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Original Research Article

Analysis of intraoperative frozen section consultations and audit of accuracy: a two year experience in a tertiary care multispeciality hospital in India

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ABSTRACT

Background: Diagnostic accuracy of intra-operative frozen section (FS) depends largely on quality of tissue sections backed by good clinical communication and experience of reporting pathologist. Periodic audit of this consultation in surgical pathology help in assessing the efficiency of procedure and addressing the pitfalls. In this study authors have analysed the spectrum, indications and assessed the accuracy of FS consultation in their institution.

Methods: A retrospective study of 212 consequent tissue specimens submitted for FS over two years in study centre was conducted. The FS and corresponding formalin fixed paraffin embedded (FFPE) tissue section with their final histopathological examination (HPE) reports were studied and analyzed. The results were classified in concordant, discordant and deferred categories. Accuracy rates and discordant frequencies were calculated and comparison with other similar studies was done. Reasons for inaccuracies were deduced.

Results: A total of 212 tissue specimens for FS were reported over two years in this study institute, six of which showed discordant results. Most common site of FS in this study centre was from central nervous system (CNS) lesions (28.77%). Indications for intra-operative consultation were mainly for establishment of tumor diagnosis (66.51%) and status of margins (29.25%). The accuracy rate was found to be 97.17% with error rate of 2.83%. On analysis of discordant cases; the reason for inaccuracy was mainly due to interpretation error (83.33%).

Conclusions: The audit of FS consultation established that accuracy rates of this study institution are comparable with most international quality control statistics for FS. The discordant cases were mostly false positive hence emphasising that a variable degree of reservation is required while interpreting and communicating the FS results. The closest possible diagnosis should be communicated on FS and definitive diagnosis should be deferred to HPE in case of doubt.

Keywords: Accuracy, Histopathology, Frozen section, Intra operative, Margin, Tumor

INTRODUCTION

Intra-operative frozen section (FS) consultation is a real time, rapid and important clinical communication

between operating surgeon and reporting surgical pathologist. It guides surgeon in planning and managing the extent of surgery. Both pathologist and operating surgeon should be aware about the indications and

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limitations of the procedure.¹ Most common indications for FS include identification of presence or absence of primary tumor or metastasis, surgical margins status and confirmation of representative tissue samples for final paraffin section diagnosis.² Other methods like squash smears for central nervous system (CNS) tumors, Imprint smears mainly for lymph nodes and fluid cytology are used uncommonly to guide the surgeon intra-operatively.³ Internationally published studies have established the overall accuracy of Intra- operative FS examination.⁴ Periodic institutional audit of this consultation is required to help overcome the pitfalls and augment the accuracy of the procedure.^{5,6}

The aim of the present study was to audit the indications for intra-operative FS consultation at the tertiary care institute, study the spectrum and assess the diagnostic accuracy of FS consultation of various anatomical sites. In addition, the study addressed the discordant and deferred cases to find underlying causes for future applications for improvement.

METHODS

A retrospective study was carried out in the histopathology section of department of pathology of the study institute in which all the consequent intra-operative FS reported from 1st January 2018 till 31st December 2019, were analyzed along with the corresponding formalin fixed paraffin embedded (FFPE) tissue sections and their final histopathological examination (HPE) reports. The data was obtained from the compiled manual records and stored sections on glass slides were retrieved for the study.

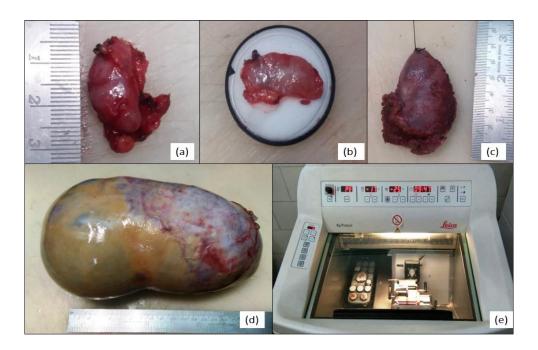


Figure 1: (a) Lymph node specimen for FS; (b) Tissue embedded on specimen disc, (c) Glossectomy specimen for Margin status, (d) ovarian mass for tumor identification, (e) Cryostat machine (Leica, CM1950).

As per the protocol for intra-operative FS followed in study institute, the appointment for consultation is taken one day prior, barring occasional emergency cases. The clinical details and radiological results are shared with the Pathologist along with the indication for FS. Fresh unfixed tissue specimen is received from operation theatre (OT) which is immediately grossed by the pathologist and representative tissue is submitted for FS analysis (Figure 1). Multiple representative tissues are taken from different sites depending on the indication of FS consultation. The tissue is embedded on specimen disc using optimal cooling temperature (OCT) compound and sections are cut on Cryostat machine (Leica, CM1950) at a temp between -18°C to -24°C depending on the nature of the tissue (Figure 1). The cut sections are rapidly fixed and stained with Leishman Giemsa (LG)

and hematoxylin and eosin (H and E) stain. The prepared FS are preferably seen by two pathologists. The FS report is conveyed to the operating surgeon telephonically and also endorsed in writing for future reference. The remaining tissue of FS and the parent specimen are formalin fixed and routinely processed for paraffin embedding and section cutting.

Retrospective data was obtained from intra-operative FS done and reported at this study institute in last two years. Data was analyzed for indications and organ/tissue system involved. The results of the study were grouped into concordant, discordant and deferred categories. Site wise accuracy rates, concordant and discordant frequencies were calculated. Discordant and deferred

cases were reviewed for the sites involved and reasons of short comings.

RESULTS

During period of two years (1st January 2018 to 31st December 2019), total number of specimens received for intra-operative FS in the study institute were 212. The age range of patients varied from 3 months (for Hirschsprung's disease) to 80 years. Out of 212 cases, female patients outnumbered the male patient and constituted 57% of cases (Figure 2).

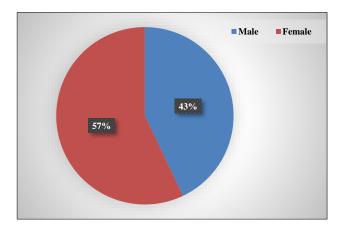


Figure 2: Gender distribution.

The commonest indication for intra operative FS in study institute was establishment of tumor diagnosis (66.5%), primary or metastatic followed by margin status (29.3%). Other indications for FS included tissue identification for Parathyroid tissue and for ganglion cells in case of Hirschsprung's disease (Figure 3).

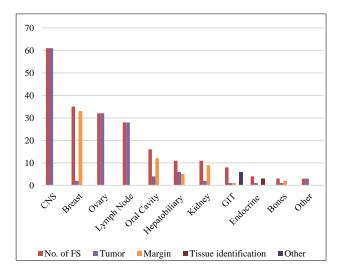


Figure 3: Distribution of FS according to the site and indications for intra-operative consultations.

Commonest site to be sampled in the study institute for FS was CNS constituting 61 cases (28.8% of total cases); four cases were from spinal lesions, rest from brain

SOLs. Glioma made bulk of CNS lesions to be sampled for intra-operative FS (45.9%) with Meningiomas being second most common (29.5%). Other CNS lesions which were sampled for FS included schwannoma, metastatic malignancy, pituitary adenoma, abscess etc. Breast lesions were the second most common sampled site for FS (16.5%) with indication mainly being for surgical margin status (Figure 3). Most cases were of invasive ductal carcinoma (85.7%) with two cases each being of invasive papillary and mucinous carcinoma of breast.

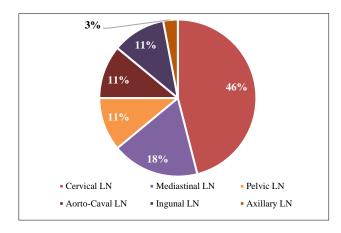


Figure 4: Distribution of group of lymph nodes subjected to FS.

Intra-operative FS for ovarian tumors made 15.1% of total cases and out of total of 32 ovarian lesions; twenty were surface epithelial tumor (62.5%) and rest included germ cell tumors, sex cord-stromal tumor and endometriotic cysts. Breast and ovarian tissue together made 31.6% of total cases of FS in this study.

Total of 28 lymph node (LN) specimens were submitted intra-operatively for metastatic nodal status. Cervical group of lymph nodes were the commonest to be sampled followed by mediastinal, pelvic, aorto-caval, inguinal and axillary LNs (Figure 4). Cervical LNs were commonly assessed for metastasis of squamous cell carcinoma followed by metastasis of thyroid carcinoma.

The overall accuracy rate for intra-operative FS consultation over two years was found to be 97.17% with discordant rate of 2.83%. Site wise the minimum accuracy rate was seen in GIT (87.5%) but it was due of less number of total cases from the site. Out of total 212 FS, only 8 were from GIT and amongst them only one case had discordant result on final HPE. Sites like breast and oral cavity sampled more for margin status had accuracy of 100% (Table 1).

In cases of intra-operative FS for tumor diagnosis, out of total 141 cases, four cases were discordant with concordance rate of 97.16% (Table 2). Two cases were in category of discordant out of 62 cases sampled for margin status resulting in concordance rate of 96.77% (Table 2). Out of total six discordant cases, four were

false positive and one was false negative for malignancy due to interpretation error and second false negative case was due to sampling error (Table 3).

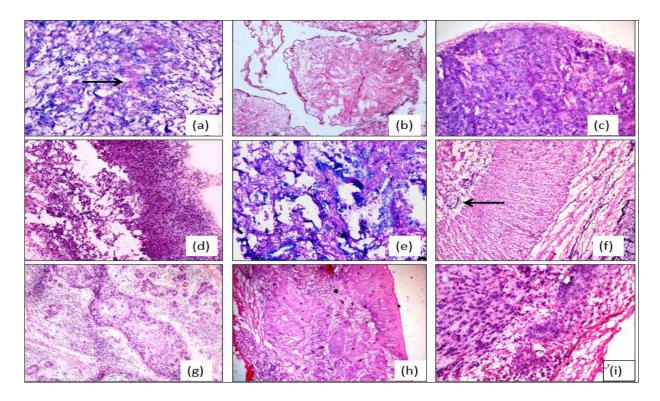


Figure 5: Histomorphology on FS (10x), (a) Schwannoma with nuclear palisading, (b) Serous cystadenoma, (c) Reactive lymph node, (d) High grade glial tumor, (e) Adenocarcinoma, (f) Arrow pointing ganglion cells, (g) Presence of high grade DCIS in surgical margin of mastectomy specimen, (h) Squamous cell carcinoma involving surgical margin, (i) Involved surgical margin with high grade tumor.

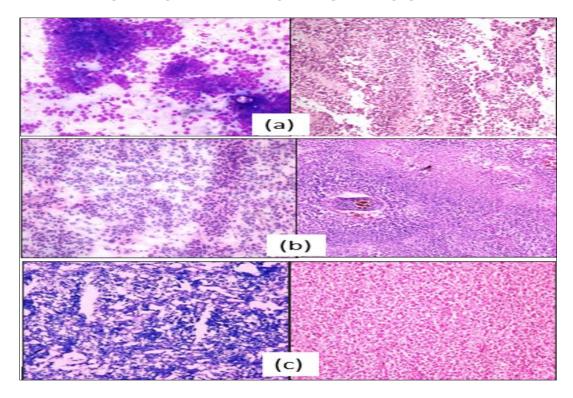


Figure 6: Histomorphology of deferred cases on FS and corresponding FFPE section (10x), (a) Anaplastic ependymoma (b) Diffuse large B cell lymphoma, (c) Juvenile granulosa cell tumor.

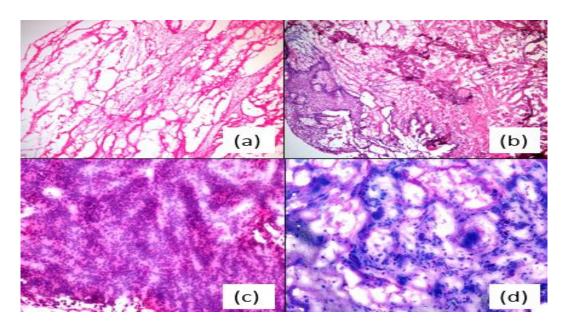


Figure 7: Technical problems on FS, (a) Freezing artefact, (b) Sectioning artefact, (c) Folding and staining artefact, (d) Cellular bloating.

Table 1: Distribution of concordant and discordant cases along with comparative accuracy rates of intra-operative FS from different sites.

Site of specimen for FS	No. of FS	Concordant	Discordant	Accuracy
CNS	61	60	1	98.36%
Breast	35	35	0	100%
Ovary	32	31	1	96.87%
Lymph node	28	27	1	96.43%
Oral cavity	16	16	0	100%
Hepatobiliary system	11	10	1	90.90%
Kidney	11	10	1	90.90%
GIT	8	7	1	87.5%
Endocrine glands	4	4	0	100%
Bone	3	3	0	100%
Other	3	3	0	100%
Total	212	206 (97.17%)	6 (2.83%)	97.17%

Table 2: Comparative diagnostic accuracy rates as per FS indications for tumor detection and margin status.

Site of frozen section	Tumor detection	Concordant	Discordant	Margin status	Concordant	Discordant
CNS	61	60	1	-	-	-
Breast	2	2	-	33	33	-
Ovary	32	31	1	-	-	-
Lymph node	28	27	1	-	-	-
Oral cavity	4	4	-	12	12	-
Hepatobiliary system	6	5	1	5	5	-
Kidney	2	2	-	9	8	1
GIT	1	1	-	1	-	1
Endocrine glands	1	1	-	-	-	-
Bone	1	1	-	2	2	-
Other	3	3		-	-	-
Total	141	137 (97.16%)	4 (2.84%)	62	60 (96.77%)	2 (3.22%)

Out of total cases (n=212), true positive = 146, false positive = 4, false negative = 2 and true negative = 60.

On calculation, the sensitivity and specificity of the intraoperative FS consultations were 98.64% and 93.75% respectively. The calculated positive predictive value (PPV) and negative predictive value (NPV) were 97.33% and 96.77% respectively.

Five cases were deferred for final opinion on FFPE sections; three out of five were from high grade CNS lesions. Intra-operative FS of these CNS lesions showed

high cellularity and dyscohesive large cells with pleomorphic nuclei. Overlapping morphological picture made it difficult to commit to definitive diagnosis intra operatively. Rest two deferred cases included papillary lesion of breast of undetermined malignant potential and the other was uncommon Juvenile granulosa cell tumor of ovary (Table 4).

Table 3: Details of FS with discordant results on final HPE and the cause of the error.

Type of sample	FS report	Final HPE report	Cause of error	
Brain tumor for primary	Glial tumor (false positive)	Abscess with reactive	Interpretation	
diagnosis	Onar tumor (raise positive)	gliosis	error	
Ovarian tumor for	Borderline serous cyst adenoma	Serous	Interpretation	
primary diagnosis	(false negative)	cystadenocarcinoma	error	
Gall bladder for primary	No infiltrative malignancy, mucosal dysplasia	Adenocarcinoma	C 1'	
diagnosis	present (false negative)	Adenocarcinoma	Sampling error	
Lymph node for	Suspicious of metastasis	Magativa for matastasis	Interpretation	
metastasis	(false positive)	Negative for metastasis	error	
Partial gastrectomy for	Positive for adenocarcinoma	Negative for	Interpretation	
margin status	(false positive)	malignancy	error	
Partial nephrectomy for	Atypical cells seen suspicious of malignancy	Inflammatory changes,	Interpretation	
margin status	(false positive)	no malignancy	error	

Table 4: Details of cases with deferred FS reports to final HPE.

Type of sample	FS report	Final HPE report	Remark
Brain tumor for primary diagnosis	High grade tumor; probably medulloblastoma	Anaplastic ependymoma	Presence of hyperchromatic round cells without differentiation
Brain tumor for Primary diagnosis	High grade tumor	Diffuse large B- Cell lymphoma	Large pleomorphic cells without differentiation
Brain tumor for primary diagnosis	High grade tumor	Metastatic carcinoma	Large pleomorphic cells without differentiation
Breast tumor for primary diagnosis	Papillary lesion of indeterminate malignant potential	Infiltrating papillary carcinoma, grade 1	Invasion difficult to comment with surety on FS
Ovarian mass for primary diagnosis	Malignant tumor	Juvenile granulosa cell tumor	Uncommon diagnosis; limited experience

Table 5: Comparative analysis of present study with other studies in same field over last two decades.

Authors	Countries	Study duration (year)	No. of	Concordance rate	Discordance rate
			cases	(%)	(%)
Geramizadeh et al ¹²	Iran	4	759	96.7	3.3
Mahe E et al ⁵	Canada	1/2	812	98	2
Farah-Klibi F et al ²⁸	France	3	1207	97.5	2.5
Patil P et al ⁹	India	2	100	96.9	3.1
Shreshtha et al ²⁶	India	5	404	94.6	5.4
Selvakumar et al ⁸	India	5	132	98.65	1.35
Present study	India	2	212	97.17	2.83

DISCUSSION

Early diagnosis and correct staging of malignant disease by the clinician help the patient in getting the proper treatment on time. The stage of the cancer at the time of diagnosis is a key factor that defines prognosis and is critical for determining the appropriate treatment. Intraoperative FS is one of the well-established tools to help the surgeon to know and confirm the nature of lesion and its extent resulting in better surgical outcomes.¹⁻⁴ FS is technically sophisticated procedure requiring necessary equipment, sufficient skilled staff and experienced pathologist. On average the total time taken from receipt of the intra-operative sample for FS to reporting varies from 20 to 25 minutes as also found true in this study setup.⁷

The accuracy of FS consultation at study institute is found to be comparable with most international quality control statistics for frozen sections. Different studies all over the world show accuracy rates for intra-operative FS consultations range from 92% to 97.7%.^{6,8-10} The accuracy rate for intra-operative FS consultation in the present study was 97.17% with discordant rate being 2.83%. The discordant frequency for FS diagnosis in written literature ranges from 1.1% to 5%.^{2,6}

The most common indication found in present study for FS consultation was for establishment of tumor diagnosis, primary or metastatic. Out of total of 212 intra-operative FS consultations, 141 cases (66.51%) were done for establishment of tumor diagnosis. Status of surgical margins was second common indication for consultation (29.25%). Uncommon indications include confirmation of absence or presence of ganglion cells in cases of pediatric surgery (2.83%) and tissue identification like in Parathyroid gland surgeries (1.41%). The frequency of indications varies in different centers depending on bulk of surgeries as brought out by different articles in written literature.^{8,9}

The most common site to be sampled at the study centre for FS reporting was CNS (28.77%) and indication being diagnosis of tumor type. The advances in neuro-imaging has strengthened the hands of neurosurgeon but intra operative FS continue to be important diagnostic tool to confirm the target lesion and in planning of surgery. 11 Out of all CNS lesions sampled, bulk was formed by Glial tumors followed by benign spindle cell lesions comprising of Meningioma and Schwannoma (Figure 1 and Figure 5a). The common problem of differentiating neurogenic tumor and fibroblastic Meningioma is already addressed in written literature.¹² In cases of overlapping features on FS and lack of distinguishing features like whorling, nuclear palisading, hyalinization of vessel wall or presence of psammoma bodies etc., the diagnosis of benign spindle cell tumor was rendered on FS to be confirmed later on FFPE tissue section.

Differentiation of low-grade glioma from reactive gliosis is one of the most difficult challenges in neuropathology and more so on FS, an experienced pathologist usually relies on FFPE sections for final opinion in cases of overlapping morphological features. Hypercellularity, irregular cell distribution, nuclear pleomorphism, micro cysts and perineural satellitisms are seen more in neoplastic lesions, features better appreciated on FFPE sections. In present study a case of abscess with surrounding gliosis was reported as glial tumor on FS due

to interpretational error and lack of neuro-imaging details.

The second commonest site sampled was breast (16.50%) but the indication for FS was more for margin status. In many cases of breast carcinoma, margins were revised in this study institute on FS consultation leading to better outcome (Figure 5g). Studies in this field have established the importance of FS in avoiding reoperations.¹³ One of the grey zones includes ductal carcinoma breast with extensive intra-ductal component (EIC); in spite of clear margins on FS initial conservative surgery was revised later to modified radical mastectomy due to presence of EIC.14 Following breast, oral cavity was the second common site to be consulted intraoperatively for margin status (Figure 5h, i). Information about margins helps the operating surgeon to limit the tissue loss without compromising the adequacy of the procedure. Adequate margin clearance is must as the recurrence is usually aggressive with poorer prognosis. 15 Studies have shown accuracy rate on FS for margin status in oral cancers varying from 71.3% to 97.5%.^{3,16} In this study centre no discordant case was reported from oral cavity during the period of audit. Most of the false negative cases on FS for margins in head and neck surgeries are reported due to staining issues, section folding, thick sections and/or lack of pathologist's experience as per related studies.¹⁷

Ovarian masses were the third commonest site for FS consultation in study centre (Figure 5b). Accurate intraoperative histologic assessment of an ovarian mass helps in selecting an appropriate surgical procedure and avoid under or over treatment of the patient. More than 50% cases were of surface epithelial tumor out of which one was reported as borderline on FS and turned out to be malignant on final FFPE sections making the accuracy rates of frozen consultation for ovarian lesions as 96.87%. Most studies have reported accuracy rates ranging from 90 to 97% for frozen section of ovarian masses. 18,19 Result of many studies have concluded that FS has high accuracy rates for the diagnosis of benign and malignant ovarian tumors but accuracy rates in borderline tumors remains low.^{20,21} Other ovarian lesions diagnosed on frozen section in the study institute were endometriotic cysts, germ cell tumors and fibroma. One of the cases deferred for final FFPE section for diagnosis turned out to be Juvenile granulosa cell tumor (Figure 6c).

For staging of malignancy, lymph nodes are commonly subjected to FS intra-operatively to confirm about the metastasis (Figure 5c). The presence of cervical lymph node metastasis is the most important adverse prognostic features for most cancers of head and neck and similarly assessment of sentinel lymph node in cases of breast carcinoma helps in therapeutic planning.²² In present study the most common lymph nodes subjected to FS were from cervical region for staging of oral cancers (Figure 4) followed by mediastinal, pelvic, aorto-caval,

inguinal and axillary LNs. Pelvic lymph nodes were usually sampled in cases of carcinoma cervix while aortocaval LN in pancreatobiliary surgeries. The accuracy rates for LN frozen section were 96.43% with one discordant case out of 28 specimens which was reported false positive for metastasis due to interpretational error.

Most of the cases of renal carcinoma subjected to margin assessment on FS had undergone partial nephrectomy. One of the renal cases was falsely reported as positive for surgical margin on FS due to presence of cellular bloating and vacuolization resulting from surrounding dense inflammation. Uncommon indication of tissue diagnosis on FS has been used for parathyroid tissue.²³ FS examination is not a very reliable method for differentiating parathyroid adenoma from multiple glands hyperplasia and routine use of FS is not recommended.²⁴

Analyzing cases with discordant results, four were the cases for tumor diagnosis and two were for margin status (Table 3). Out of four discordant cases for tumor, two each were reported as false positive and false negative for malignancy. Usually false positive diagnoses are rare on FS because pathologists tend to defer to FFPE sections for final diagnosis in case of discrepancy. Common cause of discordant result was due to interpretation error and uncommonly due to sampling error. 1,25 Interpretation errors were compounded by technical problems due to freezing artefact, poorly cut and stained section (Figure 7). Poor quality of morphological details due to cellular bloating leads to misinterpretation by reporting pathologist. Both discordant cases for margin status were reported false positive due to interpretation error resulting from bloated cell morphology and presence of dense inflammation (Table 3). Experienced pathologist rely more on the architectural details rather than on the misleading cellular features on high power during reporting intra operative FS.

The FS diagnosis sometimes can be really tricky. It should be the policy of pathologist to give closest possible diagnosis intra-operatively and avoid giving definitive diagnosis in case of doubt. The deferral rate varies from 0.65% to 6.1% in written literature. 9,25,26 The commonest reasons for deferral includes sampling mistake by surgeon, technical imperfections of FS, lack of clinical details and overlapping histomorphology features. In this study the deferral rate was 2.36%. Out of five deferred cases three were from CNS, these were all high-grade lesion with poor differentiation (Table 4; Figure 6). Large cell lymphomas, poorly differentiated metastatic deposits are difficult to differentiate from high grade glioma on FS as is well documented in literature. 11 Close communication between neurosurgeon, pathologist and radiologist is required to avoid potential pitfalls. Rest two cases deferred includes one from papillary lesion of breast of undetermined malignant potential which turned out to be infiltrating malignant lesion on FFPE sections and other was solid ovarian tumor, reported as malignant lesion on FS and as juvenile granulosa tumor on final

HPE (Figure 6). Good communication skills are necessary to adequately explain deferred diagnosis to the surgeon. Miscommunications can be avoided by keeping the FS diagnosis brief and by avoiding nonstandard and conflicting terminology.²⁷ On comparison of the present study with other similar studies published in literature over last two decades, the discordant rates of intraoperative FS range from 1.4% to 5.4% with concordance as high as 98.7% (Table 5).^{5,8,9,12,26,28}

Intra-operative FS consultation has time and again proved its merit however it should be done for scientific indications only and not just to satisfy surgeon's curiosity, to compensate for inadequate pre-operative evaluation or means to communicate information faster to patient or patient's family. The operating surgeon should also be educated about the limitations of FS.

CONCLUSION

Intra-operative FS is an important tool in hand of pathologist and with proper sampling, measures to reduce interpretive errors and better communication with operating surgeon the accuracy rates can be improved upon resulting in long term benefits to the patient. Periodic audit helps in enhancing the efficacy and addressing the pitfalls of the technique.

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