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INTRODUCTION

Next-generation sequencing-based liquid biopsy offers clinicians a novel cancer detection tool; however, the detection rate has been shown to vary by cancer type and extent of disease. Mast cell tumors (MCTs) are one of the most common cancers in dogs and diagnosis by a fine needle aspirate (FNA) or biopsy is typically straightforward. Liquid biopsy has been shown to have a lower detection rate for this cancer type relative to some other types.¹ The purpose of this study was to assess the performance of liquid biopsy for the evaluation of MCT by extent of disease and tumor size to help determine whether this novel testing option may be appropriate to apply in select clinical situations.

MATERIALS & METHODS

Blood samples from 49 dogs with MCTs were subjected to DNA extraction, proprietary library preparation, and next generation sequencing. Sequencing data were analyzed using an internally developed bioinformatics pipeline to detect genomic alterations associated with the presence of cancer.

All patients had complete staging and based on this information each dog was assigned to a category representing their extent of disease. (Figure 1) Localized disease was defined as a single primary tumor or multiple primary tumors in the same organ; regional disease was defined as cancer that had spread to an adjacent lymph node; metastatic disease was defined as cancer with distant lymph node/organ involvement.

In addition to extent of disease, each subject had the size of their tumor documented and categorized as either <2 cm, 2-5 cm, or >5 cm. This measurement represented the longest diameter of the largest lesion present in the patient.

Analysis of liquid biopsy detection rates was performed using a Chi-square test, with $p < .05$ considered significant; 95% confidence intervals were calculated using the Wilson score interval method.

REFERENCE

[1] Flory A, Kruglyak KM, Tynan JA, McLennan LM, Rafalko JM, Fiaux PC, et al. Clinical validation of a next-generation sequencing-based multi-cancer early detection “liquid biopsy” blood test in over 1,000 dogs using an independent testing set: The CANcer Detection in Dogs (CANDID) study. PLOS One. 2022;17(4):e0266623.

KEY TAKEAWAYS

- The detection rate of liquid biopsy has been shown to vary by cancer type and extent of disease
- In the context of a small, localized mast cell tumor that is accessible for FNA or biopsy, liquid biopsy may offer limited utility
- Liquid biopsy may be helpful in evaluating or staging patients with MCTs where metastatic disease is suspected

Figure 1 - Extent of disease categories and definitions

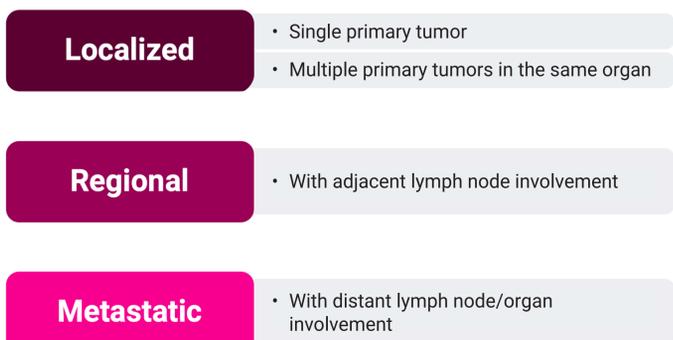
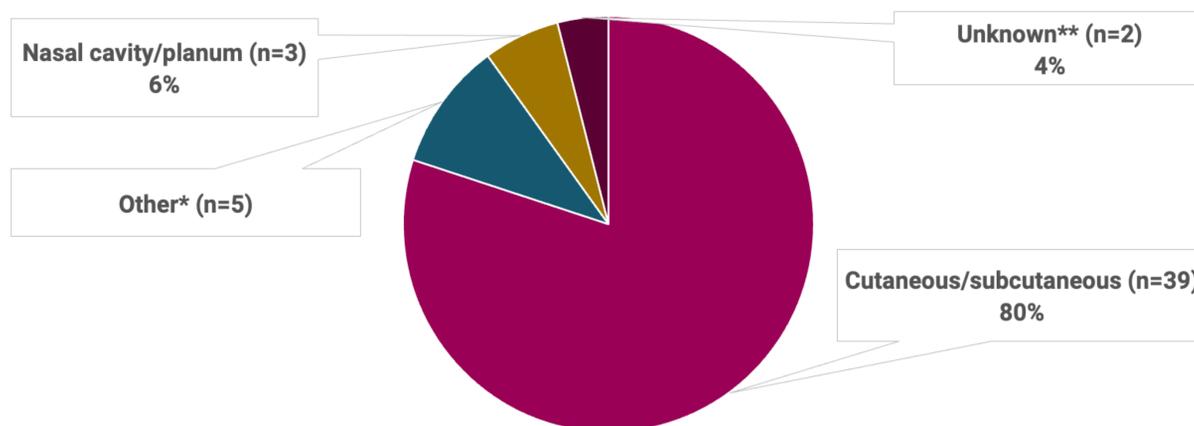


Table 1 - Demographics and characteristics of patients diagnosed with mast cell tumors (n=49)

Subjects with a diagnosis of mast cell tumor		
Breed	Purebred	51% (n=25) Representing 14 distinct breeds*
	Mixed-breed	49% (n=24)
Sex	Male	47% (n=23)
	Female	53% (n=26)
Neuter status	Spayed/neutered	90% (n=44)
	Intact	10% (n=5)
Age	Range	4.0 – 15.1 years
	Mean	8.8 years
	Median	8.4 years
Weight	Range	9.2 – 58.1 kg
	Mean	27.0 kg
	Median	27.6 kg

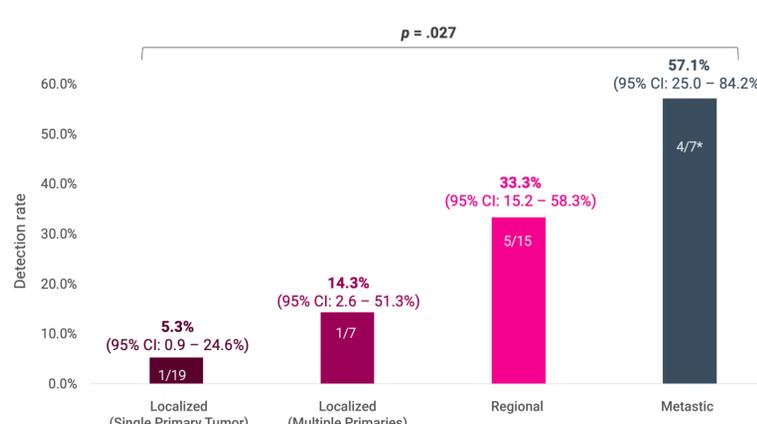
* Beagle, Bernese Mountain Dog, Boston Terrier, Boxer, Bull Terrier, Cocker Spaniel, Doberman Pinscher, French Bulldog, German Shepherd, Golden Retriever, Greater Swiss Mountain Dog, Labrador Retriever, Scottish Terrier, Vizsla

Figure 2 - Primary tumor site in patients with MCT



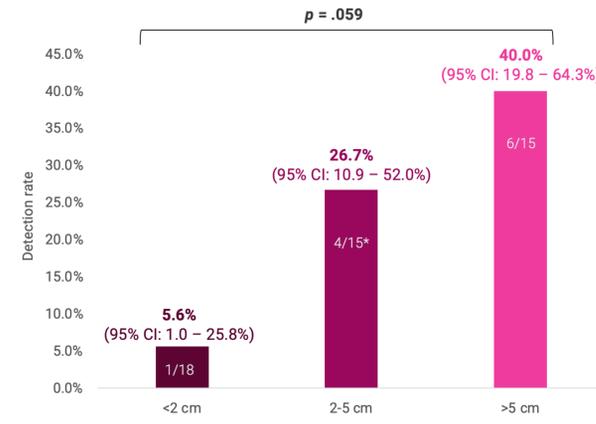
* Includes one each of the following: muscle, mouth/oral cavity, small intestine, lip, and muscle + subcutaneous
** Primary site not determined

Figure 3 - Detection rate of liquid biopsy by extent of disease in patients with MCT



* One additional dog received an Indeterminate result from liquid biopsy testing and was excluded from analysis

Figure 4 - Detection rate of liquid biopsy by longest diameter of the largest lesion measured in patients with MCT



* One additional dog received an Indeterminate result from liquid biopsy testing and was excluded from analysis

RESULTS

The median age of MCT-diagnosed subjects was 8.4 years and the median weight was 27.6 kg. The study population comprised 53% females and 47% males, and 90% of subjects were spayed or neutered. There was an approximately equal representation of purebred and mixed-breed dogs in the study, and the purebred population represented 14 distinct breeds. (Table 1) The distribution of primary tumor sites for the study subjects is shown in Figure 2; approximately 80% of patients had cutaneous and/or subcutaneous tumor(s).

Liquid biopsy returned a *Cancer Signal Detected* (positive) result in 11 of 48 patients, for an overall detection rate of 23%. One additional patient received an *Indeterminate* result, in which genomic alterations were identified but their significance was unclear.

The MCT detection rate of liquid biopsy varied significantly based on extent of disease, with a detection rate of 5.3% for localized disease involving a single primary tumor, 14.3% for localized disease involving multiple primary tumors, 33.3% for regional disease, and 57.1% for metastatic disease ($p = .027$). (Figure 3) There was no statistically significant difference in the detection rate of liquid biopsy based on the size of the largest lesion ($p = .059$); however, a trend was evident and significance may be achieved in the future with the addition of more data. (Figure 4)

The specificity of the assay, determined in a separate cohort of over 500 cancer-free dogs, was 98.5% - corresponding to a false positive rate of 1.5%.¹

CONCLUSIONS

Liquid biopsy detected nearly 60% of advanced stage MCT, and offers a novel tool to understand the clinical picture of dogs with MCT. The lower detection rate of liquid biopsy in patients with localized and regional MCT suggests that tissue cytology/biopsy should be prioritized over liquid biopsy in all cases where the suspected mass can be accessed by FNA or biopsy. However, liquid biopsy testing may be helpful in evaluating or staging cases where metastatic disease is suspected. For example, if a MCT is identified and liquid biopsy reveals a *Cancer Signal Detected* result, additional imaging and work up for advanced staging may be useful in determining the dog’s extent of disease, as metastatic disease is more likely to be present in the patient. The use of liquid biopsy for the evaluation of malignancy should always be considered in the context of each patient’s unique clinical presentation, as test performance can vary by tumor type and extent of disease.