number of cases.

Table IV. Relationship between Lymph Nodal Involvement and Result of Pleural Lavage Cytology

Cytology	Lymph nodal involvement				
	n0[a]	n1	n2	n3	Total
Positive	8	3	4	1	16
Negative	120	34	54	4	212
Total	128	37	58	5	228[b]

a) n0, no lymph-node metastasis; n1, metastasis in hilar nodes; n2, metastasis in mediastinal nodes; n3, metastasis in contralateral mediastinal and/or supraclavicular nodes.
b) Two cases were excluded for lack of pathological examination of mediastinal lymph nodes.

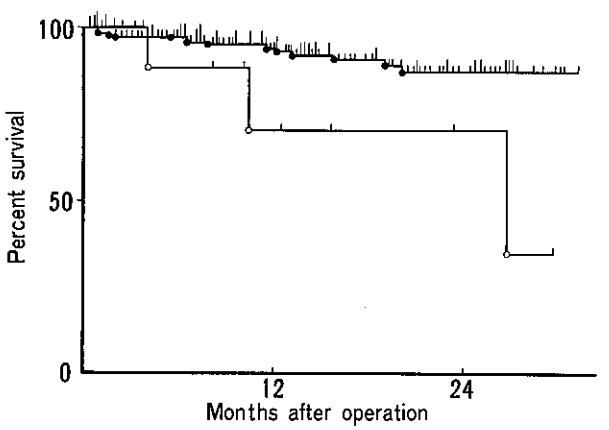

Fig. 1. Survival of patients with positive and negative lavage cytology immediately after opening of the chest (Kaplan-Meier's method); ○, 16 cases with positive cytology; ●, 214 cases with negative cytology.

out of 79 cases without vascular involvement showed positive lavage cytology, while 13 out of 143 with vascular involvement showed positive cytology. There was no statistical significance with regard to the v-factor. As for the ly-factor, 14 out of 124 cases with ly(+) had positive lavage results and only 1 out of 98 cases with ly(−) had a positive result. There was a significant correlation between the results of lavage cytology and the ly-factor ($P<0.01$).

Although the follow-up period was only 30 months at the longest, as shown in Fig. 1, at 2 years and 3 months after surgery, the survival rate for patients with positive cytology was 35% and that for patients with negative cytology was 87%. Three patients with positive cytology died of the disease and one of them had pleural dissemination. This patient had a p1 disease. One survivor with positive cytology has a recurrence in the pleural cavity. There was a strongly suggestive difference ($P=0.052$) in survival rate between the group with positive cytology and that with negative cytology. Multivariate analysis suggested that the result of pleural lavage cytology at opening of the chest is available as a postoperative prognostic factor in lung cancer patients (Table V).

Table V. Results of Multivariate Analysis (Proportional Hazards General Linear Model Procedure)

Variable	Beta	Standard error	Chi-square	*P*
Lavage cytology	1.082	0.645	2.82	0.093
Cell type	−0.317	0.548	0.33	0.564
Stage	0.559	0.165	11.47	0.007

DISCUSSION

A positive cytological result of pleural lavage cytology at opening of the chest is not synonymous with malignant pleural effusion or pleural dissemination of cancer cells, so it should not be counted in the TNM staging system (malignant pleural effusion is T4 disease).[7]

In this series, positivity of pleural lavage cytology immediately after thoracotomy was correlated with the presence of cancer involvement to the pleura and lymphatic permeation of cancer, and the incidence of positive lavage cytology was higher in microscopical disseminated diseases in which there was little pleural effusion or no gross disseminated nodule found on the pleural surface.

Several theories have been proposed concerning the mechanism of development of pleural dissemination[10]: hematogenous metastasis to the pleura, endolymphatic spread in the subpleural layer, implantation of free cancer cells in the pleural effusion, and so on. The third seems to be the major mechanism, and it is thought to occur via several steps as follows: 1) exfoliation of cancer cells from the pleural surface into the pleural cavity, 2) cancer cells becoming viable in the pleural effusion, 3) implantation at the pleural surface, 4) colony formation in the pleura. On the other hand, the main mechanism of the formation of pleural effusion is impairment of lymphatic drainage through the intrapulmonary lymphatic channel to the mediastinal lymph nodes.[11] It has been reported that in more than 90% of the disseminated cases there are amounts of pleural effusion as large as 500 ml.[12] But there may be some discrepancy between the volume of the pleural effusion and the presence of disseminated nodules on the pleural surface.

It is considered that pleural lavage cytology can detect the following situations: 1) the tumor invades visceral

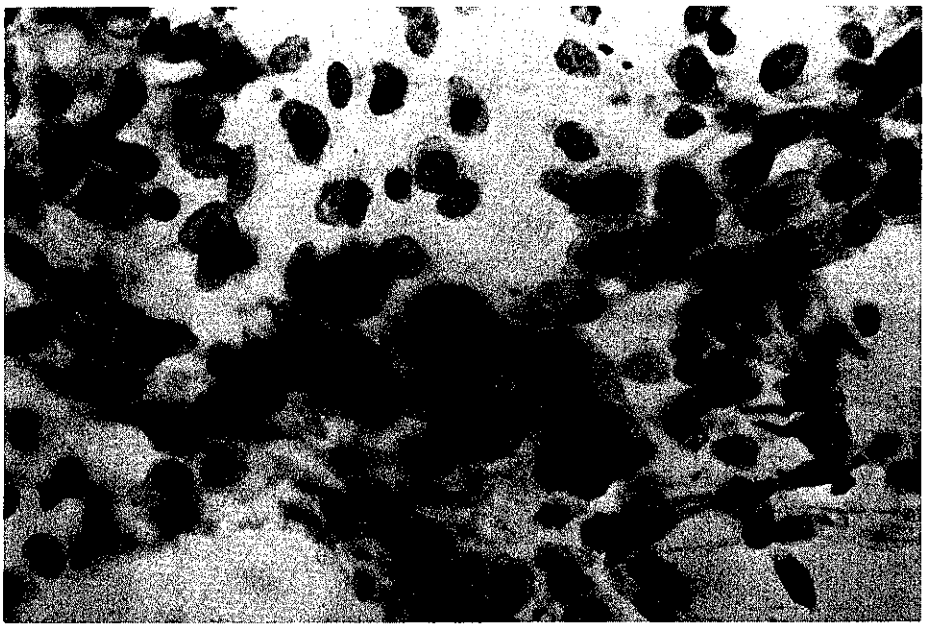

Fig. 2. A sediment obtained from pleural lavage; cancer cells are seen co-existing with desquamated mesothelial cells.

pleura and cancer cells exfoliate into the pleural cavity, 2) cancer cells spread into the subpleural lymphatics, 3) cancer cells are viable in minimal pleural effusion, 4) there is "subclinical" disseminated disease with microscopical colonies on the pleural surface. Pleural lavage cytology may become a useful indicator of cancer extension into the pleural cavity.

The fact that positive lavage cytology could occur in p1 diseases, where cancer involves the visceral pleural elastic layer but is not exposed on the surface, may reflect the ease of exfoliation of cancer cells from the mesothelial layer (Fig. 2). But it may also be suggested that cancer cells can flow out into the pleural cavity through the channels which connect the subpleural lymphatics to the pleural space and are the route of cellular exchange through the pleura.[13]

There are always some problems regarding the accuracy of cytological examination. In this series, the results of cytological examination were classified at first into three categories, i.e. negative, suspicious and positive, which were re-evaluated after the final histopathological diagnoses into two categories: negative and positive. There was no false-positive and no false-negative after the re-evaluation, and all of the suspicious cases proved to be negative. To confirm true positive results, it would be better to use immunocytological stains such as CEA, pulmonary surfactant apoprotein[14] and so on.

It appears that positivity of pleural lavage cytology is available as a postoperative prognostic factor. Further follow-up and more investigations about the mode of recurrence will be needed to answer the question of what therapy should be added in positive lavage cytology cases. In conclusion, pleural lavage cytology immediately after opening of the chest can be said to be an premonitory indicator of cancer extension into the pleural cavity, i.e., of exfoliation, dissemination or subpleural lymphatic spread of cancer cells.

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