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The use of fluorine-18-fluorodeoxyglucose-positron emission tomography/computed tomography as an effective method for staging in dogs with primary appendicular osteosarcoma

Clifton Crooks | **Elissa Randall** | **Lynn Griffin**

Department of Environmental and Radiological Health Sciences (ERHS), Colorado State University, Fort Collins, Colorado, USA

Correspondence
Clifton Crooks, 300 W. Drake Rd., Fort Collns, CO 80523-1620.
Email: jccrooks@colostate.edu

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Abstract

Fluorine-18-fluorodeoxyglucose (^{18}F-FDG) positron emission tomography/computed tomography (PET/CT) has been utilized in veterinary medicine to improve the detection and characterization of primary, recurrent, and secondary neoplasms; but its use as a staging tool for dogs diagnosed with appendicular osteosarcoma has not been published. The purpose of this retrospective, case series, descriptive study was to detail the use of ^{18}F-FDG PET/CT for staging a population of dogs with appendicular osteosarcoma, report the detection rate of secondary neoplastic lesions, and compare findings with published detection rates for other historically used imaging modalities. Seventy-one client-owned dogs with a confirmed diagnosis of appendicular osteosarcoma and staged with a whole-body ^{18}F-FDG PET/CT scan near the time of initial diagnosis were included. Each PET/CT study was re-evaluated for malignancy distinct from the primary disease entity based on a collective qualitative and quantitative assessment of ^{18}F-FDG uptake, CT appearance, and contrast enhancement characteristics. Following re-evaluation of each study, information pertaining to tissue sampling performed on identified lesions was retrieved from the medical record when available. Staging with ^{18}F-FDG PET/CT identified 17 of 71 (23.9%) and 12 of 71 (16.3%) dogs with a high suspicion or confirmation of a metastatic or comorbid malignant neoplasm respectively, with eight of 71 (11.3%) having both metastatic and comorbid lesions. The results of this study are suggestive that ^{18}F-FDG PET/CT is effective in identifying both osseous and soft tissue secondary neoplastic lesions in dogs afflicted with appendicular osteosarcoma, yielding an increased detection rate of all lesions compared those previously reported for skeletal scintigraphy or whole-body CT.

KEYWORDS
^{18}F-FDG, canine, comorbid neoplasia, metastasis, PET-CT

1 | INTRODUCTION

Canine osteosarcoma is a locally aggressive and highly metastatic primary bone tumor, most commonly originating in the metaphyseal regions of long bones.[1] Metastasis of osteosarcoma in the dog often occurs early in the disease process.[2] The dissemination of canine

Abbreviations: ^{18}F-FDG, fluorine-18-fluorodeoxyglucose; CE-WBCT, contrast-enhanced whole-body CT; HDP, hydroxymethylene diphosphonate; IRU, increased radiopharmaceutical uptake; PET, positron emission tomography; SUV_{max}, maximum standard uptake value.

osteosarcoma beyond the primary lesion most frequently involves the lungs or other bones, with additional less common sites of spread, such as lymph nodes and visceral organs, also being described.[3–8] It has been reported that subclinical micro-metastasis, primarily pulmonary, has occurred in an estimated 85-90% of dogs with osteosarcoma by the time of initial diagnosis, with a reported detection rate ranging from 1.4% to 28% of patients.[3,5,9–11]

Staging of this disease utilizes diagnostic imaging and tissue sampling to help characterize the primary disease entity and determine the distribution of disease within the body. Results from staging procedures can help determine prognosis and influence treatment recommendations.[3] In both humans and dogs, the presence of metastasis at the time of diagnosis is considered one of the most important single prognostic parameters.[10,12–14]

In addition to the determination of metastasis, staging can potentially detect concurrent incidental or confounding neoplastic disease that is unrelated to the known or suspected primary malignancy. Rebhun and Thamm examined the occurrence of multiple tumor types, finding that 53 (3.1%) of the 1722 dogs that presented during the enrollment period were diagnosed with multiple tumor types. When focusing on specific subpopulations, nine of the 278 dogs (3.2%) evaluated for osteosarcoma were noted to have at least one additional tumor type.[15]

Traditional diagnostic imaging modalities employed for the staging of appendicular osteosarcoma in the dog include long bone and thoracic radiography, abdominal ultrasound, whole-body skeletal scintigraphy, and/or contrast-enhanced whole-body CT (CE-WBCT).[3] Positron emission tomography (PET) is utilized in both human and veterinary medicine to improve the diagnosis of primary, recurrent, metastatic, and comorbid malignancies, and to monitor tumor response to treatment.[16–20] Like all forms of diagnostic nuclear medicine, PET imaging provides physiologic information with a high degree of sensitivity, but is limited in its ability to yield a high degree of specificity and precise anatomic information. To increase spatial resolution and overall diagnostic specificity, positron emission tomographic imaging can be combined with CT (PET/CT) that offers enhanced anatomic information.

PET imaging most commonly utilizes the glucose analog fluorodeoxyglucose (FDG) labeled with the radioisotope fluoride-18 (^{18}F). ^{18}F-FDG is incorporated and trapped in cells proportional to the rate of glycolysis of those cells..[18–20] This degree of uptake (or avidity) can subsequently be detected, quantified, and illustrated in three-dimensions. Quantification of radiopharmaceutical uptake is commonly reported using the maximum standard uptake value (SUV_{max}), which is a semi-quantitative value that measures the maximum radioactivity concentration within a region of interest.[18] In some tissues, such as the brain and heart, there is normally a high rate of glucose metabolism, and thus increased ^{18}F-FDG uptake.[18,20] In other tissues however, a high rate of glycolysis is abnormal and often reflects active pathology, such as neoplasia or inflammation.

The use of ^{18}F-FDG PET or ^{18}F-FDG PET/CT imaging to evaluate canine and feline neoplasia is well described in the veterinary medical literature .[18,21–26] As a staging tool, ^{18}F-FDG PET/CT has been demonstrated as an effective and promising imaging modality for the staging of dogs diagnosed with multiple different primary neoplasms, including lymphoma, mast cell tumor, mammary tumors, histiocytic sarcoma, Sertoli cell tumor, gastrointestinal stromal cell tumor, and pulmonary carcinoma.[23–25] Reports describing the use of ^{18}F-FDG PET/CT in dogs and cats diagnosed with osteosarcoma, particularly appendicular osteosarcoma, are more limited. Recently, the prognostic value of primary tumor SUV_{max} measurements was investigated in dogs with appendicular osteosarcoma, however, the use of ^{18}F-FDG PET/CT to fully stage a population of dogs diagnosed with appendicular osteosarcoma has not been reported.[26] The primary objectives of this study were to detail the use of ^{18}F-FDG PET/CT for staging a population of dogs with confirmed appendicular osteosarcoma and to report the rate of detection of metastatic and comorbid neoplastic lesions in this population. A secondary objective was to compare these findings with the published detection rates of skeletal scintigraphy and CE-WBCT.

2 | MATERIALS AND METHODS

2.1 | Study design and inclusion criteria

This was a retrospective, case series, descriptive study. All methods were approved by the university institutional animal care committee and informed consent was obtained by the Veterinary Teaching Hospital director prior to enrollment. The medical database at the Colorado State University–Veterinary Teaching Hospital was reviewed to identify client-owned dogs diagnosed with primary appendicular osteosarcoma from December 2009 to November 2017. Sample size for the study was based on convenience sampling (ie, the number of dogs meeting the study criteria during this period). Dogs were included if they had a confirmed diagnosis of appendicular osteosarcoma on cytology or histology, and were initially staged using whole body ^{18}F-FDG PET/CT near the time of initial diagnosis and prior to initiation of advanced treatments, such as surgery, chemotherapy, or radiation therapy. Dogs were excluded if they were diagnosed with primary osteosarcoma in an axial or extra-skeletal location or if the patient had advanced treatment(s) performed prior to ^{18}F-FDG PET/CT staging. Final decisions for subject inclusion/exclusion were made per design constructed by two authors, one dual-board certified veterinary radiologist and veterinary radiation oncologist (L.G.; American College of Veterinary Radiology) and one board-certified veterinary radiologist (E.R.; American College of Veterinary Radiology).

2.2 | Clinical data

For dogs meeting the inclusion criteria, the following medical data was recorded by the primary author (C.C.; third-year veterinary radiology

TABLE 1 Imaging characteristics used to score abnormal regions of radiopharmaceutical uptake

	High concern for malignancy	Moderate concern for malignancy	Low concern for malignancy
^{18}F-FDG PET/CT (quantitative)	High avidity (> 2.5)	Moderate avidity (1.5-2.5)	Low avidity (< 1.5)
^{18}F-FDG PET/CT (qualitative)	Strong subjective IRU Round Discrete, with short zone of transition to adjacent non-avid tissues	Moderate subjective IRU Ovoid to irregularly shaped Moderately blurred margination of uptake	Low subjective IRU Irregularly shaped Non-discrete with long progressive transition to regional non-avid tissues
CT characteristics (soft tissue)	Strong CE Enlargement of organ Obvious asymmetry to the contralateral side Mass-effect Regions of suspected necrosis Neoangiogenesis	Abnormal CE Moderate asymmetry Focal deformation of organ margins	Mild abnormal CE Mild asymmetry
CT characteristics (bone)	Lysis and moderate to severe irregular periosteal reaction Long zone of transition between normal and abnormal bone	Smoothly-marginated lysis and/or mild to moderate periosteal reaction	Mild smooth periosteal reaction and/or sclerosis with no lysis

Abbreviations: **^{18}F-FDG PET/CT,** fluorine-18-fluorodeoxyglucose-positron emission tomography/computed tomography; **CE,** contrast enhancement; **IRU,** increased radiopharmaceutical uptake.

resident) from the medical records: breed, age at the time of initial ^{18}F-FDG PET/CT imaging, gender, location of primary lesion, and method of sampling employed to confirm osteosarcoma. Following image assessment, information pertaining to the sampling of retrospectively identified secondary areas of increased radiopharmaceutical uptake (IRU), including method of sampling and diagnosis, was recorded if available.

2.3 | Image evaluation

Image assessment was performed retrospectively by all three authors (C.C., L.G., E.R.) on a computer workstation (Dell Inspiron, Dell Computer Company, Round Rock, TX) and calibrated LCD monitor using DICOM image processing application software (IntelliSpace Portal Version 8, Philips Medical System, Nederland BV). SUV_{max} of the primary tumor and any potential secondary neoplastic lesions were measured by the software and recorded. Pre- and postcontrast whole-body CT scans without a PET overlay, ^{18}F-FDG PET-only images, and then CE-WBCT fused with an ^{18}F-FDG PET overlay were evaluated for the presence of any abnormal areas of IRU. At the time of image assessment, the authors were only aware that all ^{18}F-FDG PET/CT studies were performed for staging purposes on patients that fit the inclusion parameters.

All potential lesions were determined by the authors as having a low, moderate, or high level of concern for malignancy by consensus evaluation based on the qualitative assessment of ^{18}F-FDG uptake (evaluating size, shape, margination, distribution, and location), semi-quantitative measurement of radiopharmaceutical avidity (SUV_{max}), and CT characteristics of the area of interest. These variables relative to the three established levels of concern for malignancy are defined in Table 1. With respect to assessing areas of abnormal IRU on PET imaging, a qualitative approach, in combination with quantitative measurements, was chosen based on human literature that suggests that visual inspection of a lesion by an experienced evaluator can be just as reliable as SUV values in determining malignancy, and the fact that specific thresholds have not been definitively established in veterinary medicine to differentiate malignant from non-malignant pathology.[18] For the quantitative aspect of our evaluation criteria, a SUV_{max} value of greater than 2.5 was selected for our design as the lower limit of the high concern category. This is based on published reports in the human literature stating that although there is a wide range of SUVs reported for similar diseases, an SUV of 2.5 or higher is generally suggestive of malignancy.[27] An SUV_{max} of less than 1.5 was chosen to differentiating a moderate suspicion of malignancy from low, based on evidence in humans which suggest that a SUV_{max} of 1.5 or greater (depending on lesion size) is a sensitive and specific threshold for differentiating benign from malignant behavior in some nodules (particularly pulmonary).[28–30] Differentiation of metastatic versus comorbid malignancy was based, in part, on anatomic location of abnormal uptake relative to the primary osteosarcoma lesion, the tissue involved relative to the "expected" behavior of osteosarcoma, and degree of avidity compared to that of the primary lesion.

3 | RESULTS

3.1 | Study population

Seventy-one patients met the selection criteria. The median age was 10 years (range: 2-17 years). There were 38 castrated males, 32 spayed females, one intact male, and one intact female. Twenty-one different breeds were represented, the most common being mixed breed (11), Golden Retriever (10), Labrador Retriever (8), Rottweiler (8), Great Pyrenees (6), Saint Bernard (5), and Staffordshire Terrier/American Pitbull (3). Primary tumor sites included the distal radius (32), proximal humerus (14), distal femur (9), distal tibia (7), proximal tibia (4), distal ulna (2), mid-radius (1), scapula (1), and metatarsus II (1). The mean and median SUV_{max} of the primary lesion was 9.26 and 7.80 respectively (range: 2.01-34.87).

3.2 | Imaging protocol and acquisition parameters

Animals were fasted for a minimum of 6 h prior to PET/CT. Blood glucose was evaluated at the time of ^{18}F-FDG PET/CT scheduling, and rechecked the morning of the imaging study. All imaging was performed with a Philips Gemini TF Big Bore 16-slice PET/CT scanner (Philips North America, Cambridge, Massachusetts) under general anesthesia. Anesthetic protocols were determined by a board-certified veterinary anesthesiologist based on individual patient needs, but most commonly involved a combination of an opioid and an alpha-2 agonist premedication, with induction using propofol (Zoetis; Kalamazoo, Michigan) and maintenance using an inhalant anesthetic. An arterial catheter and instrumentation for physiological monitoring, an indwelling urinary catheter, and a second intravenous catheter specifically used for ^{18}F-FDG injection were placed in all dogs.

All scans were performed by a dedicated CT technologist with experience in PET imaging, under the guidance of a veterinary diagnostic imaging resident and board-certified veterinary radiologist. A focused pre- and postcontrast CT centering on the primary lesion was performed on most patients for the purposes of radiation therapy planning prior to injection of the radiopharmaceutical. In these patients, 350 mg I/ml iohexol (Omnipaque™ 350; GE Healthcare, Princeton, NJ, USA) was administered intravenously via power injector (Medrad Stellant dual head power injector) at a dose of 1.5 mL/kg (525 mg I/kg) followed by 8-25 mL 0.9% NaCl infused via the same power injector. CT parameters: 120 kV, 100 mAs/slice, 0.75 sec rotation time, 0.813 pitch, matrix 768 × 768, FOV 600, and 0.75-1.5 mm (depending on patient size) × 16 detector width; data volume acquisitions were reconstructed in detail and soft tissue algorithms at 1 mm and 2 mm slice thickness respectively. Following this, ^{18}F-FDG (Cardinal Health) was injected intravenously at a dose of 5.18 - 6.29 MBq/kg (0.14-0.17 mCi/kg). For those subjects who did not have a radiation therapy planning CT performed, ^{18}F-FDG was administered intravenously at the same dose immediately following anesthetic induction.

Uptake of ^{18}F-FDG is allowed for 1 h. A whole-body pre- and postcontrast CT scan with the patient in dorsal recumbency (using the same acquisition parameters described for the focused CT scan) was obtained on all patients during the ^{18}F-FDG uptake period (with contrast administered at a dose of 1.5 mL/kg [525 mg I/kg] if CT scan for radiation planning was performed prior or at a dose of 2 mL/kg [700 mg I/mL] if radiation planning scan was not performed) for anatomic correlation and attenuation correction. At 60 minutes post-^{18}F-FDG administration, a whole-body PET scan was performed. Patients were scanned in a caudal to cranial direction, typically using eight to nine 18.0 cm frames with 1.5-3 min per bed; FOV 576. Computed tomography attenuation-corrected images and non-attenuation-corrected images of the PET data were reconstructed using the line-of-response row-action maximum likelihood algorithm method.

3.3 | Characterization of regions of IRU and correlation with tissue sampling

Results of consensus scoring are summarized in Table 2. Sixty-one of 71 dogs (85.9%) had 129 regions of IRU in areas other than the primary tumor, of which 51 (39.5%) were sampled at the time of staging. Of these 129 areas of IRU, 43 (33.3%) were considered to be highly suspicious for a second malignant lesion (28/43 were thought to most likely represent metastasis, and 15/43 of were thought to be a comorbid malignancy). Fifteen of these 43 regions were sampled at the time of staging (8/28 potential metastatic lesions and 7/15 potential comorbid neoplasms). Three of eight potential metastatic lesions were confirmed as metastasis, three were diagnostic of a comorbid neoplasm, and two were consistent with inflammatory lesions. Of the seven sampled potential comorbid lesions, three were confirmed as a comorbid malignancy, one was diagnosed as metastatic osteosarcoma, and three were consistent with a nonmalignant lesion. Figures 1 and 2 illustrate confirmed and unconfirmed areas of IRU that were considered highly suspicious for metastatic osteosarcoma on imaging.

Thirty-five of 129 areas of IRU (27.1%) were considered to be moderately suspicious for a neoplastic process. Fifteen of these 35 regions were sampled; three of 15 were confirmed neoplastic (2 metastatic, 1 comorbid) and 12 of 15 were inflammatory/non-malignant. Fifty-one areas of IRU (39.5%) were considered to have a low level of suspicion for a neoplastic process, 21 of which were sampled. Two of 21 were confirmed as comorbid neoplasms and 19 of 21 were nonmalignancies. Figure 3 shows one of the two confirmed metastatic lesions that were characterized as moderately concerning for metastasis on imaging.

After exclusion of cases that were highly concerning for metastasis, but were cytologically or histologically diagnostic of an inflammatory lesion or comorbid malignancy, a total of 17 of 71 (23.9%) dogs had a confirmed or highly suspicious region for metastatic disease. Sites of metastasis include bones distant from the primary lesion (7 dogs; 9.9% of subject population), lungs (5; 7.0%), regional lymph nodes (4; 5.6%), and abdominal viscera (1; 1.4%). Metastasis to distant lymph nodes or involving multiple tissue types were not noted in our patient population. SUV_{max} values for these sites varied depending on the specific tis-

TABLE 2 Level of suspicion for secondary neoplasia: 129 regions of increased radiopharmaceutical uptake

Level of suspicion (number of sites)	High(43)	Medium(35)	Low(51)
Suspected metastatic	28 (8 sampled; 3 M, 3C, 2I/B)	29 (11 sampled; 2 M, 0C, 9I/B)	47 (18 sampled; 0 M, 1C, 17I/B)
Suspected comorbid	15 (7 sampled; 1 M, 3C, 3I/B)	6 (4 sampled; 0 M, 1C, 3I/B)	4 (3 sampled; 0 M, 1C, 2I/B)
Total neoplastic lesions confirmed	10/15 (67%)	3/15 (20%)	2/21 (9.5%)

Abbreviations: **C,** comorbid neoplasm; **I/B,** inflammatory/benign; **M,** metastatic.

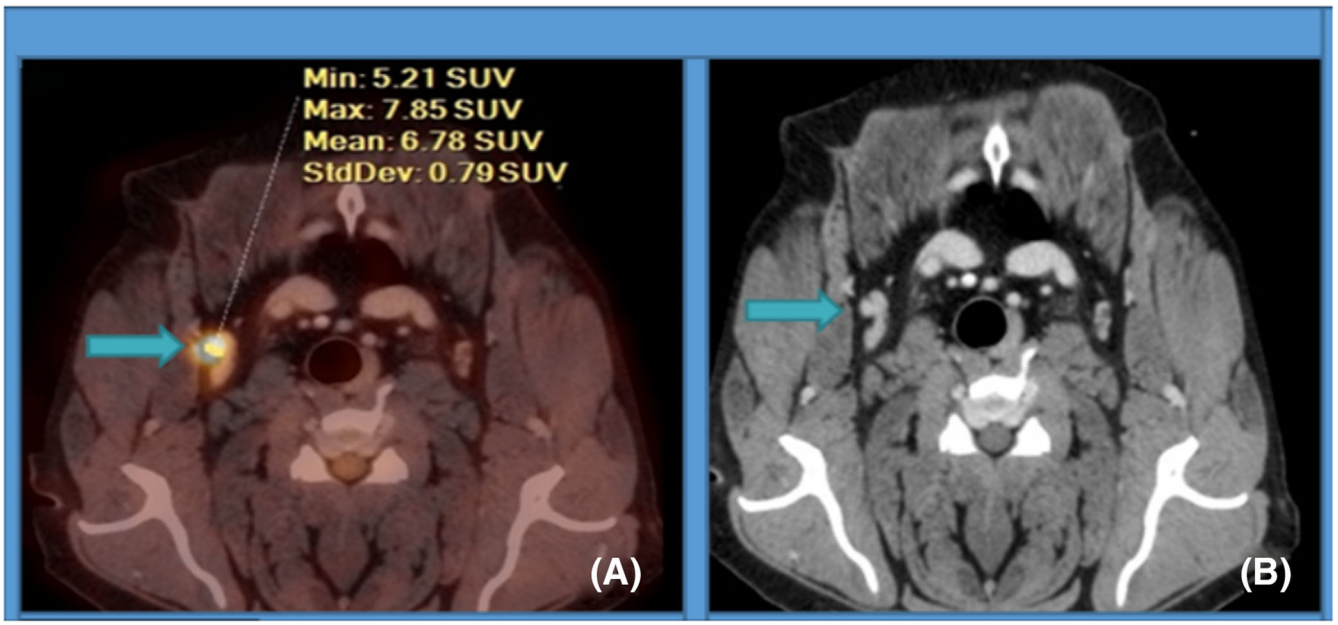

FIGURE 1 Primary osteosarcoma in the right proximal humerus. On these images, the right axillary LN (arrows) is enlarged, hypermetabolic (SUV_{MAX} – 7.85) (**A**), and contrast enhancing (**B**). This lesion was considered highly concerning for metastasis. **Diagnosis**: Histology: metastatic osteosarcoma

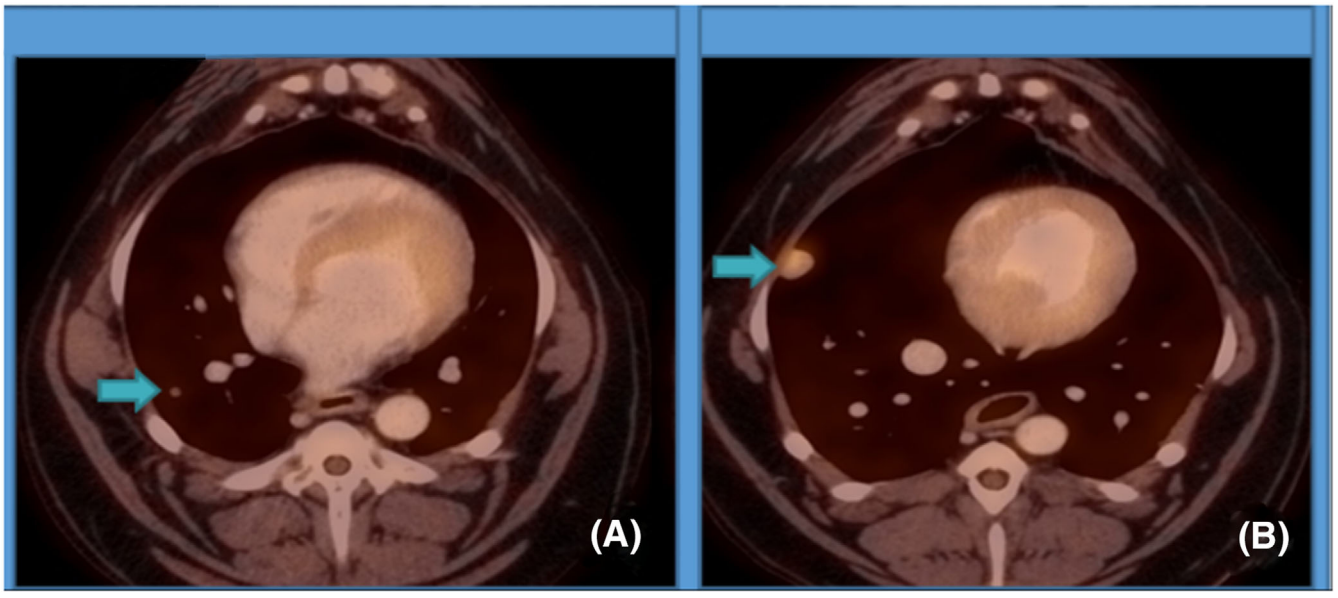

FIGURE 2 Multiple, well-defined, round, soft tissue attenuating, variably hypermetabolic nodules in multiple lung lobes (arrows), the largest (**B**) in the right middle lobe (SUV_{max} - 2.9). **Primary Differential**: metastatic neoplasia. Pulmonary granulomas are also considered [Color figure can be viewed at wileyonlinelibrary.com]

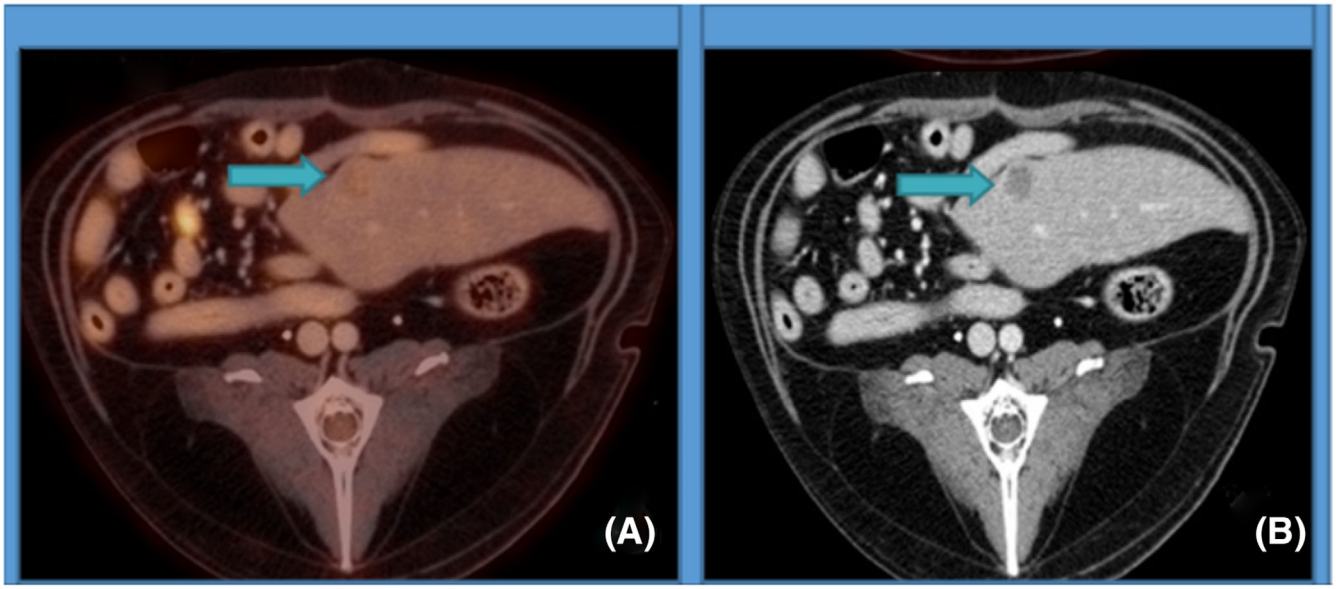

FIGURE 3 A capsular deforming mildly avid (SUV_{MAX} - 1.69) (**A**), non-contrast enhancing, hypoattenuating (**B**) splenic nodule. **Differentials**: metastatic or comorbid neoplasia, lymphoid hyperplasia or extramedullary hematopoiesis. **Diagnosis**: Cytology: sarcoma – suspected osteosarcoma metastasis [Color figure can be viewed at wileyonlinelibrary.com]

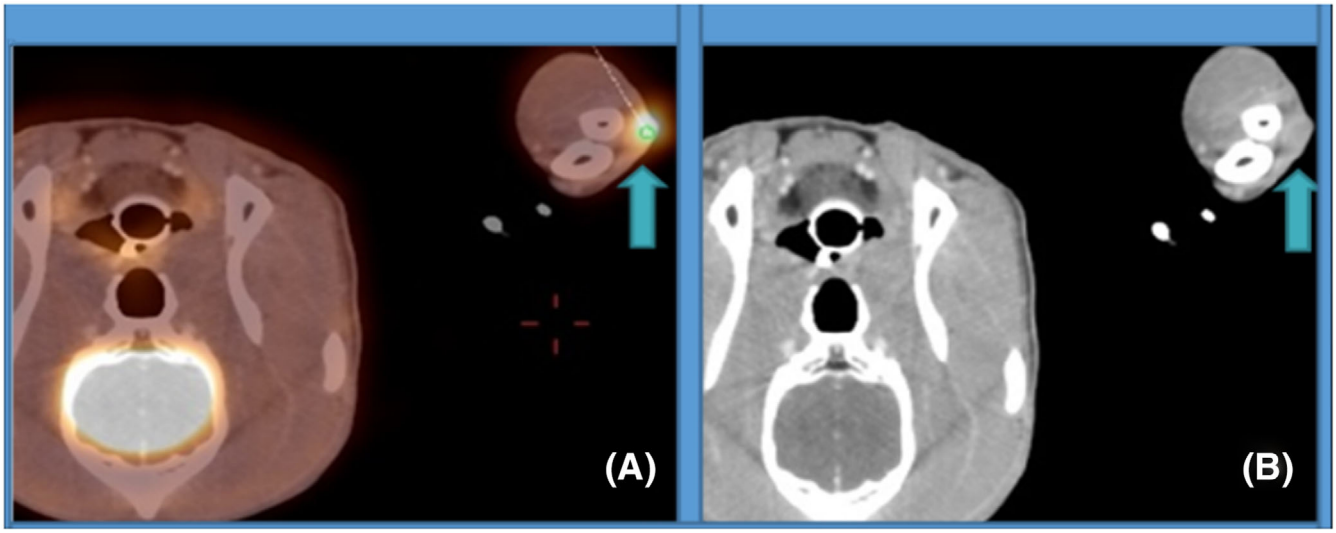

FIGURE 4 A hypermetabolic cutaneous nodule (SUVmax – 12.17) (**A**) in the left antebrachium that is ill-defined on CE-WBCT (**B**). Due to the intensity of FDG uptake, this is concerning for neoplasia such as MCT or melanoma. **Diagnosis**: Histology: basosquamous carcinoma

sue type (range: 1.37-9.82; mean: 4.37; median: 3.4), and are further stratified in Table 3A, 3B.

A total of 12 of 71 (16.9%) dogs had regions confirmed or highly suspicious of comorbid neoplasia. Figure 4 illustrates a small, ill-defined, minimally contrast-enhancing, markedly avid soft tissue nodule in the left proximal antebrachium, initially thought to represent a comorbid neoplasia such as mast cell tumor or melanoma. This finding was diagnosed as a basosquamous carcinoma on histopathology. Other confirmed comorbid malignancies were lymphoma (1), soft tissue sarcoma (2), nasal adenocarcinoma (1), meningioma (1), mast cell tumor (2), and pulmonary adenocarcinoma (1). Overall, there were 21 of 71 (29.6%) dogs that had at least one highly suspected or confirmed malignant neoplastic lesion, with eight of 71 (11.3%) patients having both metastatic and comorbid lesions.

3.4 | Tissue sampling

Some potential metastatic or comorbid lesions detected on ^{18}F-FDG PET/CT were aspirated for cytological examination at the time of ini-

TABLE 3A Stratification of confirmed or highly suspicious metastatic lesions identified on ^{18}F-FDG PET/CT staging by location and SUV_{max}

Location of primary lesion (no. of dogs total)	SUV_{max} of primary lesion	Dogs with metastasis (% of group total)	SUV_{max} of primary lesion in dogs with metastasis	Tissue type of metastatic lesions (no. of dogs)[*specific location*]	SUV_{max} of metastatic lesions
Distal radius (32)	μ: 9.34 M: 7.80 R: 3.11-23.20	5 (15.6%)	μ: 10.37 M: 8.19 R: 5.19-22.18	**Osseous (1)**: [*rib & contralateral scapula*] **Pulmonary (3)** **Regional LN (1)**: [*Ipsilateral axillary LN*]	2.70, 4.91 respectively Up to 1.90, 3.45, 1.91 3.40
Proximal humerus (14)	μ: 9.13 M:7.37 R: 3.10-19.20	3 (21.4%)	μ: 12.78 M: 11.83 R: 7.30-19.20	**Osseous (1)**: [*Contralateral humerus*] **Regional LN (2)**: [*Ipsilateral axillary LNs in both dogs*]	5.30 8.91, 6.90
Distal femur (9)	μ: 9.58 M: 9.40 R: 2.01-18.00	1 (11.1%)	μ: 10.54 M: 10.54 R: N/A	**Osseous (1)**: [*Rib*]	9.70
Distal tibia (7)	μ: 12.18 M: 5.76 R: 4.98-34.87	2 (28.6%)	μ: 20.1 M: 20.1 R: 5.33-34.87	**Osseous (2)**: [*Contralateral humerus*] [*Spine & ipsilateral radius*]	9.82 6.24, 3.61 respectively
Proximal tibia (4)	μ: 6.46 M: 6.20 R: 4.80—8.64	4 (100%)	μ: 6.46 M: 6.20 R: 4.80—8.64	**Osseous (1)**: [*5 rib & spine lesions*] **Pulmonary (1)** **Regional LN (1)**: [*Ipsilateral MILN*] **ESST (1)**: [*Spleen*]	2.28 - 5.01* Up to 1.37 3.01 1.69
Distal ulna (2)	μ: 6.87 M: 6.87 R: 4.74 - 9.00	2 (100%)	μ: 6.87 M: 6.87 R: 4.74 - 9.00	**Osseous (1)**: [*Ipsilateral metatarsal II*] **Pulmonary (1)**	6.60 2.90
Mid-radius (1)	2.98	0 (0%)	N/A	N/A	N/A
Scapula (1)	7.82	0 (0%)	N/A	N/A	N/A
Metatarsus (1)	7.05	0 (0%)	N/A	N/A	N/A

TABLE 3B Summary of stratification for confirmed or highly suspicious metastatic lesions identified on ^{18}F-FDG PET/CT staging

SUV_{max} of primary lesion(71 dogs)	Dogs with metastasis (% of group total)	SUV_{max} of primary lesion in dogs with metastasis	Location of metastatic lesions (no. of dogs)	SUV_{max} of metastatic lesions
μ: 9.26 M:7.80 R: 2.01 - 34.9	17 (23.9%)	μ: 10.62 M: 8.19 R: 4.7-34.9	Osseous (7) Pulmonary (5) Regional LN (4) ESST (1) Distant LN (0)	μ: 6.80; M: 6.24 μ: 2.31; M: 1.91** μ: 5.56; M: 5.15 μ: 1.69; M: 1.69 μ: N/A; M: N/A **Combined SUV max of all metastatic lesions**** μ: 4.37 M: 3.4 R: 1.37-9.82

Abbreviations: **μ**, population mean; **M**, median; **R**, range; **LN**, lymph node; **MILN**, medial iliac lymph node; **ESST**, non-pulmonary extra-skeletal soft tissue; **Ipsilateral/contralateral** is relative to side of primary lesion.*μ and M of the five osseous lesions: 3.23, 2.98.**In the event that multiple pulmonary lesions were noted in the same dog, the lesion with the highest measured SUV_{max} was used for the calculation of SUV_{max} mean and median of the pulmonary and combined metastatic groups.

tial staging if deemed appropriate by the primary clinician with owner consent. Tissue sampling was performed by the attending clinician (most commonly a veterinary oncology or internal medicine resident or board-certified oncologist/internist) or using ultrasound-guidance (by a veterinary diagnostic imaging resident or board-certified radiologist) depending on the accessibility of the target. In addition, histopathology of tissues obtained from surgical biopsy, surgical resection/amputation, or postmortem examination was performed on some patients. All tissues obtained for cytology and histopathology following initial ^{18}F-FDG PET/CT staging were submitted to the CSU Veterinary Diagnostic Laboratory and evaluated by a board-certified veterinary pathologist.

4 | DISCUSSION

In the current study, ^{18}F-FDG PET/CT identified 23.9% of appendicular osteosarcoma patients with a confirmed or highly suspected metastatic lesion and 16.3% with a second unrelated malignancy. The incidence of detected comorbid neoplasms is higher than that previously reported (3.2%).[15] These results suggest that ^{18}F-FDG PET/CT is effective in identifying both osseous and soft tissue neoplastic lesions in dogs afflicted with appendicular osteosarcoma at or near the time of initial diagnosis.

Seven of 36 (19.4%) sampled regions of IRU highly concerning for malignancy or thought to represent a non-malignancy were erroneously categorized. False-positive regions of IRU for malignancy included two of eight sampled areas initially classified as highly suspicious for metastasis and three of seven sampled areas initially characterized as highly suspicious for a comorbid neoplasm. All five of these areas were moderately to strongly avid, confirmed inflammatory lesions involving cutaneous, subcutaneous, pulmonary, or splenic tissues. This is somewhat expected as it is well documented that focal inflammation can have a high degree of FDG uptake and is the most common cause of false positive identification of cancer on ^{18}F-FDG PET/CT.[18,31]

False negatives in this study included two of 21 sampled areas of IRU (in two separate dogs) that were initially considered more consistent with a benign/inflammatory process based on their imaging characteristics, but determined to be comorbid soft tissue sarcomas on cytology. Both lesions were soft tissue attenuating, mildly contrast-enhancing, and mildly avid. One of these was thought to represent focal trauma. The other was a subcutaneous mass that was sampled after a follow-up PET/CT performed 1 year later, where the mass was mildly larger, but exhibited similar avidity. The grade of a particular soft tissue sarcoma is known to affect the degree of FDG uptake in people, with low-grade tumors having a significantly lower SUV_{max} in comparison to those that are higher grade.[31,32] The grade of these masses was not histologically determined, but it is possible that they were low grade, slow growing entities with relatively lower glycolytic requirements, resulting in only mild, and in the instance of one, non-progressive ^{18}F-FDG uptake.

Sixty-seven percent of the sampled sites that were highly concerning for malignancy based on the imaging characteristics were confirmed to be neoplastic, however, cytology/histopathology revealed that some of the sampled lesions in our study were mischaracterized. Overall, it is difficult to determine the accuracy of this modality in truly identifying and correctly characterizing suspected metastatic and comorbid lesions in dogs afflicted with appendicular osteosarcoma without sampling a greater number of categorized regions of IRU.

For staging of canine osteosarcoma, radiography is often the initial diagnostic modality chosen. Radiographs are relatively inexpensive, non-invasive, and time-efficient. One disadvantage of radiographs as a staging tool is their relative inherent insensitivity. For example, studies looking at skeletal metastasis from multiple different primary malignancies in humans have shown that more than 50% to 70% of bone must be destroyed to be reliably detected by survey radiographs.[33]

As a staging tool for osteosarcoma, CT has been utilized in veterinary medicine mainly to screen for pulmonary metastasis. One study found evidence of pulmonary metastasis in 23% of dogs with appendicular osteosarcoma that had no evidence of pulmonary spread on thoracic radiographs.[34] More recent studies have investigated the utility of CE-WBCT, both as a sole staging modality and in combination with other modalities.[3,5] Talbott et al reported that CE-WBCT detected pulmonary metastasis in 5% of dogs with appendicular osteosarcoma evaluated near the time of diagnosis. Evidence of extra-pulmonary metastasis was not noted in any patient enrolled in that study.[5] By way of comparison, ^{18}F-FDG PET/CT in the current study identified metastatic lesions involving the pulmonary tissue at a similar rate (7%) to that reported by Talbott, but noted confirmed or highly suspected metastatic lesions outside of the lungs in an additional 16.9% of subjects. In addition, the incidence of comorbid lesions identified on ^{18}F-FDG PET/CT (16.9%) compared to that reported using CE-WBCT (5%) was noticeably higher.[5] Reports in the human literature support the highlighted similarities and differences between these two studies. Quartuccio et al noted that ^{18}F-FDG PET/CT proved more accurate than CE-WBCT for the detection of secondary malignant bone lesions and was complimentary to CT in evaluating metastatic lung nodules in children with osteosarcoma.[35]

Whole-body skeletal scintigraphy, using methylene diphosphonate or hydroxymethylene diphosphonate (HDP) labeled with the radioisotope technetium-99 m (^{99m}Tc), identifies regions of increased osteoblastic activity and has been shown to be a highly sensitive, but relatively less specific imaging modality for the initial characterization of primary bone tumors and detection of metastatic osseous lesions.[4,9] Jankowski et al reported that ^{99m}Tc-HDP skeletal scintigraphy detected secondary osseous lesion in 72/399 (18.1%) of dogs diagnosed with osteosarcoma, 31 of which (7.8% of the subject population) were considered highly suspicious for metastasis.[9] Whole-body ^{99m}Tc-HDP/^{99m}Tc-methylene diphosphonate scintigraphy has also been shown to be useful in detecting soft tissue sites of metastasis associated with osteosarcoma that have osteoid production.[4] Investigations focusing on this modality's ability to identify soft tissue metastasis associated with osteosarcoma in the dog are limited, however, likely because scintigraphy is primarily utilized for the detection of osseous lesions.

^{18}F-FDG PET imaging without CT fusion has been shown to be less sensitive in comparison to bone scintigraphy for the detection

TABLE 4 Reported detection rates of secondary neoplastic lesion during staging of canine osteosarcoma: skeletal scintigraphy versus CE-WBCT versus PET/CT

Article(Subject Population)[Study Design]	Modality	Percentage of Dogs with High Suspicion or Definitive Metastasis	Comorbid Malignancy
Jankowski, et al. (2003) (399) [Retrospective case series]	^{99m}Tc-HDP Skeletal Scintigraphy	7.8% osseous	Not reported
Talbott, et al. (2017) (39) [Retrospective case series]	CE-WBCT	5% pulmonary 0% osseous	5%
Present Study (2020) (71) [Retrospective case series]	^{18}F-FDG PET/CT	23.9% (multiple sites) • 9.9% osseous • 7.0% pulmonary • 5.6% regional LNs • 0% distant LNs • 1.4% extra-skeletal soft tissue/abdominal viscera	16.9%

Abbreviations: **LN,** lymph node.

of osseous metastatic lesions from osteosarcoma in humans.[16] It is only after ^{18}F-FDG PET imaging is fused with CT that this modality was shown to be more sensitive and accurate than skeletal scintigraphy for diagnosing bone metastasis associated with osteosarcoma in people.[36] This suggests that the improved spatial resolution achieved from the CT fusion with PET may be the biggest difference between the two modalities when assessing for osseous metastasis. In our study, ^{18}F-FDG PET/CT identified suspected or confirmed osseous metastasis in 9.9% of our patient population, which is similar to the rate of detection of osseous lesions noted by Jankowski et al (7.8%). Detection of soft tissue lesions by ^{99m}Tc-HDP was not reported by Jankowski et al.[9]

As illustrated in Table 4, our findings show that ^{18}F-FDG PET/CT was able to identify concurrent skeletal and extra-skeletal metastatic lesions from appendicular osteosarcoma at a rate higher than that reported using whole-body skeletal scintigraphy or CE-WBCT alone.[5,9] In addition, ^{18}F-FDG PET/CT also detected comorbid malignant neoplasia at a higher rate than previously reported using other imaging modalities.[5,15] It remains uncertain, however, how many metastatic or comorbid lesions identified on ^{18}F-FDG PET/CT would have been missed or underestimated or if any lesion(s) not identified or mischaracterized by ^{18}F-FDG PET/CT would have been detected or more accurately classified utilizing these other imaging modalities in our patient population.

The present study has several limitations aside from its retrospective design and discussed limitations inherent to PET/CT. Not all regions of IRU that we deemed highly concerning for malignancy were sampled due to inability to access the particular region, inability to receive owner consent, and the fact that necropsies were not performed in many of these patients. Consequently, a gold standard reference for metastasis or comorbid neoplasia could not be established, and the sensitivity, specificity, positive predictive value (PPV), or negative predictive value (NPV) of ^{18}F-FDG PET/CT for staging appendicular osteosarcoma could not be determined in this population. Although this is a weakness of this descriptive study, the lack of tissue sampling and confirmation of malignancy is also true for the two main reports we are comparing our results with. Another limitation of this study lies with the possibility of selection bias for cases undergoing ^{18}F-FDG PET/CT, as an animal with previously known advanced pulmonary metastasis, severe geographic cortical lysis +/- pathologic fracturing at the site of the primary lesion, or other debilitating comorbidities may not have been staged with a relatively expensive imaging modality. As a result, the true rate of metastasis for appendicular osteosarcoma potentially detected by ^{18}F-FDG PET/CT may be higher than discussed in this study.

In conclusion, we have taken the initial steps towards understanding a potential role that ^{18}F-FDG PET/CT may play in the staging of canine appendicular osteosarcoma by establishing ^{18}F-FDG PET/CT as a promising diagnostic imaging modality for the identification of metastatic and comorbid malignant lesions in dogs afflicted with this disease. This descriptive study also lays the groundwork for future studies, including designs comparing the rate of detecting potential secondary malignant lesions by ^{18}F-FDG PET/CT versus other less expensive and more readily available staging modalities in the same patient population.

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LIST OF AUTHOR CONTRIBUTIONS

Category 1

(a) Conception and Design: Crooks, Randall, Griffin
(b) Acquisition of Data: Crooks, Randall, Griffin
(c) Analysis and Interpretation of Data: Crooks

Category 2

(a) Drafting the Article: Crooks
(b) Revising Article for Intellectual Content: Randall, Griffin

Category 3

(a) Final Approval of the Completed Article: Crooks, Randall, Griffin

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