

Evaluation of a blood-based liquid biopsy test in canine patients with difficult-to-diagnose cancers

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INTRODUCTION

Cancer in dogs may occur in anatomic locations that are difficult or are deemed too risky to access by fine needle aspiration (FNA) or surgical biopsy, making confirmation of malignancy difficult or in some cases impossible. These "difficult to diagnose" (DTD) cases may include: endocrine, bone, central nervous system (CNS), intra-thoracic, intra-abdominal, upper respiratory tract, and thymic tumors. (Figure 1) In such cases, a noninvasive 'aid in diagnosis' test may prove valuable in narrowing the differential diagnosis.

METHODS

As part of ongoing research and development programs at one commercial laboratory, blood samples were collected from over 1,700 canine subjects; and a subset was used for this analysis. A set of 200 subjects (54 cancer-diagnosed and 146 presumably cancer-free) was used to train the algorithms, and a set of 377 subjects (191 cancer-diagnosed and 186 presumably cancer-free) was used to validate the assay and establish test performance.

The cancer-diagnosed subjects in the training and validation sets were an all-comers cohort with a definitive diagnosis of malignant tumor, representing over 40 cancer types and the full spectrum of cancer stages. The cancer-free subjects were presumed to be cancer-free due to no history of cancer and no suspicion of cancer on clinical evaluation at the time of study enrollment; other clinical conditions (trauma, inflammation, infection, other diseases, etc.) were not considered exclusion criteria for the study, and fasting was not required prior to the blood collection.

Blood samples 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 data reviewers were blinded to the cancer status and type of cancer in these patients until after test results were issued.

RESULTS

In an all-comers cohort of 191 cancer-diagnosed subjects, 33% (n=63) were designated as DTD cases, comprising over 20 distinct cancer types from previously defined anatomical locations. (Figure 2)

Of these 63 dogs with a DTD cancer, 65% were purebred (representing 20 different breeds) and 35% were mixed-breed; 59% were male and 41% were female; the median age was 9 years (range: 4 – 15 years); and the median weight was 30.7kg (range: 4.5 – 61kg). (Table 1) The distribution of subjects with a DTD cancer is shown in Figure 3.

The blood-based 'liquid biopsy' test returned a *Cancer Signal Detected* (positive) result in 25 of the 63 subjects, for an overall detection rate of 40% across the difficult to diagnose cancers. (Figure 2) The demographics of the patients with a *Cancer Signal Detected* result compared to patients with a *Cancer Signal Not Detected* result and to the overall cohort are shown in Table 1.

In the broader validation cohort, the specificity of the assay was determined to be 97% (180/186).

Figure 1: Difficult to diagnose cancer types by anatomical location

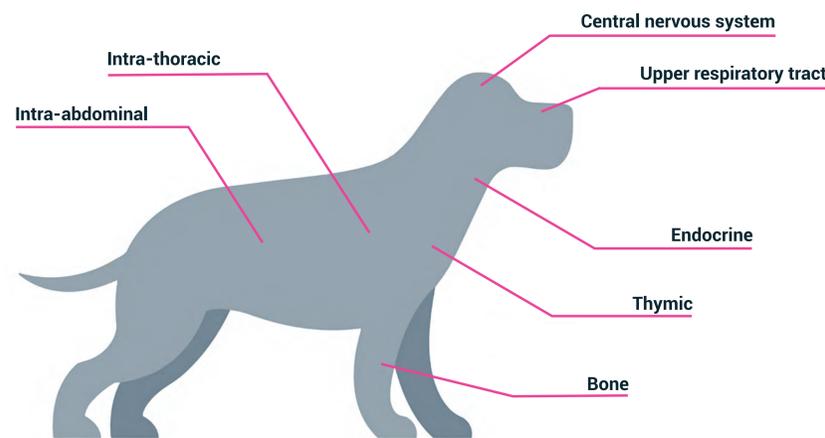


Figure 3: Distribution of subjects with a difficult to diagnose cancer type (n=63)

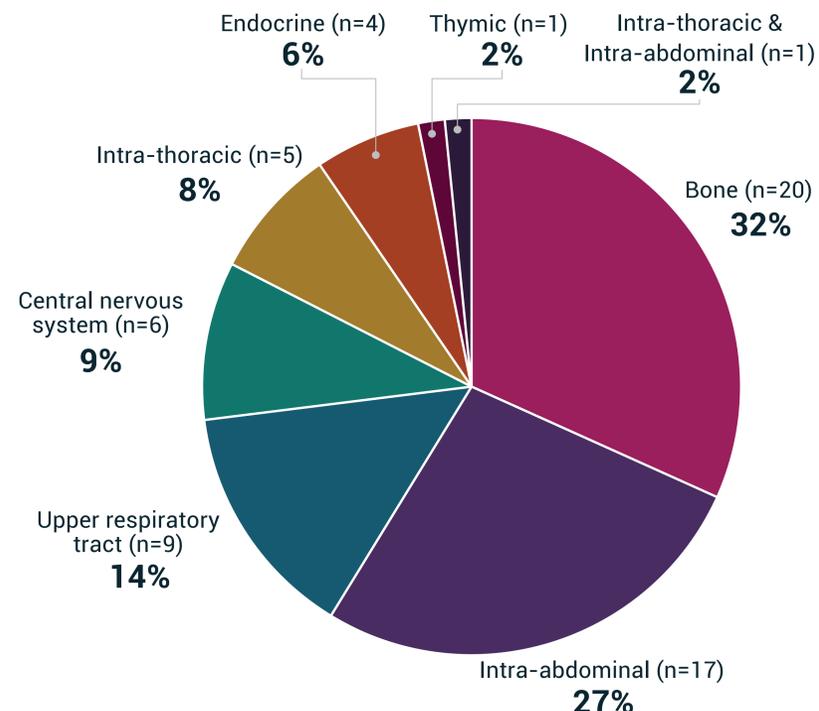


Figure 2: Distribution of cancer types in an all-comers cohort of cancer-diagnosed subjects & performance of liquid biopsy testing for a subgroup of dogs with difficult to diagnose cancers

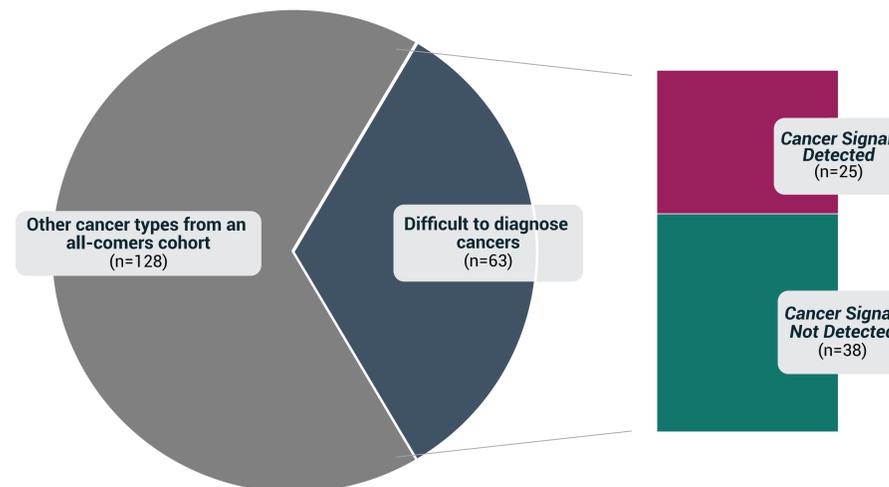


Table 1: Demographics of subjects diagnosed with a "difficult to diagnose" cancer

		Total subjects with one of the predefined "difficult to diagnose" cancers (n=63)	Subjects with <i>Cancer Signal Detected</i> (n=25)	Subjects with <i>Cancer Signal Not Detected</i> (n=38)
Breed	Purebred	41	15	26
	Mixed breed or Unknown	22	10	12
Sex	Male	37	16	21
	Female	26	9	17
Age	Range	4 – 15 years	5 – 14 years	4 – 15 years
	Mean (years)	9.4	9.9	9.1
	Median (years)	9	10	9
Weight	Range	4.5 – 61 kg	4.5 – 57.5 kg	15.2 – 61 kg
	Mean	30.2 kg	28.6 kg	32.1 kg
	Median	30.7 kg	28 kg	31.5 kg

CONCLUSION

A noninvasive, blood-based cancer detection test has potential to serve as an aid-in-diagnosis for patients with DTD masses. This type of testing may provide clinical utility in situations where tissue-based diagnosis may not be available or achievable for masses that are suspected to be malignant.

KEY POINTS

- 1 Certain cancers occur in anatomic locations that are difficult or deemed too risky to access by FNA or surgical biopsy.
- 2 Alternative means of detecting cancer in patients with "difficult to diagnose" masses would be beneficial to clinicians and pet owners when traditional diagnostic approaches are not feasible.
- 3 A blood-based 'liquid biopsy' test using cell-free DNA has the potential to noninvasively detect the presence of cancer-associated genomic alterations in patients with "difficult to diagnose" masses.