

## Intraoperative Fine Needle Aspiration Biopsy and Rapid Nuclear DNA Content Analysis of Lung Tumors

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**Intraoperative fine needle aspiration biopsy using 21 gauge needle was performed on 16 patients with suspected lung cancer during exploratory thoracotomy, and results were obtained from pathologists in about 20 minutes. The biopsy demonstrated 100% accuracy in differentiating malignant from benign tumors. This technique, which can be applied even to tumors located deep within the lung parenchyma with technical easiness and permits quick and accurate biopsy, was evaluated as a useful diagnostic tool.**

**Rapid nuclear DNA content analysis was performed for 13 of the 16 patients concurrently with the intraoperative fine needle biopsy. Results were obtained in about 10 minutes. Decision of surgical procedures was made referring to the result of the analysis in one patient. Importance of nuclear DNA content analysis as well as intraoperative fine needle aspiration biopsy is thus emphasized.**

**Key words:** Intraoperative fine needle aspiration biopsy, Suspected lung cancer, DNA content

### Introduction

While the accuracy of preoperative diagnosis in the area of thoracic surgery, particularly that for lung cancer, has drastically improved in these years, several percent of patients still undergo undiagnosed operation. Surgeons usually perform exploratory thoracotomy for suspected lung cancer even in cases not confirmed by preoperative histological diagnosis and wait for the result of intraoperative rapid diagnosis. Such intraoperative rapid diagnosis includes histological diagnosis by tumor wedge resection and cytological diagnosis by fine needle aspiration biopsy (FNAB). The latter diagnostic tool has some merits including technical simplicity, rapid diagnosis, and easiness in sampling of materials even from deep lesions.<sup>1,2)</sup> We have, therefore, attempted intraoperative rapid cytological diagnosis using FNAB specimen and compared the diagnosis with postoperative histological results.

Recent technological progress facilitates rapid analysis of nuclear DNA content from specimens obtained by intraoperative FNAB, providing new information.<sup>3,4)</sup> As we performed nuclear DNA content analysis concurrently with cytological diagnosis in our patients, we report the results including those of nuclear DNA content analysis and discuss the usefulness of FNAB specimen in diagnosing lung cancer.

### Materials and Methods

Sixteen patients who were suspected of lung cancer by diagnostic imaging and otherwise (by TBLB and other examinations) and thus underwent exploratory thoracotomy, though not confirmed by histological diagnosis, are the subjects of this study. The patients, twelve males and four females, ranged from 28 to 80 years in age with the mean of 60.9 years. Nine were suspected of lung cancer, five of metastatic lung tumor of lung cancer, one of malignant lymphoma or lung cancer and one of infiltrative thymoma or lung cancer in preoperative diagnosis.

Thoracotomy was performed under general anesthesia. FNAB was performed after confirmation of lesions, using a 10ml syringe with a 21 gauge needle attached. The biopsy specimens were directly sent to laboratories for rapid cytological diagnosis by Papanicolaou-stained method. Results were reported in about 20 minutes. Histological diagnosis was later made with postoperative paraffin-embedded specimens fixed in formalin and compared with intraoperative cytological diagnosis.

Concurrent DNA content analysis was available in 13 of the 16 patients. The FNAB specimens were stained with Propidium iodide after addition of 0.1% Triton X-100, and nuclear DNA content was analyzed with a flow cytometer (FACScan, Becton-Dickinson) during operation.<sup>4)</sup> The analysis required about 10 minutes. Nuclear DNA content analysis was done also on two or three sections of several mm square each obtained from the specimen after resection and compared with that on intraoperative FNAB specimen.

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## Results

Two of the 16 patients were diagnosed as benign diseases by intraoperative FNAB. The tumors in these patients were confirmed by postoperative histological diagnosis. The remaining 14 patients were diagnosed as malignancy by both intraoperative rapid cytological diagnosis and postoperative histological diagnosis. A 100% agreement was obtained between the two diagnostic tools (Table 1). The intraoperative FNAB permitted differentiation of tissue types in nine patients, in six of which the diagnosis agreed with postoperative histological diagnosis (Table 2).

**Table 1.** Relationship between cytological diagnosis of intraoperative fine needle aspiration biopsy and histological diagnosis of postoperative tissues in 16 patients of suspected lung cancer.

Patient No.	Cytological findings	Histological findings	Age	Sex
1	Benign cells	Antraco-silicosis	70	Female
2	Benign cells	Tuberculosis	33	Female
3	Malignant cells	Mesothelioma	73	Male
4	Malignant cells	Carcinoid tumor	58	Male
5	Malignant cells	Primary lung cancer	68	Male
6	Malignant cells	Primary lung cancer	66	Female
7	Malignant cells	Primary lung cancer	70	Male
8	Malignant cells	Primary lung cancer	67	Female
9	Malignant cells	Primary lung cancer	63	Male
10	Malignant cells	Primary lung cancer	80	Male
11	Malignant cells	Primary lung cancer	60	Male
12	Malignant cells	Primary lung cancer	64	Male
13	Malignant cells	Primary lung cancer	50	Male
14	Malignant cells	Primary lung cancer	57	Male
15	Malignant cells	Primary lung cancer	67	Male
16	Malignant cells	Metastatic lung cancer	29	Female

**Table 2.** Correlation of histological typing judged intraoperative fine needle aspiration biopsy and postoperative tissues in 16 patients of suspected lung cancer.

Patient No.	Cytological diagnosis	Histological diagnosis
1	Unknown	Anthraco-silicosis
2	Unknown	Pulmonary tuberculosis
3	Mesothelioma	Diffuse Mesothelioma
4	Adenocarcinoma	Carcinoid tumor
5	Unknown	Small cell carcinoma
6	Small cell carcinoma	Small cell carcinoma
7	Small cell carcinoma	Small cell carcinoma
8	Unknown	Squamous cell carcinoma
9	Adenocarcinoma	Adenocarcinoma
10	Unknown	Adenocarcinoma
11	Squamous cell carcinoma	Squamous cell carcinoma
12	Unknown	Squamous cell carcinoma
13	Unknown	Squamous cell carcinoma
14	Large cell carcinoma	Adenocarcinoma
15	Adenocarcinoma	Adenocarcinoma
16	Adenocarcinoma	Adenocarcinoma

Thirteen patients were available for nuclear DNA content analysis, in nine (69.2%) of which DNA Index (DI) agreed completely between FNAB specimen and postop-

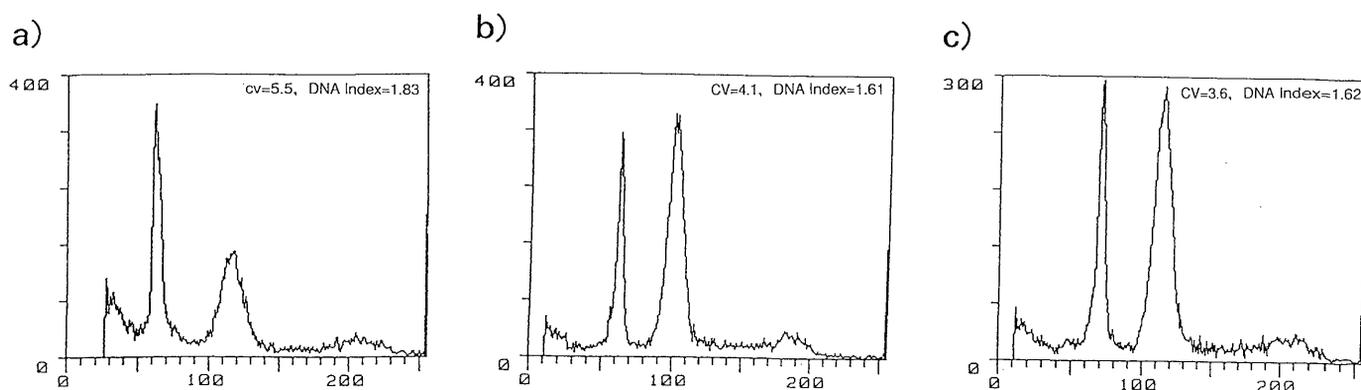
erative tissue specimen. Difference in DI between the two specimens was less than 10%, within the range of error, in the remaining four patients, demonstrating that FNAB specimen is well-available for nuclear DNA content analysis (Table 3).

**Table 3.** Comparison of nuclear DNA content obtained with intraoperative fine needle aspiration biopsy and postoperative tissues in 16 patients of suspected lung cancer.

Patient No.	Intraoperative FNAB	Postoperative Tissue
1	—	—
2	—	—
3	1.00	1.00
4	—	—
5	1.61	1.62
6	1.64	1.64
7	1.53	1.53
8	1.00	1.00
9	1.00	1.00
10	1.90	1.91
11	1.98	1.98
12	1.07	1.10
13	1.00	1.00
14	1.00	1.00
15	1.18	1.21
16	1.73	1.73

Figures showed with DNA Index.

The following is a case in which cytological diagnosis by FNAB and nuclear DNA content analysis supplied important information. The case (Patient No. 5) was 68-year-old male patient, who underwent right lower lobectomy for lung cancer (T<sub>2</sub>N<sub>2</sub>M<sub>0</sub>, Stage IIIA, Squamous cell carcinoma, DI = 1.83) on June 18, 1987, and had been under observation in outpatient clinic. The patient was hospitalized for precise examination since his chest roentgenogram taken on January 17, 1991, demonstrated abnormal shadow in the left upper lobe which gradually extended. A tumor with spicula measuring 2.7 x 2.0cm and with pleural indentation was imaged in the left segment 4b on the CT scanning. It was difficult to decide the basis of diagnostic imaging whether the tumor was primary lung cancer or it was recurrent tumor in the lung. Thoracotomy was performed on March 5, 1991, because other metastasis was not observed and the tumor was regarded as resectable. The surgical procedure was intended to perform partial resection in case of recurrence or left upper lobectomy in case of primary tumor. FNAB was performed on the tumor located in Segment 4 of the left upper lobe. The tumor was judged positive (Class V) by cytological findings based on the observation of cells with highly irregular naked nuclei and of malignant tumor cells with nuclei stained dark, however the cytological diagnosis failed to identify tissue type of the tumor. Concurrently performed nuclear DNA content analysis demonstrated DI = 1.61, which was definitely different from DI = 1.83 of the previous squamous cell carcinoma of the patient (Fig. 1). The tumor, therefore,



**Fig. 1.** DNA histogram of case 5 in patient number. a) DNA histogram obtained from paraffin-embedded tissue of first operation. b) DNA histogram obtained from intraoperative fine needle aspiration biopsy in second operation. c) DNA histogram obtained from postoperative tissue of second operation.

was diagnosed as a methachronous primary cancer, and left upper lobectomy was selected. Postoperative histological diagnosis of the tumor was small cell lung cancer of intermediate cell type, and it was judged, as estimated during operation, to be methachronous primary lung cancer.

## Discussion

Intraoperative rapid cytological diagnosis with FNAB specimen enabled accurate diagnosis with technical ease. The FNAB specimen was available also for concurrent nuclear DNA content analysis and we have experienced case where FNAB specimen provided valuable information.

Of our 16 cases, 14 were diagnosed as malignant and two as benign tumor by intraoperative FNAB, achieving 100% agreement with postoperative histological diagnosis. Such accuracy of diagnosis by intraoperative FNAB, which was reported to be 100% by McCarthy et al.<sup>1)</sup> and 96% by Pantzer et al.<sup>5)</sup> is an evidence that intraoperative FNAB is a highly reliable diagnostic tool. Shiba et al.<sup>2)</sup> reported that only two in 51 subjects were misdiagnosed by this method for malignant or benign disease, suggesting comparatively good diagnostic results. Of 30 cases in their study which had not firmly diagnosed before operation, all the seven malignant tumors were correctly diagnosed based on intraoperative FNAB for malignancy, but correct identification of specific tissue type was made only in five cases. They reported, however, that the result is satisfactory because the primary purpose of intraoperative rapid diagnosis is to determine whether the tumor is malignant or benign disease, and under conditions that the technique has limitations with respect to stainability and time given for diagnosis. FNAB, with high diagnostic accuracy as reported by them, appears to be a highly reliable diagnostic tool.

The nuclear DNA content analysis on the intraoperative FNAB specimen showed identical results to those on postoperative resection specimen, suggesting that the FNAB

specimen can well reflect the nuclear DNA content of the tumor. The incidence of DNA aneuploidy in lung cancer is about 80%<sup>6)</sup> and so a tumor can be judged as malignancy if its nuclear DNA content analysis reveals DNA aneuploidy. Nuclear DNA content analysis can thus be available for an adjuvant diagnostic tool to intraoperative rapid cytological diagnosis.<sup>3,4)</sup> In case there exist two tumors, agreement in DI between the two tumors means lung metastasis and non-agreement means synchronous lung cancer in high possibility.<sup>7)</sup> Analysis of DI in advance on previous tumor specimen, as in Case No. 5 in the present study, might permit presumption whether the tumor is lung metastasis or methachronous primary cancer. This supports the usefulness of nuclear content analysis.

In summary, intraoperative FNAB, as compared with wedge resection of lung tissue, has some merits: it is applicable to tumor of deep lesions, it scarcely disperses tumor cells, it was technical ease and it permits to obtain diagnosis in short time. In our present study, intraoperative FNAB achieved high diagnostic accuracy, and concurrent nuclear DNA content analysis provided useful information in deciding surgical procedure.

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