ABSTRACT

Objective: To determine the accuracy of frozen section diagnosis and factors associated with final pathological diagnosis upgrade in patients with mucinous ovarian tumors.

Methods: This study included 1,032 patients with mucinous ovarian tumors who underwent frozen section diagnosis during surgery. Sensitivity, specificity, and diagnostic accuracy of frozen section diagnosis was calculated. Univariate and multivariate regression analyses were performed to determine factors associated with diagnosis upgrade in the final pathology report.

Results: The sensitivity and specificity of frozen section diagnosis were 99.1% (95% CI=98%-99.6%) and 82.2% (95% CI=77.9%-85.7%), respectively, for benign mucinous tumors; 74.6% (95% CI=69.1%-79.4%) and 96.7% (95% CI=95.2%-97.8%), respectively, for mucinous borderline ovarian tumors; and 72.5% (95% CI=62.9%-80.3%) and 98.8% (95% CI=97.9%-99.3%), respectively, for invasive mucinous carcinomas. The multivariate analysis revealed that mixed tumor histology (odds ratio [OR]=2.8; 95% CI=1.3–6.3; p=0.012), tumor size >12 cm (OR=2.5; 95% CI=1.5–4.3; p=0.001), multilocular tumor (OR=2.9; 95% CI=1.4–6.0; p=0.006), and presence of a solid component in the tumor (OR=3.1; 95% CI=1.8–5.1; p<0.001) were independent risk factors for final pathological diagnosis upgrade.

Conclusions: Mixed tumor histology, tumor size >12 cm, multilocular tumor, and presence of a solid component in the tumor were independent risk factors for final pathological diagnosis upgrade based on frozen section diagnosis.

Keywords: Ovarian Neoplasm; Mucinous; Frozen Section; Diagnosis; Accuracy

INTRODUCTION

Ovarian cancer is one of the most lethal gynecologic malignancy worldwide [1]. Preoperative histological differential diagnosis among benign, borderline, and malignant ovarian tumors...
is challenging due to the lack of reliable methods for preoperative histological diagnosis. Furthermore, imaging studies and serum tumor markers for preoperative diagnosis of ovarian malignancies still exhibit low diagnostic accuracy [2,3]. Therefore, histological diagnosis of ovarian malignancy is intraoperatively performed using frozen section diagnosis, and the extent of surgery, including comprehensive staging procedures and conservative fertility-sparing surgery, is tailored based on the results of frozen section diagnosis obtained during surgery. Previous studies have extensively argued that the rationale behind the use of frozen section diagnosis is to obtain a precise diagnosis to guide the therapeutic decision for the most appropriate treatment [4]. Frozen section diagnosis during surgery is considered a readily available and reliable diagnostic procedure for assessing ovarian tumors [4].

Although the accuracy of intraoperative frozen section diagnosis of ovarian tumors is relatively high (range, 86%–97%), recent studies have reported an increase in the inaccuracy of frozen section diagnosis of mucinous ovarian tumors [5-15]. Unlike other histological types of ovarian tumors, mucinous ovarian tumors can contain lesions of varying severity, including benign, borderline, and invasive carcinomas, within one tumor. In addition, mucinous ovarian tumors are very large at times. Therefore, diagnosis achieved by frozen section analysis of mucinous ovarian tumors is substantially upgraded in final pathology reports. This study aimed to determine the accuracy of frozen section diagnosis and clinicopathological parameters that were associated with diagnosis upgrade in final pathology reports of patients with mucinous ovarian tumors.

MATERIALS AND METHODS

1. Study Population
In this retrospective study, clinical and pathological electronic databases of Asan Medical Center (AMC; Seoul, Korea) from January 1997 to December 2010 were reviewed to identify all patients who had undergone intraoperative frozen section diagnosis among those who had undergone primary surgical procedure and had received a final pathological diagnosis of primary mucinous ovarian tumor. However, patients with pseudomyxoma peritonei arising from the appendix, those with metastatic mucinous adenocarcinoma, those who had received radiation or chemotherapy before surgery, and those who were referred to AMC for complete staging surgery for ovarian tumor after incomplete surgery at another hospital were excluded from the study. The study flowchart is shown in Fig. 1. This study was conducted with the approval of the Institutional Review Board of Asan Medical Center (2017-1241).

Following parameters were retrieved from the medical records: patient age, menopause, parity, the International Federation of Obstetrics and Gynecology (FIGO) stage, differentiation grade, tumor bilaterality, tumor size, presence of associated tumor, intraoperative tumor rupture, peritoneal cytology, peritoneal biopsy, ovarian surface involvement, spread beyond the ovary, lymph node involvement, presence of septation in tumor, presence of a solid component in tumor, mode of surgery (laparotomy or laparoscopy), and type of adnexal surgery.

2. Frozen section diagnosis
All frozen section specimens were examined by pathologists who took turns on a weekly basis to perform frozen section diagnoses. To maximize diagnostic accuracy, an expert gynecologic pathologist was available for consultation at all times.
All specimens were submitted to the pathology department for histopathological diagnosis using frozen sections as well as standard paraffin section techniques to obtain a final histological diagnosis. Highly suspicious areas were selected by the attending pathologists using touch printing and gross examination. One to three of the most representative samples were frozen in a cryostat, and 5-µm sections were obtained and stained with hematoxylin and eosin. All slides were examined by the attending pathologist and were diagnosed as a benign mucinous tumor, mucinous borderline ovarian tumor (MBOT), or mucinous carcinoma.

### 3. Final histological diagnosis

For final histological diagnosis, blocks used for frozen sectioning were fixed in formalin and embedded in paraffin. Tumors were sliced to obtain a minimum of one section per centimeter in full thickness. Final histological diagnosis was achieved by two expert gynecologic pathologists. Histological typing was performed according to the FIGO recommendations [16]. In cases of a discrepancy between the frozen section diagnosis and the final histological diagnosis, the slides were reassessed by the pathologists to ascertain whether the errors were due to gross sampling or interpretation. Mucinous tumors were diagnosed using the criteria defined by the World Health Organization [17].

### 4. Statistical analysis

To evaluate the agreement between frozen and final histological diagnoses, sensitivity and specificity were calculated with 95% confidence intervals (CIs). Clinicopathological factors associated with frozen diagnosis upgrade in the final pathology report were assessed using chi-squared test or Fisher’s exact test. A multivariate analysis was performed including significant factors in univariate analysis using a binary logistic regression model. Statistical
significance was assumed at p<0.05 in two-sided tests. SPSS software (version 11.0; SPSS Inc., Chicago, IL, USA) was used for all statistical analyses.

RESULTS

1. Study population
During the study period, 1,032 patients with mucinous ovarian tumors who satisfied the eligibility criteria were included (Fig. 1). All patients underwent primary surgical treatment for primary mucinous ovarian tumors and were evaluated using intraoperative frozen section diagnosis for ovarian tumors at AMC. Patient characteristics are shown in Table 1. Among the 1,032 patients, the final pathological diagnosis was benign mucinous tumor in 662 (64.1%) patients, MBOT in 272 (26.4%), and invasive mucinous carcinoma in 98 (9.5%).

Table 1. Characteristics of patients (n=1,032)

| Variables                      | Values  |
|--------------------------------|---------|
| **Age (yr)**                   |         |
| Mean±SD                        | 40.5±14.9 |
| ≤40*                           | 561 (54.4) |
| >40*                           | 471 (45.6) |
| **Parity**                     |         |
| Nulliparous                    | 324 (31.4) |
| Parous                         | 708 (68.6) |
| **Menopause**                  |         |
| No                             | 804 (77.9) |
| Yes                            | 228 (22.1) |
| **Histology of tumor**         |         |
| Pure mucinous tumor            | 981 (95.1) |
| Mixed tumor                    | 51 (4.9)  |
| **Tumor size (cm)**            |         |
| Mean±SD                        | 12.4±7.1 |
| ≤12*                           | 592 (57.4) |
| >12*                           | 440 (42.6) |
| **Bilaterality of tumor**      |         |
| Unilateral                     | 997 (96.6) |
| Bilateral                      | 35 (3.4)  |
| **Septa in tumor**             |         |
| Unilocular                     | 359 (34.8) |
| Multilocular                   | 673 (65.2) |
| **Solid portion in tumor**     |         |
| No                             | 808 (78.3) |
| Yes                            | 224 (21.7) |
| **Type of adnexal surgery**    |         |
| Cystectomy                     | 146 (14.1) |
| Oophorectomy or salpingo-oophorectomy | 886 (85.9) |
| **Results of frozen biopsy**   |         |
| Benign mucinous tumor          | 722 (72.0) |
| Borderline mucinous tumor      | 228 (22.1) |
| Invasive mucinous carcinoma    | 82 (7.9)  |
| **Results of final pathology** |         |
| Benign mucinous tumor          | 662 (64.1) |
| Borderline mucinous tumor      | 272 (26.4) |
| Invasive mucinous carcinoma    | 98 (9.5)  |

Values are presented as number (%).
SD, standard deviation.
*Divided by mean values.
2. Accuracy of frozen section diagnosis

Table 2 shows the accuracy of frozen section biopsy in mucinous ovarian tumors. Using frozen section biopsy, 1% (6/662; 95% CI=0.4%–2.0%) of benign mucinous tumors were overdiagnosed as MBOTs, 21.3% (58/272; 95% CI=16.9%–26.6%) of MBOTs were underdiagnosed as benign mucinous tumors, 4.0% (11/272; 95% CI=2.3%–7.1%) of MBOTs were overdiagnosed as invasive mucinous carcinomas, and 27.6% (27/98; 95% CI=19.7%–37.1%) of invasive mucinous carcinomas were underdiagnosed as benign mucinous tumors or MBOTs. Overall, frozen section biopsy led to underdiagnosis in 8.2% of the patients (85/1,032; 95% CI=6.7%–10.7%) and overdiagnosis in 1.6% of the patients (17/1,032; 95% CI=1.0%–2.6%). The sensitivity and specificity of frozen section diagnosis were 99.1% (95% CI=98%–99.6%) and 82.2% (95% CI=77.9%–85.7%), respectively, for benign mucinous tumors; 74.6% (95% CI=69.1%–79.4%) and 96.7% (95% CI=95.2%–97.8%), respectively, for MBOTs; and 72.5% (95% CI=62.9%–80.3%) and 98.8% (95% CI=97.9%–99.3%), respectively, for invasive mucinous carcinomas.

The causes of misdiagnosis by frozen section biopsy were defined as misinterpretation of the frozen section pathology slide and sampling errors during frozen sectioning. The revision of final pathological diagnosis based on the review of frozen section pathology slides during final pathological diagnosis was categorized as the misinterpretation of the frozen section pathology slide, which was identified in 21 cases (20.6%). Conversely, the revision of final pathological diagnosis based on the evaluation of additional pathology slides that were prepared during final pathological diagnosis was categorized as sampling errors during frozen sectioning, which was identified in 81 cases (79.4%).

3. Factors associated with frozen section diagnosis upgraded in the final pathology report

Among 722 patients who were diagnosed with benign mucinous tumor by frozen section biopsy, the final pathological diagnosis was upgraded to MBOT in 8% (58/722; 95% CI=6.3%–10.2%) of the patients and to invasive mucinous carcinoma in 1% (8/722; 95% CI=0.6%–2.2%) of the patients (Table 2). Among 228 patients who were diagnosed with MBOT by frozen section biopsy, the final pathological diagnosis was upgraded to invasive mucinous carcinoma in 8.3% (19/228; 95% CI=0.54%–12.6%) of the patients. Together, among 950 patients who were diagnosed with benign mucinous tumor or MBOT by frozen section biopsy, the final pathology diagnosis was upgraded in 9% (85/950; 95% CI=7.3%–10.9%) of the patients.

Next, a univariate analysis was performed to determine factors associated with the frozen section diagnosis upgraded in the final pathology report in these patients (Table 3). Age, parity, menopause, and tumor bilaterality were not significantly associated with final pathological diagnosis upgrade. In contrast, mixed tumor histology, tumor size >12 cm, multilocular tumor, presence of a solid component in the tumor, and type of adnexal surgery

Table 2. The accuracy of frozen biopsy in mucinous ovarian tumors (n=1,032)

| Variables          | Frozen biopsy | Final pathology | Total |
|--------------------|---------------|-----------------|-------|
|                    | Benign tumor  | Borderline tumor| Invasive carcinoma |
| Frozen biopsy      | 656           | 58              | 8     | 722  |
| Benign tumor       | 6             | 203             | 19    | 228  |
| Borderline tumor   | 0             | 11              | 71    | 82   |
| Invasive carcinoma | 0             | 11              | 71    | 82   |
| Total              | 662           | 272             | 98    | 1,032|
were significantly associated with final pathological diagnosis upgrade. The multivariate analysis that included these significant factors revealed that mixed tumor histology (OR=2.8; 95% CI=1.3–6.3; p=0.012), tumor size >12 cm (OR=2.5; 95% CI=1.5–4.3; p=0.001), multilocular tumor (OR=2.9; 95% CI=1.4–6.0; p=0.006), and presence of a solid component in the tumor (OR=3.1; 95% CI=1.8–5.1; p<0.001) were independent risk factors for final pathological diagnosis upgrade. However, the type of adnexal surgery was not revealed to be a significant factor (OR=1.5; 95% CI=0.6–3.9; p=0.443) in the multivariate analysis.

A subgroup analysis was performed to determine factors associated with the frozen section diagnosis updated to invasive mucinous carcinoma in the final pathology report in these patients (Table 4). In univariate analysis, age, parity, mixed tumor histology, and tumor bilaterality were not significantly associated with final pathological diagnosis upgrade to invasive mucinous carcinoma. However, menopause, tumor size >12 cm, multilocular tumor, presence of a solid component in the tumor, and type of adnexal surgery were significantly associated with final pathological diagnosis upgrade to invasive mucinous carcinoma. In multivariate analysis including these significant factors, only presence of a solid component in the tumor (OR=16.2; 95% CI=6.1–43.4; p<0.001) was independent risk factor for final pathological diagnosis upgrade to invasive mucinous carcinoma. However, menopause (OR=2.2; 95% CI=0.9–5.3; p=0.066), tumor size >12 cm (OR=1.1; 95% CI=0.4–2.8; p=0.816), multilocular tumor (OR=2.5; 95% CI=0.5–11.8; p=0.237), and type of adnexal surgery (OR=141022; 95% CI=0–0; p=0.996) were not revealed to be a significant factor in the multivariate analysis.
DISCUSSION

The findings of the current study revealed that the rates of underdiagnosis and overdiagnosis of mucinous ovarian tumors by frozen section biopsy were 8.2% and 1.6%, respectively. Furthermore, by frozen section biopsy, MBOTs were underdiagnosed in 21.3% of the patients and overdiagnosed in 4.0% of the patients, whereas mucinous carcinomas were underdiagnosed in 27.6% of the patients. In contrast, 8% and 1% of all patients in the current study who were diagnosed with benign mucinous tumor by frozen section biopsy were upgraded to MBOT or invasive mucinous carcinoma, respectively, in the final pathology report. Moreover, 8.3% of the patients who were diagnosed with MBOT by frozen section biopsy were upgraded to invasive mucinous carcinoma in the final pathology report. Therefore, overall, 9% of the patients diagnosed with benign mucinous tumors or MBOTs by frozen section biopsy were upgraded in the final pathology report. Mixed tumor histology, tumor size >12 cm, multilocular tumor, and presence of a solid component in the tumor were independent risk factors for final pathological diagnosis upgrade.

The accuracy of frozen section diagnosis in discriminating mucinous ovarian tumor is significantly lower for mucinous ovarian tumors than for other histological types [13] because mucinous ovarian tumors are larger than other histological types [18]. In addition, unlike other histological types of ovarian tumors, mucinous ovarian tumors exhibit diverse histological features, with a spectrum of coexistent morphologically benign, borderline,

| Table 4. Factors associated with upgrade to invasive mucinous carcinoma of frozen diagnosis at final pathology report (n=950) |
| Variables | Final pathology report | p-value |
|-----------|------------------------|---------|
|           | Not upgraded | Upgraded |         |
| Age (yr)  | ≤40* | 519 (97.7) | 12 (2.3) | 0.224 |
|           | >40* | 404 (96.4) | 15 (3.6) |         |
| Parity    | Nulliparous | 289 (97.0) | 9 (3.0) | 0.823 |
|           | Parous | 634 (97.2) | 18 (2.8) |         |
| Menopause | No | 740 (97.8) | 17 (2.2) | 0.028 |
|           | Yes | 183 (94.8) | 10 (5.2) |         |
| Histology of tumor | Pure mucinous tumor | 876 (97.1) | 26 (2.9) | 0.745 |
|           | Mixed tumor | 47 (97.9) | 1 (2.1) |         |
| Tumor size (cm) | ≤12* | 559 (98.6) | 8 (1.4) | 0.001 |
|           | >12* | 364 (95.0) | 19 (5.0) |         |
| Bilaterality of tumor | Unilateral | 894 (97.2) | 26 (2.8) | 0.869 |
|           | Bilateral | 29 (96.7) | 1 (3.3) |         |
| Septa in tumor | Unilocular | 348 (99.4) | 2 (0.6) | 0.001 |
|           | Multilocular | 575 (95.8) | 25 (4.2) |         |
| Solid portion in tumor | No | 796 (99.3) | 6 (0.7) | <0.001 |
|           | Yes | 127 (85.8) | 21 (14.2) |         |
| Type of adnexal surgery | Cystectomy | 146 (100.0) | 0 (0) | 0.015 |
|           | Oophorectomy or salpingo-oophorectomy | 777 (96.6) | 27 (3.4) |         |

Values are presented as number (%). *Divided by mean values.
and malignant components in individual tumors [19]. Only few studies have reported the accuracy of frozen section diagnosis for mucinous ovarian tumors in the literature. In a study by Storms et al, the sensitivity and specificity of frozen section diagnosis of mucinous ovarian tumors were 71.4% and 93.3%, respectively, for benign tumors; 91.6% and 55.1%, respectively, for MBOTs; and 28.6% and 100%, respectively, for invasive mucinous carcinomas [20]. Pongsuwareeyakul et al. [21] have reported the sensitivity and specificity of frozen section diagnosis of mucinous ovarian tumors as 99.2% and 78.5%, respectively, for benign tumors; 67.2% and 94.8%, respectively, for MBOTs; and 55.6% and 98.9%, respectively, for invasive mucinous carcinomas. In the current study, the sensitivity and specificity of frozen section diagnosis of mucinous ovarian tumors were 99.1% and 82.2%, respectively, for benign tumors; 74.6% and 96.7%, respectively, for MBOTs; and 72.5% and 98.8%, respectively, for invasive mucinous carcinomas. Altogether, these findings highlight that a substantial number of MBOTs and invasive mucinous carcinomas are misdiagnosed by frozen section diagnosis.

In our study, the most frequent cause for the misdiagnosis of mucinous ovarian tumors by frozen section biopsy was sampling error. This finding also reflects the innate characteristics of mucinous ovarian tumors [18,19]. Therefore, to improve the diagnostic accuracy of frozen section diagnosis of mucinous ovarian tumors, higher number of frozen section slides would be beneficial. However, the relationship between the accuracy of frozen section diagnosis and the number of frozen section slides has not been evaluated. Although at least one section per centimeter is recommended for the final histological diagnosis of mucinous ovarian tumors, this may be impossible for frozen section diagnosis. The other cause for misdiagnosis by frozen section biopsy was misinterpretation of the frozen section pathology slides, which is associated with the experience or expertise of the pathologists performing the frozen section diagnosis. Bige et al. [22] and Brun et al. [13] have reported that the accuracy of diagnosis of ovarian tumors by frozen section biopsy is improved by expert pathologists specialized in gynecology compared with those not specialized in gynecology. Although it may not be possible to ensure the attendance of only expert pathologists specialized for frozen section diagnosis of all ovarian tumors, consultation with such experts after initial pathological examination by the attending pathologists not specialized in gynecology should be considered. All the significant factors which are associated with histologic diagnosis upgrade should be considered during frozen diagnosis, and if the result is unclear, it should be referred to the gynecologic oncology specialized pathologist to improve the diagnostic accuracy.

The overdiagnosis of mucinous ovarian tumors is challenging as it leads to overtreatment. However, the underdiagnosis of these tumors pose a more urgent problem as the underdiagnosis of MBOTs and invasive mucinous carcinomas frequently lead to restaging surgeries. In addition, delays in staging surgeries with appropriate coverage due to inaccurate histological diagnosis by frozen section biopsy may lead to upstaging of early disease and poor survival outcomes [23]. Therefore, it is critical to identify factors associated with frozen section diagnosis upgraded during final pathology diagnosis. In the current study, mixed tumor histology, tumor size >12 cm, multilocular tumor, and presence of a solid component in the tumor were independent risk factors for final pathological diagnosis upgrade. Previous studies have rarely evaluated factors associated with the diagnostic accuracy of frozen section biopsy for mucinous ovarian tumors. Storms et al have analyzed factors associated with the accuracy of frozen section diagnosis and found that age, tumor size, endometriosis, tumor bilaterality, and disseminated disease outside the ovary are not significant factors [20]. Pongsuwareeyakul et al. [21] have reported that although tumor size >13 cm is associated with the accuracy of frozen section diagnosis of mucinous ovarian tumors, it is not a significant factor, as revealed in a multivariate analysis.
Although recent studies have reported the inaccuracy of frozen section diagnosis of mucinous ovarian tumors [9,24], few studies have reported the accuracy of frozen section diagnosis of mucinous ovarian tumors alone [20,21]. As mucinous ovarian tumors mostly occur in Asian women [25], an adequate number of patients could not be recruited in previous studies to support the results. Under these circumstances, the present study is the largest one that focused on mucinous ovarian tumors to report independent factors associated with frozen section diagnosis upgrade.

In conclusion, a substantial number of mucinous ovarian tumors were misdiagnosed by frozen section diagnosis in this retrospective analysis that included a large cohort. Mixed tumor histology, tumor size >12 cm, multilocular tumor, and presence of solid components in the tumor were independent risk factors for frozen section diagnosis upgraded in the final pathological report.

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