RESEARCH ARTICLE

Intra-Operative Frozen Sections: Experience at A Tertiary Care Centre

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Abstract

The present study was conducted to assess error rates with diagnosis using intra-operative frozen sections, and to indicate ways to increase overall performance. Over a period of two years, 227 cases were biopsied intra-operatively. Errors were observed in 14 cases. Four of these were sampling errors, one by a pathologist and three by surgeons. In seven cases incorrect interpretations were made. Epithelial dysplasia was observed on definitive histology in two cases which was not reported intra-operatively. One case was of ectopic thyroid. In cases of oral cancer where sentinel lymph nodes were sampled, immunohistochemistry for cytokeratin was performed to facilitate identification of micrometastasis. Only single case displayed tumor deposits which was not evident morphologically. Resection margins were reported in seventy eight cases. Some 18% (14/50) benefited from revision of margins; overall sensitivity of intra-operative frozen sections for marginal status was 71.4%, with a specificity of 90.3%. Overall sensitivity was 75% and specificity was 97.5%. Careful observation, pathologist experience and knowledge of limitations help in improving the overall diagnostic outcome.

Keywords: Intra-operative Frozen section- accuracy- micrometastasis- margin status- sensitivity

Asian Pac J Cancer Prev, **17 (12)**, 5057-5061

Introduction

Intra-operative histology consultation was introduced for rapid examination of surgical specimens in 1905 by Dr.Louis B Wilson on request of Dr. William Mayo now widely used for guidance of surgeons for surgical treatment (Wilson LB, 1905). The different modalities used for intra-operative consultation are squash smear cytology, frozen sections and fluid cytology. These investigations help to provide a preliminary diagnosis, enabling the surgeon to decide further management at the operating table. The ideal method for providing intra-operative diagnosis, apart from being rapid and accurate, should also allow tissue to be preserved for paraffin embedding and other ancillary studies if required. Intra-operative histology has long been applied as an effective diagnostic method for neoplastic as well as non-neoplastic lesions for multiple reasons namely organ identification, confirmation of clinical diagnosis of malignancy, determining per-operative extent of disease and margin status (Patil P et al., 2015). Accurate intra-operative diagnosis requires clinical correlation and correlation with preoperative findings as well as familiarity of the pathologist with the known pathological entities and knowledge of limitations.

In the present study we reviewed our Intra-operative histology data for the previous two years with the following aims: (i) to assess its diagnostic accuracy, (ii) to evaluate the common reasons for misdiagnosis, including disagreements and partial disagreements with remnant frozen tissue and definitive non frozen tissue (iii) to evaluate the impact of misdiagnosis on immediate surgical management of the patient, and hence calculate overall sensitivity and specificity.

Materials and Methods

This was a retrospective study of all histological specimens received for intraoperative frozen section from May 2014 to May 2016 in the Department of Pathology, King George's Medical College, Lucknow, India, which is a tertiary care referral center. Histopathological confirmation by remnant frozen and non frozen tissue of the lesions was available in all cases.

Technique of frozen sectioning

Unfixed fresh biopsy material sent in normal saline from the operating theatre was examined grossly and sampling was performed as per requirement. In cases of peritoneal nodules, lymph nodes the samples tissue was bisected half frozen rest half was transferred in formalin for definitive histopathology. A minimum of two sections were examined for each frozen tissue, with 10-20µm

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distance. The remaining tissue after frozen was fixed in formalin and processed for paraffin embedding.

Diagnostic categories

The frozen sections were evaluated for their morphological agreement with remnant frozen tissue and definitive non-frozen tissue in haematoxylin and eosin (H&E) stained sections. Assessment of impact of intraoperative opinion on surgical management of patient and co-relation was performed in terms of agreement with intra-operative diagnosis.

Immunohistochemistry

Immunohistochemistry was performed for Pan-cytokeratins (CK) in sentinel lymph nodes (Methylene blue positive per operatively) to look for micro metastasis.

Results

During a period of two years, 224 cases of surgical specimens were received for intra-operative diagnosis. 78 cases were assessed for margin status, rest were either for identification of tumor deposits/ metastasis (lymph nodes, peritoneal nodules, liver nodules etc.) or organ identification (parathyroid or ganglion cells in case of Hirschsprung disease) (Table 1).

Fifteen cases were misdiagnosed on Hematoxylin and eosin stained sections of frozen tissue intra-operatively. Critical evaluation of failure of intra-operative diagnosis of the above cases we found that histomorphology of frozen H&E stained section and remnant frozen and definitive tissue section were similar in six cases and there was no agreement in nine cases (Table 2).

Most prominent reason for misdiagnosis was misinterpretation (7) followed by incorrect sampling (4) and others (3). On detailed evaluation we found apart from one (ectopic thyroid) rest 13 could have been avoided. (Table 2)

Cases where morphology of frozen and remnant tissue was similar but there was failure of intra-operative and tissue diagnosis in decision making were ovary (n=1), Thyroid (n=1), Neck mass (n=1), Lumpectomy specimen breast (n=1), resection margin in oral cancer (n=1) and sentinel blue node for oral cancer (n=1).

Cases where the frozen tissue and remnant tissue were morphologically dissimilar formed the largest group, named as 'interpretation error'. Among this group dysplasia was seen in remnant tissue (Figure 1c) resection margin section reported free intra-operatively (Figure 1d) in two cases. In a case of carcinoma gall bladder frozen diagnosis was deferred due to small biopsy and difficult interpretation; though the formalin fixed paraffin embedded tissue section of the remnant frozen tissue displayed mucin pools with few tumor cells in it.

In one case inflammatory cells were misinterpreted for malignant cells in section from superior shave margin in a lumpectomy specimen breast which resulted in unnecessary revision.

Utmost failure was seen in single case of oral cancer where even on revision tumor deposit was found on revised margin (Figure 1e). There was a small keratin pearl which was seen in revised infero-medial remnant tissue. On careful re-examination of frozen section we found a small keratin pearl (Figure 1f) which was missed and misinterpreted.

In cases of incorrect sampling frozen and remnant tissue morphology was same, however the tissue sampled was not representative. Frozen and remnant tissue were negativebut malignancy was found in the definitive specimen received after grossing of the resected specimen. Suture site recurrence occurred in a male with tumor excision of buccal mucosa cancer on seventh post-operative day. In this case the definitive specimen posterior mucosal resection margin, remnant tissue posterior resection margin and frozen section showed neither tumor deposits nor dysplasia.

Thirty six Methylene blue positive sentinel lymph nodes were received during the study period. Tumor deposits were seen morphologically as well as on IHC (n=2) and only on IHC (n=1). Figure 1a shows tumor deposit on Cytokeratin immunostain and corresponding frozen section also shows metastatic atypical squamous cell within the lymph node parenchyma (Figure 1b)

Total 49 cases of oral cancer underwent intra-operative margin evaluation during the study period. Among them only four cases had false negative results, two of them displayed high grade epithelial dysplasia, one had surgical site recurrence and in one case definite tumor deposit was missed. Accuracy in oral cancer resection was 91.8% (45/49) which is quite good. Ten patients (20%) benefited from revision of surgical margins.

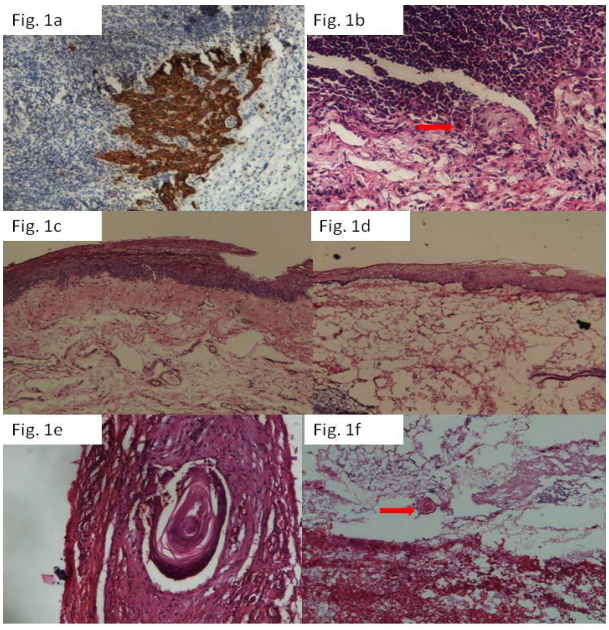

Figure 1. Microscopic Images of Cytokeratin Immunohistochemistry (a) displaying cytoplasmic expression of CK in tumor deposit, concurrent frozen section shows atypical squamous cell (1b; H and E X 200). Section from a carcinoma buccal mucosa resection margin displaying epithelial dysplasia (1c; H and E X 200), concurrent frozen section shows no dysplasia (1d; H and E X 200). Section from remnant tissue displaying a squamous pearl in shave resection margin (1e; H&E X 400) on careful search a small keratin pearl was identified on frozen seciton on review (1f; H and E X 100)

Table1. List of Sites which were Sampled Intra-Operatively

Site	Number	%
Lymph Nodes	77	34.4
Oral	55	24.5
GB	15	6.7
Ovary	15	6.7
Lumpectomy specimen breast	14	6.2
Liver Nodule	10	4.5
Peritoneal Nodule	9	4.0
CBD Margin	7	3.1
Parotid/ Thyroid	3 each	1.3 each
Cyst/ Ganglion cells/ Portahepatis tissue/ Vulval carcinoma	2 each	0.9 each
Bladder Biopsy/ Cervix/ Diaphragmatic nodule/ Esophageal margin/ Orbital cancer/ Parathyroid tissue/ Vagus Nerve/ Laryngectomy Specimen	1 each	0.4 each
	224	100.0

Table2. List of Cases which were Incorrectly Reported with Possible Reasons Behind Them

Sample type	Number of cases	Frozen reported as	Frozen tissue and remenant tissue histology	Cause of the error
Lumpectomy breast	1	Positive	Non Concurrent	Interpretation error
Lumpectomy breast	1	Negative	Concurrent	Sampling error
Gall bladder	1	Negative	Non Concurrent	Interpretation error
Neck mass/ LN	1	Positive	Concurrent	Ectopic thyriod
Oral cancer for margin	2	Negative	Non Concurrent	Dysplasia present
Oral cancer for margin	1	Negative	Concurrent	Sampling error
Oral cancer for margin	1	Negative	Non Concurrent	Interpretation error
Ovary	1	Positive	Non Concurrent	Interpretation error
Ovary	1	Negative	Concurrent	Sampling error
Thyriod	1	Negative	Concurrent	Sampling error
CBD margin	1	Negative	Non Concurrent	Interpretation error
Liver nodule	1	Positive	Non Concurrent	Interpretation error
Mesentric Nodule	1	Negative	Non Concurrent	Interpretation error

The sensitivity of frozen histology for diagnosing neoplastic lesions intra-operatively was 75% with specificity of 97.54%. Positive predictive value and negative predictive value was 90.9% and 94% respectively. There were five false-positive and ten false-negative cases among 224 surgical specimens in terms of their comparison with definitive histological diagnosis and clinical outcome. Overall accuracy was 94.2%.

Discussion

Surgeons often depend upon rapid intra-operative diagnosis for immediate surgical management. Fluid cytology may be used for cystic lesions but squash cytology and frozen sections are the two main techniques used, the choice depending on individual experience and preference. Squash cytology is mainly used in intra-operative consultation of neurosurgical specimens.

Frozen is now a worldwide method for intra-operative consultation, because it provides a rapid and reliable diagnosis, and allows tissue to be preserved for paraffin embedding. It needs technical equipment (Cryostat) as well as technical expertise for a good section and a trained histopathologist for interpretation. Intra-operative histology is indicated for confirmation of an intra-operative impression, confirmation of malignancy and assessment of surgical margins at vulnerable sites where the extent of removal directly influences the surgical outcome. According to various studies the accuracy of Intra-operative histology diagnosis ranges from 87% to 97%. (Hermanek P et al., 1981; Ferreiro JA et al., 1995; Wang KG et al., 1998; Ghauri RR et al., 1999; Pinto PB et al., 2001; Savargaonkar P et al., 2001; Khoo JJ et al., 2004; Subbian A et al., 2013). The studies with higher accuracy had a different study design in terms of type of specimen and inclusion of different categories. Some included squash smears in addition to frozen section, whereas in others, the authors only included tumours or tumours plus infections and / or other miscellaneous lesions. The overall accuracy in the present study was 94.2%, which is a little less than some of the studies in the literature; however, complete agreement between remnant histology and definite diagnosis was 99.1% and between remnant tissue histology and frozen histology was 94.1%. The above result for frozen diagnosis was lower than few other studies. The possible reasons for this

lower figure could be (i) inclusion of all types of lesions (tumours, infections and miscellaneous lesions), (ii) inclusion of tissue evaluated for both margin evaluation and tumor diagnosis, (iii) inclusion of histology and IHC for sentinel lymph node, and (v) inclusion of rare lesions (e.g. ectopic thyroid).

The entities with complete correlation with remnant diagnosis were identification of organ. Jaafar et al in 2006 has highlighted the possible reasons of misinterpretation in frozen section (Jaafar et al., 2006). In our study interpretation error was most frequently observed. Four cases which were reported as negative for tumor deposits on frozen histology had tumor deposits. These were found on remnant tissue examination and review of frozen section. These were samples from radical pancreatico-duedenectomy surgeries where sectioning artefact and small cell size of adenocarcinoma led to incorrect diagnosis on frozen. The result of wrong diagnosis at the time of surgery was that these cases underwent radical surgery which otherwise would had been withdrawn in view of peritoneal metastasis.

As far as interpretative errors are concerned careful examination, ordering additional sections help in their reduction. Second most important thing is despite of pressure of timely reporting a pathologist should never hesitate in ordering additional sections. Repercussions' of incorrect interpretation may lead to litigations. Finally, low power examination is more helpful in decision making than high power examination as the cells swell up due to freezing and may lead to spurious interpretation of nucleomegaly.

Accuracy rates of frozen section in margin interpretation in oral cancer have been reported to be as low as 71.3% in a study from Virginia (DiNardo L J et al., 2000) and high 97.5% in a study from USA. We had comparable accuracy rates (91.8%).

Oral cancer is more frequent in our region because of widespread addiction to tobacco in form of gutka and khaini. We come across a high number of oral cancer cases being operated. As a routine we process two samples intraoperatively 1) tumor bed separately and 2) shave/ radial margin from excised tumor. The above practice might be reason for better results. In study by Olson et al in 2011 three section from oral resection margins were recommended to reduce error due to inadequate frozen sampling (Olson et al., 2011).

We also assessed the utility of Cytokeratin expression in sentinel lymph nodes sampled from oral cancer and found that no significant added advantage of immunohistochemistry with frozen as far as overall surgical output was concerned.

Possible causes of errors may be concisely divided as sampling, technical and interpretative. As far as sampling is concerned better interaction between the reporting pathologist and operating surgeon reduce it. Prior information of the cases being operated which might require frozen also helps to reduce it. Sampling of margins and their intraoperative interpretation gave better results when we started sampling tumour bed.

Chattering of the section and folding are frequently observed. Training of the technical staff involved in sectioning reduces the above complain.

The strength of present study is that all samples were evaluated collectively, which lead lower sensitivity and specificity. However, this represents the actual scenario of anatomical pathologists' working. We come across different types of samples at the same time with multiple specialties operating in different operation theaters in simultaneously. It is important for the operating surgeons to have the knowledge of limitations of this procedure. Precise questions which need to be answered by frozen histology must be clear. Both pathologists and operating surgeons must work as a team for better outcome.

Careful observation, skill of the pathologist and knowledge of limitations help in improving the overall diagnostic utility of frozen section. Despite the advancements in histological and molecular techniques; this investigation, clearly remains a valuable tool during operative procedures.

Conflict of interest: Nil

Acknowledgement

Authors are thankful to King Georges Medical University, Lucknow, for providing necessary resources. We want to thank our technical team members especially Mr. Kamlesh Sharma and Miss Nidhi Varma for their support. The authors have no other relevant affiliations or financial involvements.

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