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Evaluation of Accuracy of Intraoperative Frozen Section and Imprint Cytology in Gynecological Neoplasms—A Descriptive Cross-Sectional Study of 50 Cases in Tertiary Care Center

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Abstract

Aims and Objectives Gynecological neoplasms are among the most common cancers in female population of India and worldwide. Various new advances have been made to diagnose gynecological pathologies which include imprint cytology and frozen sections in addition to the histopathological techniques. The aim of the study was to assess the diagnostic accuracy of intraoperative diagnostic procedure with gold standard histopathology.

Method The study included 50 patients who had suspected gynecological neoplasm in a time period of 12 months. Their intraoperative diagnosis was made on frozen section and imprint cytology to rule out benign or malignant lesions and compared with gold standard histopathology.

Results Our study concludes that maximum cases of female genital tract neoplasms belonged to the age group of 19 to 76 years, with 60% cases in postmenopausal age group. Overall diagnosis of 62, 52, and 76% malignancies were made on imprint cytology, frozen section, and histopathological examination, respectively.

Conclusion The study concludes that diagnostic accuracy by intraoperative imprint cytology is higher (80%) than frozen section (76%). True positive cases were maximally reported by histopathology. True negative and false positive cases were equally reported by both frozen and imprint cytology. False negative cases were reported by frozen section more than by imprint cytology. The kappa statistical value was lesser in frozen versus histopathology and more in imprint versus histopathology. The sensitivity, specificity, positive predictive value, and negative predictive value for imprint cytology were 77.5, 90.0, 96.9, and 50%, respectively, whereas for frozen section, it was 72.5, 90.0, 96.7, and 45.0%, respectively.

Keywords
- frozen
- imprint cytology
- sensitivity

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Introduction

The female reproductive system is a very significant part of a body system. It definitely has the capability to function with nearly all others organs of body for the purpose of reproduction.

Gynecological neoplasms are among the most common cancers in female population of India and worldwide. There is approximately 60% of cancer burden among women which include breast, ovary, corpus uteri, and cervical cancers. Mostly women report in advanced stage of cancer in India. Being a developing country, there is a lack of awareness, varying pathology of tumor, and shortage of proper screening facilities. Ovarian and cervical cancers are the most common female genital organ cancers affecting women worldwide and in India. According to GLOBOCAN 2020 data, total numbers of new cases are 313,959 and deaths due to ovarian carcinoma are approximately 207,252. Total number of new cases in corpus uteri and cervix uteri cancers are 417,367 and 604,127, respectively.[1] Various new advances have been made to diagnose gynecological pathologies which include imprint cytology and frozen sections in addition to the gold standard histopathological techniques.

Imprint cytology is a popular technique for pathological assessment. The excised tissue is sent fresh to the pathologist who processes it immediately. It provides rapid diagnosis (within 20 minutes) without losing the architecture of the tissue. This procedure can be done in less developed setting with minimal trained technicians.[2]

Frozen sections are used in gynecological practices mostly to help in differentiating between benign and malignant diseases. The reason of performing this procedure is to provide the doctors with information which enables them to perform the most apt treatment. The diagnostic accuracy of intraoperative rapid frozen procedure in gynecological neoplasms is assessed to be approximately 91 to 97%. This technique is performed by embedding a fragment of tissue in Tissue-Tek OCT compound and cutting.[3] The present study was therefore conducted to assess the accuracy of intraoperative diagnostic techniques and their comparison with histopathological diagnosis which was considered as gold standard for tumor diagnosis.

Materials and Methods

The observational study was performed in the department of pathology over a period of 12 months with a sample size of 50 samples which were included for statistical purpose by convenient sampling. The subjects were recruited after obtaining written informed consent and ethical clearance. Sampling methods included all the female patients who came with complaints related to benign and malignant lesion of female genital tract. Patients with prior chemotherapy or radiotherapy and tumors with extensive hemorrhage or necrosis were excluded from our study. After noting all the gross features including capsular integrity of the received specimen, it was sectioned and imprints were made from the cyst wall and solid areas of the fresh specimen, avoiding lateral movements, by applying a slight but firm pressure against the glass slides. A minimum of four slides were prepared and were fixed in 90% isopropyl alcohol for 5 minutes and stained with pap/Giemsa as well as were kept air dried. Isopropyl alcohol was used as a fixative in our study as per our departmental standard operating procedures. It was preferred over 95% ethyl alcohol in view of better fixation, cost effectiveness, accessibility, and better results in staining of slides in our department. For pap staining, the fixed smears were hydrated, dipped in Harris' alum hematoxylin, decolorized, and differentiated using 1% acid alcohol. These differentiated smears were then put into running tap water for bluing following which they were dehydrated and stained with OG-6 and EA-50. Air-dried or isopropyl alcohol-fixed smears were also stained with May–Grunwald working solution followed by Giemsa stain which was washed with 6.8 pH buffer and mounted. The smears were evaluated for cellularity, arrangement of epithelial cells, cellular features of malignancy, necrosis, and background. Benign category included few number of cells, arranged in sheets with no overlapping, absent tumor diathesis, no nuclear atypia, and small nuclei with abundant cytoplasm whereas large cell clusters with overlapping, tumor diathesis, marked nuclear atypia, and enlarged nuclei with scant cytoplasm were included in malignant category. Any cells having mild nuclear atypia with moderately overlapping clusters and having features not fitting into clear benign or malignant categories on imprint cytology were labeled as borderline category. Blinding was applied for evaluation of these criteria for classifying tumors in various categories on imprints. Frozen sectioning (at –20°C to –30°C on a cryostat instrument) of the same specimen was done, fixed in 10% neutral-buffered formalin and stained in Harris' (progressive) hematoxylin followed by 1% aqueous eosin. Simultaneous hematoxylin and eosin (H&E) sections of the main specimen were prepared at the time of frozen reporting and compared with findings on frozen. With all the data in hand, a correlative study of imprint cytology, frozen sectioning, and histopathology was performed, taking histopathology as the gold standard.

Results

The study included 50 cases of suspected gynecological neoplasms ranging from 19 to 76 years of age with mean age of patients being 53.14 ± 14.17 years and median of 53.50 years. Majority of the cases were married (98%), had complaint of pain in abdomen (70%), were multigravida (96%), and were postmenopausal (60%). Maximum number of specimens retrieved were from ovaries (68%) followed by uterus (26%) and cervix (6%). Thirty-four cases (68%) were labeled as malignant on radiological investigations which included contrast-enhanced computed tomography scan and ultrasound of pelvis and abdomen.

Imprint cytology, frozen sections, and histopathology were done in all the cases which were categorized into benign, malignant, and borderline. Thirty-eight (76%) cases were diagnosed as malignant using histopathology whereas $n = 31$ (62%) and $n = 26$ (52%) were diagnosed as malignant

using imprint cytology and frozen section, respectively. Borderline cases of ovary which were reported as $n = 1$ (2%) on imprint cytology and $n = 4$ (8%) on frozen section were actually $n = 2$ (4%) cases on histopathology. Among histopathology, 30% cases belonged to serous papillary cystadenocarcinoma of ovaries whereas among imprint cytology 24% cases were diagnosed as endometrioid adenocarcinoma and ovarian adenocarcinoma. Similarly, 52% cases were diagnosed as malignant on frozen section.

►**Table 1** shows that there was a significant statistical difference between the various groups in terms of distribution of histopathological diagnosis and frozen section diagnosis (chi-square = 13.646, $p = 0.004$), hence proving their association, similarly ►**Table 2** also shows that there was a significant statistical difference between the histopathological diagnosis and imprint diagnosis (chi-square = 27.427, $p \leq 0.001$), thus proving their association.

►**Table 3** shows that sensitivity of imprint cytology was 77.5% which was slightly greater than sensitivity of frozen section. The diagnostic accuracy of cases reported by intraoperative imprint cytology was seen to be much higher, that is, 80% than frozen section which was 76%.

►**Fig. 1** shows concordant cases having similar findings in imprint cytology, frozen section, and histopathology, whereas ►**Fig. 2** shows discordant cases as findings of imprint cytology and frozen section were not similar on comparison with gold standard histopathology.

For calculating predictive values from imprint cytology, frozen section, and histopathology, all borderline and malignant cases were grouped under malignant/borderline to calculate predicting impression. Maximum true positive cases were diagnosed by histopathology as it being the gold standard. True negative and false positive cases were equally reported by both frozen and imprint cytology. False negative cases were reported by frozen section than imprint cytology.

A statistical significance (p-value < 0.05) was observed in correlation of CA-125 with histopathology, comparison of

Table 1 Association between diagnosis: frozen section and diagnosis: histopathology ($n = 50$)

Diagnosis: Histopathology	Diagnosis: Frozen section				Fisher's exact test	
	Benign	Borderline	Malignant	Total	Chi-square	*p*-Value
Benign	9 (45.0%)	0 (0.0%)	1 (3.8%)	10 (20.0%)	13.646	0.004
Borderline	1 (5.0%)	0 (0.0%)	1 (3.8%)	2 (4.0%)		
Malignant	10 (50.0%)	4 (100.0%)	24 (92.3%)	38 (76.0%)		
Total	20 (100.0%)	4 (100.0%)	26 (100.0%)	50 (100.0%)		

Note: Fisher's exact test.

Table 2 Association between diagnosis: imprint and diagnosis: histopathology ($n = 50$)

Diagnosis: Histopathology	Diagnosis: Imprint				Fisher's exact test	
	Benign	Borderline	Malignant	Total	Chi-square	*p*-Value
Benign	9 (50.0%)	1 (100.0%)	0 (0.0%)	10 (20.0%)	27.427	< 0.001
Borderline	2 (11.1%)	0 (0.0%)	0 (0.0%)	2 (4.0%)		
Malignant	7 (38.9%)	0 (0.0%)	31 (100.0%)	38 (76.0%)		
Total	18 (100.0%)	1 (100.0%)	31 (100.0%)	50 (100.0%)		

Note: Fisher's exact.

Table 3 Primary diagnostic parameters (frozen section and imprint cytology impression)

Variable	Sensitivity	Specificity	PPV	NPV	Diagnostic accuracy
Impression: Imprint	77.5% (62–89)	90.0% (55–100)	96.9% (84–100)	50.0% (26–74)	80.0% (66–90)
Impression: Frozen section	72.5% (56–85)	90.0% (55–100)	96.7% (83–100)	45.0% (23–68)	76.0% (62–87)

Abbreviations: NPV, negative predictive value; PPV, positive predictive value.

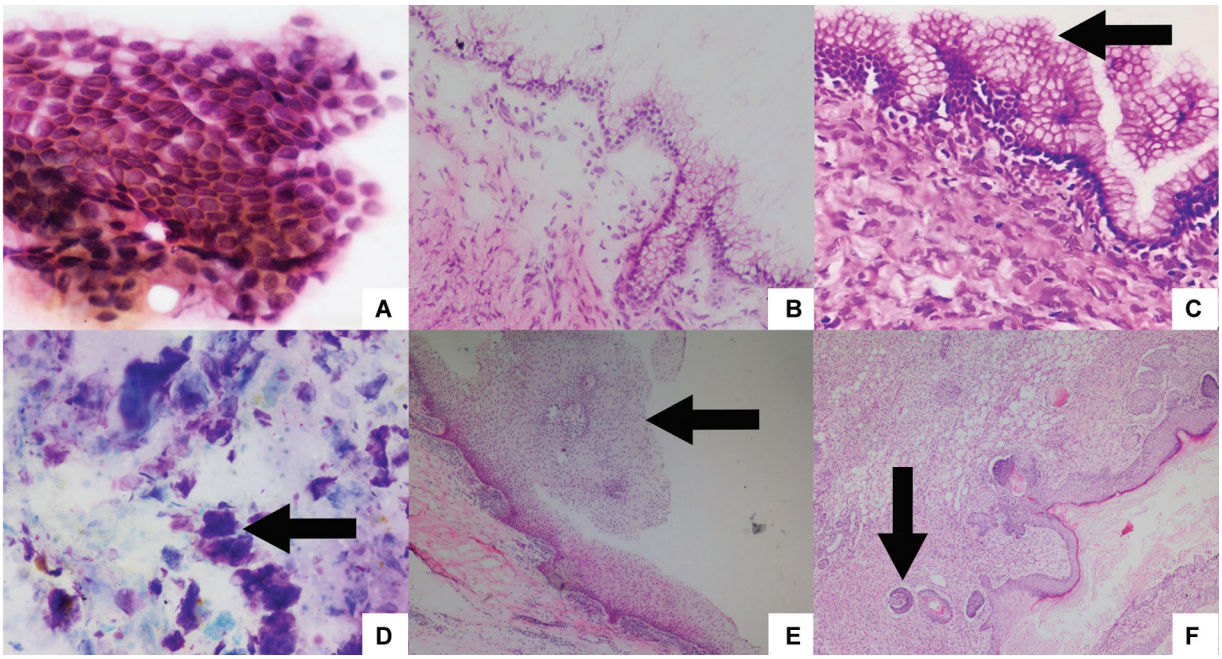

Fig. 1 Photomicrographs of concordant cases suggesting similar findings in imprint cytology, frozen section, and histopathology sections. (**A**) Imprint cytology showing benign sheets of cells in ovarian cyst adenoma (hematoxylin and eosin [H&E] 40 ×, 10 ×). (**B**) Frozen section showing mucinous cystadenoma of ovary lined by stratified columnar epithelium (H&E 20 ×, 10 ×). (**C**) Histopathological examination of mucinous cystadenoma of ovary lined by stratified columnar epithelium with apical mucin (H&E 20 ×, 10 ×). (**D**) Imprint cytology showing anucleate squames in teratoma (May Grunwald-Giemsa [MGG] 20 ×, 10 ×). (**E**) Frozen section of mature cystic teratoma showing nest of squamous epithelium (H&E 10 ×, 10 ×). (**F**) Histopathological examination of mature teratoma showing hair follicle (H&E 10 ×, 10 ×).

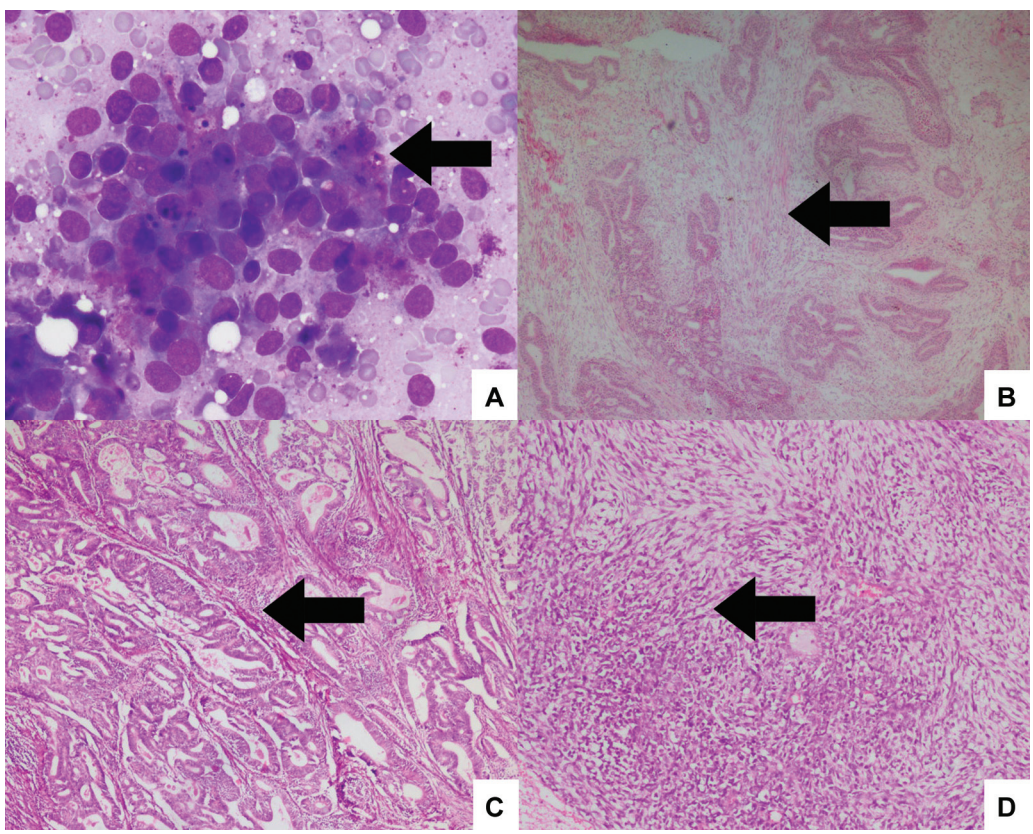

Fig. 2 Photomicrographs of discordant cases with findings not similar when compared with gold standard histopathology. (**A**) Imprint cytology of endometrium showing adenocarcinoma with tumor cells forming vague acini (May Grunwald-Giemsa [MGG] 40 ×, 10 ×). (**B**) Frozen section showing infiltrating endometrial carcinoma into the stroma (H&E 10 ×, 10 ×). (**C**) Histopathology examination of endometrium showing epithelial component in mixed Mullerian tumor (H&E 4 ×, 10 ×). (**D**) Histopathological examination of endometrium showing sarcomatous component in mixed Mullerian tumor (H&E 20 ×,10 ×).

histopathology with imprint diagnosis, comparison of histopathology with frozen section, and comparison of diagnosis of imprint cytology with frozen section. However, the kappa statistical value, deciding the agreement between two methods, was lesser in frozen versus histopathology and more in imprint versus histopathology.

Discussion

The present observational study was done in a tertiary care hospital. Therefore, cases taken were mainly patients suffering from critical illness or suspicious female genital tract neoplasms.

Female gynecological neoplasms have global distribution, but vary from one region to another. Cervical, ovarian, and endometrial carcinoma are the most common type of cancers in female genital system. Cervical cancer is the fourth most frequently diagnosed cancer and the fourth leading cause of cancer death in women, with an estimated 604,000 new cases and 342,000 deaths worldwide in 2020. Similarly, total number of new cases in ovarian, corpus uteri, and cervix uteri cancers are 313,959, 417,367, and 604,127, respectively.[1] Dudgeon and Patrick were the first to describe the imprint smears of fresh tissues in the rapid microscopic diagnoses of tumors.[2] Hence, we found from our research that intraoperative imprint cytology procedure was better and a rapid method for diagnosis of lesions which helps in making surgeon quick decision for surgery. In the past, many studies were done on imprint cytology and frozen section in intraoperative diagnosis of various tumors especially ovarian neoplasms. But we undertook this study as there was no much emphasis given on intraoperative diagnostic role in gynecological tumors which can further help surgeons in early and accurate diagnosis.

The total numbers of cases included in the present study were 50. The studies done by Bokhman et al,[4] Lee,[5] and Kumar et al[6] included more cases since they studied the cases for a longer duration of time and these studies were done for suspected ovarian neoplasm. The present study was done for a limited duration of 1 year and it comprises all those suspected female genital tract lesions whose frozen and imprint were done in our hospital.

Bokhman et al study[4] was one of the most comparable studies to our research as the age group in the former was 13 to 96 years and the mean age was 54.5, whereas in comparison to our study, the age ranged from 19 to 76 years with a mean age of 53.14 years and a median age of 53.50 years. In one of the previous studies by Terzic et al, they have observed immature teratoma in a 17-year-old female,[7] similarly we had reported one such case in a 19-year-old and findings were comparable to the study. According Morice et al, endometrial cancers were more prevalent in patients above 55 years of age.[8] We had 8 such cases of endometrioid carcinomas in which females were mostly 56 years or above. The findings in our study were comparable with Platz and Benda which showed that most malignant and aggressive cancers were seen with increasing age group and TNM staging.[9]

Shahid et al assessed the role of intraoperative cytology in ovarian neoplasms. They studied 50 cases, out of which 25 were labeled as benign, 24 as malignant, and 1 was reported as inconclusive. This intraoperative cytology was compared with histopathology which resulted in 25 malignant lesions and 25 benign lesions resulting in a sensitivity, specificity, and diagnostic accuracy of 95.8, 96.0, and 95.8%, respectively.[10] In our research, we included all female genital tract lesions, comprising of 34 ovarian lesions as the most common intraoperative diagnosis. Imprint diagnosis showed that there were 17 benign cases, 16 malignant cases, and 1 borderline case. On comparison with histopathology, these 17 benign cases on imprint cytology, actually turned out to be less (10 benign lesions). There were 5 cystadenomas on imprint smears which were reported as 1 mucinous and 4 serous cystadenocarcinoma on H&E stained sections. Two teratoma cases turned out to be immature teratoma which has a high malignant potential. All malignant imprint smears were reported as same on histopathology and borderline tumor was labeled as mucinous cyst adenoma. Hence, our statistics resulted in a sensitivity of 77.5%, specificity of 90.0%, positive predictive value (PPV) of 96.9%, negative predictive value (NPV) of 50%, and diagnostic accuracy of 80%. Our study showed discordance among these values as we took samples from all parts of female genital tract that came for intraoperative diagnosis whereas most studies included ovarian samples for imprint cytology.

According to Khan et al, diagnostic accuracy was assessed in 54 patients by intraoperative frozen section in pelvic neoplasms which also included ovarian malignancy. These frozen sections were later compared with histopathological examination. Their frozen diagnosis included 20 benign cases, 6 malignant cases, 1 borderline, and 3 deferred cases. On paraffin-embedded sections, 17 cases were benign, 1 was borderline, and 2 were malignant. The malignant and borderline cases were reported as same on histopathology. Out of the 3 deferred cases 2 were reported as malignant and 1 as borderline. There diagnostic accuracy was 92.6% with sensitivity of 75%, specificity of 97.6%, 90% PPV, and 93.2% NPV.[11] This study was compared with our present study in which 20 cases were diagnosed as benign lesions, 4 were diagnosed as borderline, and 26 were diagnosed as malignant cases. Histopathology was done which resulted in diagnosing 10 benign lesions into malignant, that is, 4 benign ovarian cysts turned out to be serous and mucinous cyst adenocarcinomas, 5 endometrioid carcinomas were reported on frozen as endometrial polyp, high-grade dysplasia, and complex endometrial hyperplasia, and 1 was diagnosed as teratoma which was diagnosed as immature teratoma on histopathology. Hence, our study statistics showed diagnostic value of frozen sections in terms of sensitivity, specificity, NPV, and PPV as 72.5, 90.0, 45.0, and 96.7%, respectively. However, the diagnostic accuracy of frozen sections remained low, that is, 76.0% versus 80% of imprint cytology. There was discordance in diagnostic accuracy as number of borderline and benign cases reported on frozen were wrong.

The study done by Negri et al emphasized the role of family history in cases of cervical cancers[12] and similarly

Cramer et al studied the significance of family history in ovarian cancers and correlated with follicular phase hormone levels.[13] Both the studies proved their importance as there is twice the risk of cancers in cases of positive family history. We studied such 7 cases in which 6 ovarian tumors had a positive family history. Out of them 4 had first degree relative on maternal side and 2 cases had second degree relative on maternal side and first degree relative on paternal side, respectively. There was one case of cervical cancer which reported as poorly differentiated squamous cell carcinoma and had a positive history of breast cancer in patient's mother who had mastectomy. Thus, our findings were in concordance with the earlier studies.

An important limitation of the present study is that lesser number of cases were included which may have resulted in statistical bias. Difficulty faced while preparing imprints was that yield obtained was low in cases, especially stromal tumors, and care had to be taken not to cause capsular rupture in cystic lesions.

Conclusion

Our study concludes that the maximum cases of female genital tract neoplasms belonged to the age group of 19 to 76 years, with 60% cases in the postmenopausal age group. In most of the ovarian neoplasms initially complaints were abdominal distension associated with pain. Abnormal uterine bleeding was seen in cervical and endometrial carcinomas. In many cases of suspicious ovarian, cervical, and endometrial neoplasms, per vaginal discharge were present along with foul smelling in some which favors a malignant outcome. Majority of specimens taken were ovary ($n = 34$, 68%), as intraoperative diagnosis is more required in ovarian lesions. Serum tumor markers CA-125 and CEA were helpful in making diagnosis in ovarian neoplasms. Positive family histories were relevant in cases of ovarian and cervical carcinoma. Radiology played its vital role in making diagnosis of various benign and malignant lesions on cytology and H&E stained sections. The intraoperative imprint cytology showed sensitivity of 77.5%, specificity of 90.0%, PPV of 96.9%, NPV of 50%, and diagnostic accuracy of 80%. Diagnosis by intraoperative frozen section showed sensitivity, specificity, NPV, and PPV as 72.5, 90.0, 45.0, and 96.7%, respectively. The kappa statistical value, deciding the agreement between two methods, was lesser in frozen versus histopathology and more in imprint versus histopathology.

Authors' Contributions

Conceptualization: A.K., N.S.
Data curation: A.K.
Critical and intellectual evaluation: N.S., R.N., A.K.
Drafting of manuscript: A.K., N.S.
Approval of final manuscript: All authors.

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Conflict of Interest

None declared.

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