The Accuracy of Frozen Section Diagnosis of Pulmonary Nodules

Evaluation of Inflation Method during Intraoperative Pathology Consultation with Cryosection

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Introduction: Intraoperative frozen section diagnosis (FSD) is a very important pathologic examination that can determine the extent of the subsequent surgical procedure. However, FSD is especially difficult in small pulmonary nodules due to severe architectural distortion during cryosection. This study was undertaken to determine the accuracy of FSD of pulmonary nodules and to evaluate the inflation method during cryosection.

Methods: We retrospectively reviewed FSD of 229 consecutive pulmonary nodules and evaluated the diagnostic accuracy and efficacy of inflation method during cryosection. Since August 2006, all frozen sections (165, 72.1%) were made after inflation with optimally diluted embedding medium.

Results: The FSD were as follows: nonneoplastic lesions (29, 12.7%), benign neoplasms (28, 12.2%), and malignant neoplasms (172, 75.1%). The proportion of the lesions smaller than 2 cm was 60.3% (138 of 229). The frozen section quality of lung tissue was excellent after inflation with diluted embedding medium. Inflated lung specimens harboring minute lesion displayed distinct gross appearance, which could not be palpated. Histologically, open air spaces and normal parenchymal architectures were well preserved. Minute precancerous foci such as atypical adenomatous hyperplasia and bronchioloalveolar carcinoma could be readily identified. After using inflation method during cryosection, both the sensitivity and specificity reached 100%, and the incidence of intraoperative pathology consultation increased markedly, especially small impalpable lesions.

Conclusions: The accuracy of FSD and histologic qualities were excellent by using inflation method, especially in cases of impalpable small precancerous lesions. The pathologist could guide with confidence the surgeon in planning the surgical management.

Key Words: Lung neoplasm, Frozen section, Accuracy, Inflation.

The present paradigm is that most of untreated lung cancers are rapidly fatal, and consequently early surgical intervention is mandatory.1 With recent advances in computed tomography (CT) screening for lung cancer, the detection of small peripheral lung cancer has been increasing. Most of these CT-detected small lesions are adenocarcinomas (ADCs), which frequently show ground-glass opacity (GGO) on high-resolution CT.2,3 Recently, limited surgery has been tried for the small GGO nodules including Noguchi types A and B, and the result of limited resection for these lesions was promising.4 As a successful limited resection is based on accurate intraoperative pathology diagnosis, the importance of frozen section diagnosis (FSD) is more emphasized than ever. However, the surgical pathologists are presented with a dilemma during intraoperative pathology consultation by minute peripheral lung nodules, which are not diagnosed preoperatively.5,6 Patients with these small peripheral lesions frequently receive their initial diagnosis at the time of intraoperative pathology consultation with frozen section (FS).7 It is difficult to get enough materials with biopsy to be diagnosed as carcinoma or precancerous lesion due to small size of the lesion or its deep location. The accuracy of FSD without preoperative pathologic examination is very important, which guide the extent of surgical treatment in these patients. However, it is difficult to interpret FS of the lung tissue due to severely distorted architecture, ice crystal formation, and complete collapse of the alveolar spaces during cryosection. The diagnosis such as “Atypia, defer to permanent sections” when examining minute pulmonary lesions on FS is often made by the surgical pathologist, as it avoids possible diagnostic errors and potential medico-legal exposure. However, this equivocal FSD
delays the correct diagnosis and may subject the patients to the second operation after permanent pathologic diagnosis.\textsuperscript{6}

In the previous study, we reported a simple and quick inflation technique using diluted embedding medium and excellent morphologic preservation with high diagnostic accuracy.\textsuperscript{8} It is necessary to confirm the efficacy of the inflation method using a large scale because of limited number of patients in the previous study. In the context, we aimed to determine the diagnostic accuracy of intraoperative pathology consultations in our institution and to evaluate the merit of inflation method during cryosection of the lung by comparison of the diagnostic accuracy before and after inflation.

PATIENTS AND METHODS

We retrospectively reviewed the pathology reports of 1014 pulmonary nodules, which were surgically resected at Seoul National University Bundang Hospital between January 2004 and December 2008. Among them, 229 lung specimens (22.6%) were submitted to intraoperative pathology consultation, and 165 lung specimens (72.1%) were inflated with embedding medium from August 2006.

To evaluate the effect of embedding medium (Tissue-Tek OCT, Sakura Finetek-USA, CA) injection, serial dilutions (1:2, 2:1, 2:3, and not diluted) of embedding medium were attempted. Infusion of the medium with 2:3 dilution resulted in easier penetration into the lung tissue, reduced leakage, and more complete microscopic expansion than other groups. Therefore, all the lung specimens were inflated with 2:3 diluted embedding medium for cryosection using 23-gauge needles through the pleura until the lung tissue swelled enough.\textsuperscript{8} Each sample was embedded in Cryometrix and frozen at −25°C using Leica-CM3050S cryostat. Samples were cut into 5-μm thick sections, treated with 95% alcohol, and subsequently stained with hematoxylin and eosin. After FSD, the specimens were immersed in 2:3 diluted embedding medium from August 2006.

Table 1. Intraoperative Pathology Diagnoses of 229 Pulmonary Nodules

\begin{tabular}{|c|c|c|c|c|c|}
\hline
Diagnoses & ≤1 cm No. (BI/AI) & ≤2 cm No. (BI/AI) & ≤3 cm No. (BI/AI) & >3 cm No. (BI/AI) & Total (BI/AI) \\
\hline
Nonneoplastic lesion & 4 (3/1) & 7 (1/6) & 4 (1/3) & 7 (0/7) & 22 (5/17) \\
AAH & 6 (1/5) & 0 (0/0) & 0 (0/0) & 0 (0/0) & 6 (1/5) \\
BAC & 11 (3/8) & 7 (0/7) & 1 (1/0) & 2 (1/1) & 21 (5/16) \\
Adenocarcinoma & 27 (6/21) & 33 (9/24) & 14 (2/12) & 19 (6/13) & 93 (23/70) \\
Squamous cell carcinoma & 1 (1/0) & 8 (1/7) & 8 (3/5) & 13 (0/13) & 30 (5/25) \\
Non-small cell carcinoma & 2 (0/2) & 8 (4/4) & 10 (4/6) & 3 (1/2) & 23 (9/14) \\
Neuroendocrine carcinoma & 1 (0/1) & 2 (1/1) & 0 (0/0) & 1 (0/1) & 4 (2/2) \\
Lymphoma & 0 (0/0) & 0 (0/0) & 0 (0/0) & 0 (0/0) & 1 (0/1) \\
Hamartoma & 8 (2/6) & 10 (6/4) & 4 (4/0) & 4 (2/2) & 26 (14/12) \\
Sclerosing hemangioma & 0 (0/0) & 2 (0/2) & 1 (0/1) & 0 (0/0) & 3 (0/3) \\
Total & 60 (16/44) & 77 (22/55) & 42 (15/27) & 50 (11/39) & 229 (64/165) \\
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\textbf{TABLE 1. Intraoperative Pathology Diagnoses of 229 Pulmonary Nodules}

\textbf{RESULTS}

\textbf{Clinicopathologic Characteristics of 229 Pulmonary Nodules for FSD}

This respective study (January 2004–December 2008) enrolled 229 patients (146 men and 83 women), with ages ranging from 20 to 85 years. The clinicopathologic characteristics were summarized in Table 1. The diagnoses were nonneoplastic lesions in 28 cases (12.2%), benign tumors in 29 cases (12.7%), and malignant tumors in 172 cases (75.1%). The specimens were subdivided into four groups according to their sizes: 60 lesions equal or smaller than 1 cm in diameter, 77 between 1 and 2 cm, 42 between 2 and 3 cm, and 50 larger than 3 cm. The proportion of the small lesions less than 2 cm was 59.8% (137 of 229); among them, 38 cases (27.7%) were not inflated, whereas 99 cases (72.3%) were inflated. Comparing the FSD after inflation to before, atypical adenomatous hyperplasia (AAH) and bronchioloalveolar carcinoma (BAC) were 20.2% (20 of 99), which were incomparably more than 10.5% (4 of 38) despite the similar distribution of disease pattern between the two periods. The lesions smaller than 1 cm were diagnosed as four inflammation, six AAH, 11 BAC, 27 ADC, one squamous cell carcinoma, three non-small cell carcinoma (NSCLC), and eight hamartomas. Regarding surgical treatment of these small size lesions, standard lobectomy was performed in one AAH, 10 BAC, 22 ADC, and three NSCLC, and limited surgery such as wedge resection or segmentectomy was done in 20 cases (eight hamartoma, five AAH, one BAC, five ADC, and one squamous cell carcinoma). Of the 229 cases, 56 lung nodules presented as GGO lesions on high-resolution CT, and 89.3% (50 of 56) of them was smaller than 2 cm. These GGO lesions comprised six inflammatory and fibrotic lesions, six AAH, 15 BAC, 26 ADC, and three other NSCLC (Table 2).

\textbf{Pathologic Features of FS Using Inflation Method}

On the serial cut section, inflated lung specimens diagnosed as AAH displayed distinct appearance even in naked...
eyes, which could not be palpated or visualized during surgery (Figure 1A, B). Lung tissues inflated by embedding medium were easier to section, and the compression of lung tissue during cryosection was markedly reduced as the embedding medium filled up the empty alveolar spaces. The whole FS procedure needs about 7 to 12 minutes from gross examination to making slides in our department; however, it takes about 3 minutes from freezing inflated tissue to making slides. We invented metal molding plate for rapid freezing because inflated lung tissue needs more time for freezing than uninflated lung tissue. The metal molding plates were placed in the cryostat and kept frozen before using FS (Figure 2). Therefore, we could reduce working hours of FS procedure. Under the microscope, inflated lung specimens by embedding medium showed open air spaces and well-preserved normal parenchymal architecture including alveolar capillaries. In addition, alveolar walls are stretched rather than curled, and details such as alveolar breaks become readily apparent compared with uninflated lung tissue (Figures 3A, B). The small peripheral lung nodules such as AAH and BAC could be identified in the FSD (Figure 4). Nonneoplastic lesions, such as minute granulomas and/or inflammation, were also easily discernible in the FSD (Figure 5). The morphologic architectures of the lung were well preserved, and delicate cytologic changes were discriminated on the cryosection. What is better, the quality of immunohistochemical staining
using inflated lung tissue during cryosection had no differences compared with formalin-fixed lung tissues (Figure 6).

**Diagnostic Accuracy of FSD**

The correct diagnoses of malignancy and of specific subtypes were established in all the pulmonary nodules submitted for FS. After using the inflation method in the FSs, there were no false-positive or false-negative results. The overall sensitivity and specificity for a diagnosis of malignancy were 100%. However, there are two false-positive reports before using inflation method; one “alveolar cell hyperplasia with inflammation” was interpreted as “well differentiated ADC” and the other “pneumocytic hyperplasia” as “BAC.” The two specimens were not inflated at the time of FS and showed marked collapse of the alveoli, cellular crowding, and distortion of architecture, which led misinterpretation.

**FIGURE 3.** Effect of inflation during cryosection. A, Uninflated lung specimen shows collapsed markedly air spaces. B, Inflated lung tissue with embedding medium demonstrates well preserved parenchymal architecture and open air spaces. Hematoxylin and eosin (HE) staining ×400.

**FIGURE 4.** Frozen section of atypical adenomatous hyperplasia (AAH). Slightly thickened alveolar wall lined by an intermittent single layer of cuboidal cells with apical cytoplasmic snouts. Hematoxylin and eosin (HE) staining ×400.

**FIGURE 5.** Frozen section of sarcoidosis. Well formed conglomerated granulomas were readily recognized. Hematoxylin and eosin (HE) staining ×100.

**FIGURE 6.** Immunohistochemical staining of carcinoembryonic antigen (CEA) using inflated lung tissue during cryosection. The quality of CEA immunoreactivity is as good as that of formalin-fixed lung tissues. Immunostain ×400.
Moreover, the intraoperative pathology consultations have increased markedly after using inflation method in the FSs despite the similar distribution of people and diseases.

**DISCUSSION**

In this study, we demonstrated excellent morphologic preservation with high diagnostic accuracy in the FS of the lung tissue and confirmed the efficacy of inflation method using a large number of patients. Before introduction of this inflation method, AAH and BAC lesions smaller than 1 cm in size remained hard to visualize on the cut surface of sections, even after formalin fixation. This requires many pathologic sections to be made, which is time consuming and expensive. However, the inflation method enables pathologist to detect minute lesion more easily, which could reduce the number of histologic sections. In addition, injection of optimally diluted embedding medium makes lung tissue easy to be cut during gross section and cryosection. The compression or distortion of lung tissue during sectioning was markedly reduced, as the gelatinous embedding medium filled up the empty alveolar spaces. However, we also anguished over the faint and minute lesions less than 5 mm in size, which required multiple serial sections (Figures 1A, B). The important tip was that the consistency of the minute lesion was slightly firm compared with surrounding normal lung parenchyma. The embedding medium was sticky as glue, and injection of optimally diluted embedding medium makes lung tissue easy to be cut during cryosection, so that we can make more sections in a short time. There were a few cases of sampling error in lesions that were not palpable, which were usually presented as pure GGO. Despite meticulous intraoperative pathology examination, we could not find the matching lesion compared with CT finding because the lesion was not removed in the surgery. In such cases, the surgeon performed further resection, and the pathologists could find the minute lesion in the additionally resected specimens.

The peripheral small nodules include the full spectrum of preinvasive to invasive lesions under the putative hypothesis of the sequential development of pulmonary ADC. It is difficult to recognize the minute invasive focus of small ADC, especially to discriminate Noguchi type C from Noguchi type B. However, the pathologist has to distinguish BAC from early invasive ADC, because the FSD may have direct influence on the consequent surgical management. In this series, 37 lesions (13 BAC and 24 ADC) required discrimination of invasiveness in the FS. Application of inflation procedure could allow a correct pathologic diagnosis in these lesions including impalpable pulmonary nodules, even when the FSD was made by a nonexpert in pulmonary pathology. Therefore, the pathologist can provide rapid feedback on tumor histology and resection margin status and give the surgeon license to proceed with a definitive surgical procedure or termination of a procedure.

Regarding inflation method during cryosection, Gianoulis et al. introduced inflating the lung specimen with undiluted embedding medium using 18-gauge needles for FS. We modified their method and inflated lung tissue with diluted embedding medium using 23-gauge needles to prevent injury to minute impalpable lesions during injection and achieved excellent morphologic preservation with high diagnostic accuracy. Other studies also inflated lung specimens using vacuum or 30 cm H2O transpulmonary pressure during cryosection. They showed good morphologic evidence for alveolar recruitment; however, their methods required a additional equipment such as humidified chamber or vacuum chamber.

Because the pathologist can make FSD of small pulmonary nodules with confidence, the surgeon prefers operation after FSD in our institution. Certainly, the intraoperative pathology consultations have increased markedly after using inflation method in the FSs, especially in nodules smaller than 2 cm, despite similar distribution of disease category. Furthermore, a highly accurate FSD can avoid unnecessary preoperative needle biopsy, which may cause tumor contamination of needle tract and/or pneumothorax during the procedure. Even a wedge resection of the lung tissue requires a general anesthesia, and therefore, an accurate FSD is needed to avoid a second general anesthesia. The need for accurate FSD for pulmonary nodules is, however, in sharp contrast to the need for breast masses. Breast biopsies are usually done with local anesthesia, and a decision regarding further treatment is made at a postoperative discussion based on the results of permanent section.

However, this study has some limitations—we could not compare the results of inflation method in the same lesions of the same patients due to small size of the mass. We could not rule out the possibility that the longer the pathologists practice, the better their diagnostic accuracy gets. In summary, the accuracy of FSD was excellent by using inflation method, especially in cases of impalpable small precancerous lesions. The pathologist could guide with confidence the surgeon in planning the surgical management.

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