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Frozen sections compared with paraffin-embedded sections: A retrospective veterinary autopsy study

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Abstract

Histologic examination of tissues collected at autopsy are typically prepared using conventional methods (formalin fixed, paraffin-embedded tissue). In this study, twenty-five tissue specimens from twelve animals were collected at autopsy and examined using the frozen section technique. Of the 25 specimens examined, an accurate and specific diagnosis was obtained in 21 specimens; the pathologic process was correctly identified, but a specific diagnosis was not obtained in 1 specimen; the diagnosis was missed in 2 specimens, and in 1 specimen the diagnosis was deferred. Of the two incorrect diagnoses, one was due to a sampling error and one was due to an interpretation error. Overall, the use of postmortem frozen sections allowed for complete agreement with conventional methods in 21 specimens (84%) and the results from this study support the use of frozen section examination of tissue samples collected postmortem.

Key words: frozen section procedure, cryosection, histopathology, postmortem

Introduction

Postmortem examinations are performed for a number of reasons including determination of the cause of death, evaluate the extent of disease in a patient, and determine the effectiveness of a therapeutic procedure for the benefits of future patients. In a veterinary diagnostic laboratory, it usually takes at least 48-72 hours to allow for adequate tissue fixation and processing of tissues before microscopic examination of tissues can be performed by the pathologist. Given this delay, it is not uncommon for veterinary pathologists to perform touch impression cytology in order to obtain a rapid diagnosis. Although cytology is a rapid procedure, a major limitation of cytology is the inferior ability to evaluate tissue architecture (2).

The frozen section technique was first described in 1818 by De Riemer (5) and the use of frozen section analysis of intraoperative specimens is based on the procedure first published in 1905 (3). Frozen section histology can allow for the rapid diagnosis of a pathologic process; however, it is not a replacement for standard histopathology. Frozen section histology has many uses including the ability to establish the presence of a lesion, establish the nature of a lesion, and determine the adequacy of margins (5). Since its development, it is an extremely valuable method to surgical teams and in order to provide rapid interpretation of cases, a semi-automated method has been developed at one institution to determine subspecialty case distribution and prediction of intraoperative consultations in surgical pathology (4). One of the most common used for frozen section analysis is Mohs surgery; however, virtually any tissue can be analyzed using this technique.

Although uncommonly used by veterinary pathologists, examination of frozen tissue sections can be performed to allow for the rapid diagnosis of disease processes identified during the postmortem examination. Frozen sections allow for retention of tissue architecture. The successful use of the frozen section examination at autopsy has been previously described in humans (8). The purpose of this study is to investigate the usefulness of frozen section examination of tissues collected during the veterinary postmortem examination. To the author's

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knowledge, this is the first study to investigate the usefulness of frozen section examination in from tissues collect postmortem in veterinary medicine.

Material and methods

During a 6-month period, 7 dogs, 4 cats, and 1 horse that were submitted for autopsy had tissue specimens collected for frozen section histology as part of the autopsy procedure. The autopsies were performed according to the standard autopsy procedure. For each case, select organs were collected with a total of 25 specimens being collected. The tissue specimens were transferred to a histotechnologist, who subsequently froze the tissues with liquid nitrogen after embedding in OCT compound. The frozen sections were cut and stained with a hematoxylin and eosin stain. The slides were available the same day the autopsy was performed.

Simultaneous to the collection of the tissues for frozen sectioning, tissue samples were collected and fixed in 10% neutral buffered formalin. All tissue specimens were routinely processed, sectioned, and stained with hematoxylin and eosin. Using the histopathologic diagnosis as the gold standard, results were reviewed on the basis of overall agreement.

Results

A total of 25 tissue sections including twelve different tissue types were evaluated using the frozen section (Fig. 1). In eight autopsies, there was complete correlation of the anatomic diagnoses. A range of major and minor processes were identified within the tissues examined including inflammatory and neoplastic processes and several tissues did not have any significant findings in both frozen section and formalin fixed sections (Table 1). Diagnosed neoplastic processes included lymphoma, hemangiosarcoma, and a metastatic (pulmonary) carcinoma. Inflammatory processes diagnosed included pneumonia, mesenteric granulomas, meningitis, pyogranulomatous nephritis, and gastric perforation with associated suppurative inflammation. Other diagnoses include amyloidosis of Islets of Langerhans, and acute hemorrhage.

In the remaining four autopsies, there were three discrepancies and one deferral identified. Organs with discrepancies and deferrals include the lungs (n = 3), and skin (n = 1) (Table 1). One discrepancy was due to gross sampling as the suppurative inflammatory foci were not in the frozen section of lung examined. In the second discrepancy, there was an interpretation error (under call) in which the formalin fixed specimen of lung had acute pulmonary edema. The final discrepancy was due to a tissue processing error as the skin repeatedly fell apart during the cryostat cutting process in the histology laboratory. The only deferral was from a section of lung in which the process was correctly identified as a carcinoma; however, it was definitively diagnosed as a pulmonary carcinoma during evaluation of the formalin fixed tissue.

For the 12 cases, there was complete correlation in 66.6% of cases (n = 8). The use of postmortem frozen sections allowed for complete agreement with conventional methods in 21 specimens (84%), discrepancy in 3 specimens (12%), and deferral in 1 specimen (4%).

Discussion

Frozen section evaluation of tissues is most commonly perform during intraoperative procedures, although is not commonly utilized in veterinary medicine. The uncommon use of frozen section histology is likely due to the need for specialized equipment that is likely only available in veterinary diagnostic laboratories or academic institutions. Additionally, as most tissues collected at autopsy are placed directly in formalin, when performing frozen section analysis; tissues are to remain in an unfixed state as transient fixation can result in compromise of the biopsy sample (11). A search of the World Wide Web revealed one private practice in the United States that advertises the use of this procedure on its website (9). Proposed indications for use of intraoperative frozen section histology in veterinary medicine include determination of the nature of a pathologic process, determination of the extent of spread of a neoplastic process, evaluation of resection margins of a neoplastic process, and clarify situations where a discrepancy exists between the preoperative cytological or histologic diagnosis and intraoperative gross pathology (10). Rapid protocols have also been developed for immunohistochemical stains, which can further aid in the analysis of frozen sectioned tissues (1, 6).

In the current study, it has been shown that frozen section examination can be performed on tissues collected during the veterinary autopsy just as in human autopsies and can allow for definitive diagnosis of various pathologic processes. Three of the four discrepancies and deferrals were from lung tissue and were due to a variety of reasons including an error of sampling (pneumonia), a false negative result (pulmonary edema), and difficulty in fine microscopic examination of a section. In each of these instances, only a single piece of tissue was evaluated; therefore, if the numbers of lung specimens examined increased it is theorized that the missed lesions could have been observed.

Errors from frozen section examination can occur due to diagnostic misinterpretation, sections are not taken from lesional tissue, or technical issues may preclude proper evaluation (7). Multiple human studies have evaluated the rates of frozen section discrepancies and deferrals from biopsy specimens. Discrepancy rates range from 1.7% to 4.8% and deferral rates range from 1.3% to 4.8% (10). In a human study with 22 interpretation errors from 1042 frozen sections samples, there were 8 false negatives, 10 false positives, and 4 misclassifications (8).

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Table 1. Listing of frozen section and formalin fixed diagnoses.

Organ	Frozen Diagnosis	Final Diagnosis	Agree/Disagree/Deferral	Reason for Discrepancy/Deferral
Liver	Vacuolar hepatopathy, mild	Vacuolar hepatopathy	Agree	
Liver	No abnormalities	Same	Agree	
Liver	Hemorrhage	Same	Agree	
Lung	No abnormalities	Pulmonary edema	Disagree	Undercall
Lung	BALT hyperplasia	Same	Agree	
Lung	No abnormalities	Same	Agree	
Lung	No abnormalities	Suppurative pneumonia	Disagree	Gross sampling
Lung	Increased alveolar macrophages	Pulmonary edema and increased alveolar macrophages	Agree	
Lung	Bacterial pneumonia	Same	Agree	
Lung	Hemorrhage	Same	Agree	
Lung	Carcinoma	Pulmonary carcinoma	Deferral	Lack of cellular detail
Kidney	Pyogranulomatous nephritis	Same	Agree	
Kidney	Lymphoma	Same	Agree	
Kidney	Tubular necrosis and interstitial nephritis	Same	Agree	
Kidney	No abnormalities	Same	Agree	
Heart	Lymphoma	Same	Agree	
Heart	Hemangiosarcoma	Same	Agree	
Spleen	No abnormalities	Same	Agree	
Pancreas	Islet amyloid	Same	Agree	
Stomach	Transmural suppurative gastritis (perforation)	Same	Agree	
Retroperitoneum	Lymphoma	Same	Agree	
Adrenal gland	Metastatic carcinoma	Same	Agree	
Skin	Hemorrhage	Hemorrhage and gunshot residue detected	Disagree	Tissue processing
Mesentery	Granuloma	Same	Agree	
Brain stem	Pyogranulomatous meningitis	Same	Agree	

In veterinary medicine, a study evaluating 194 frozen tissue biopsy specimens had a 1% deferral rate and incorrect diagnosis were reported in 6% of cases (12). In contrast to antemortem sampling, there is only a single study evaluating the correlation and discrepancy rate in human autopsy specimens with 18 of 50 autopsies (36%) having complete correlation (11). The remaining 32 autopsies had 47 errors in the frozen section diagnoses with 38 errors due to sampling and 9 errors involved interpretation due to fine microscopic detail (11).

Overall, the results of this study support the use of frozen sections of samples collected during the autopsy. In one instance, a definitive diagnosis was not obtained due to fine microscopic detail; however, the correct process was identified and in two instances, collection of additional lung tissue would have likely resulted in identification of the pathologic lesions. Although the exact time to completion of the tissue processing was not documented, all tissues were evaluated by the end of the day when submitted to the histology laboratory. This rapid turn-around-time could allow for incorporation of results into the preliminary autopsy report.

The histologic examination of frozen sections is not new to veterinary medicine; however, it is an underutilized technique. In a veterinary teaching hospital, access to an immediate diagnosis after performing an autopsy would be beneficial to veterinary students who might not otherwise receive the final diagnosis from the autopsy since many cases are completed several weeks after the gross examination is performed. If examination of frozen sections is incorporated into the autopsy procedure, it is important to continue to collect formalin

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fixed tissues to serve as a backup in instances where pathologic processes are not detected or cannot be fully characterized during the frozen section examination. Although the numbers of specimens examined in this study were small, it has been shown that frozen section examination of postmortem samples is a feasible and potentially useful technique.

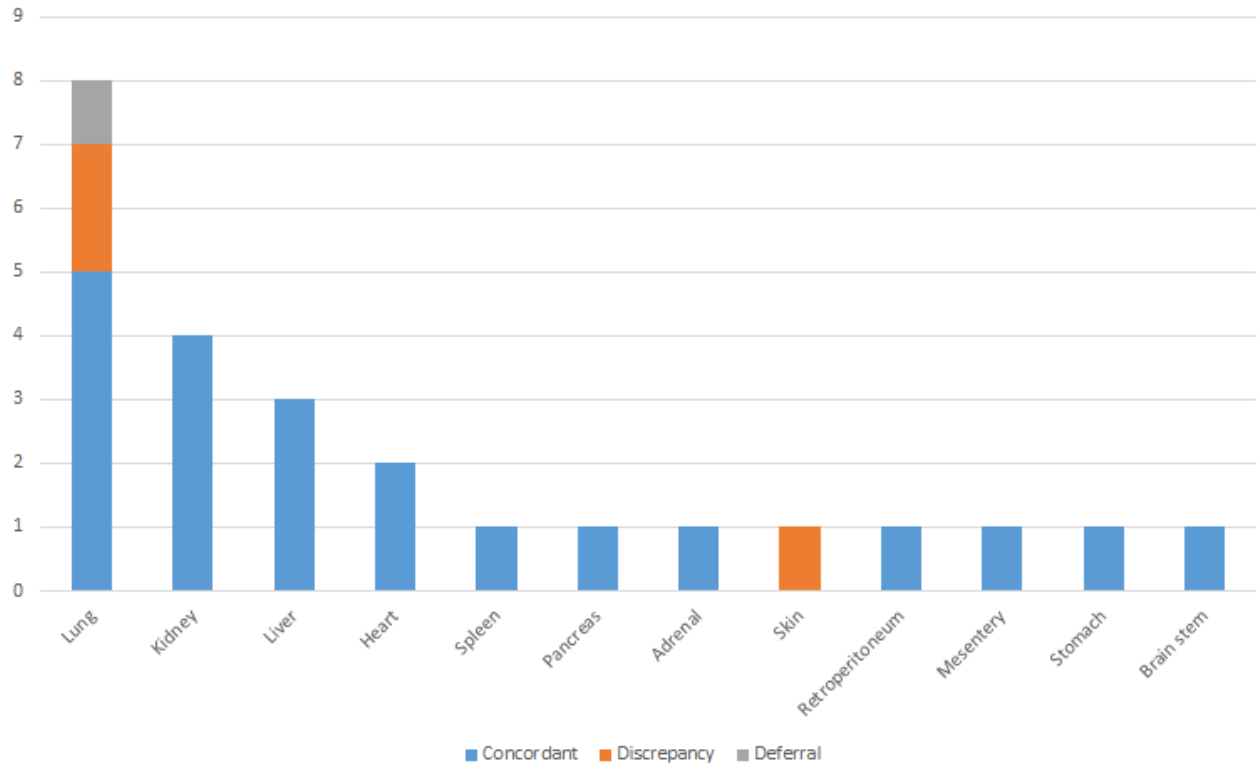

Figure 1. Concordance, discrepancy, and deferrals by specific organ.

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