

# Application of liquid biopsy technology to detect lymphoma in dogs

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## INTRODUCTION

Lymphoma is one of the most common canine cancers with an estimated incidence of 20-100 cases per 100,000 dogs<sup>1-3</sup>, and is a multicentric disease, most often affecting lymph nodes externally and internally, as well as internal organs such as liver and spleen. Historically, the detection of lymphoma has relied upon the recognition of enlarged peripheral lymph nodes by dog owners or on physical exam, and diagnosis is typically made by fine needle aspiration (FNA) cytology of enlarged peripheral lymph nodes. In some cases, however, lymphoma sites may only be internal, and thus difficult to access for FNA. Blood-based 'liquid biopsy' testing using cell-free DNA (cfDNA) offers a novel, noninvasive approach to the identification and classification of lymphoma in dogs.

## METHODS

Blood samples from 57 dogs with a confirmed diagnosis of lymphoma were subjected to cfDNA extraction from plasma and genomic DNA (gDNA) extraction from white blood cells, 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. Computational and manual reviews of the genomic data were used to detect signal patterns highly suspect for lymphoma in cases that received a positive result. The testing laboratory was blinded to the cancer status and type of cancer present in these patients until after test results were issued.

## RESULTS

In 57 dogs with lymphoma, 28 (49%) were purebred (representing 18 different breeds) and 51% were mixed-breed; 47% were male and 53% were female; the median age was 7 years (range: 2 – 15 years), and the median weight was 27kg (range: 10 – 81.5kg). Demographics of the patients with a *Cancer Signal Detected* (positive) result compared to patients with a *Cancer Signal Not Detected* (negative) result and to the overall cohort are shown in **Table 1**.

In the 57 lymphoma-diagnosed subjects, the liquid biopsy test returned a *Cancer Signal Detected* (positive) result for 47 cases, yielding an overall sensitivity of 82%. When analyzed by subtype, the test showed 78% sensitivity for B-cell lymphoma (25/32), 100% for T-cell lymphoma (9/9), 50% for both B- and T-cell (1/2), and 86% for unphenotyped cases (12/14). (**Figure 1**) Within the 47 positive cases, a 'Cancer Signal Origin' prediction of "lymphoma" was provided in 19 cases (40%). Among a larger cohort of cancer-diagnosed dogs (representing over 40 different cancer types), none had a 'Cancer Signal Origin' prediction of lymphoma.

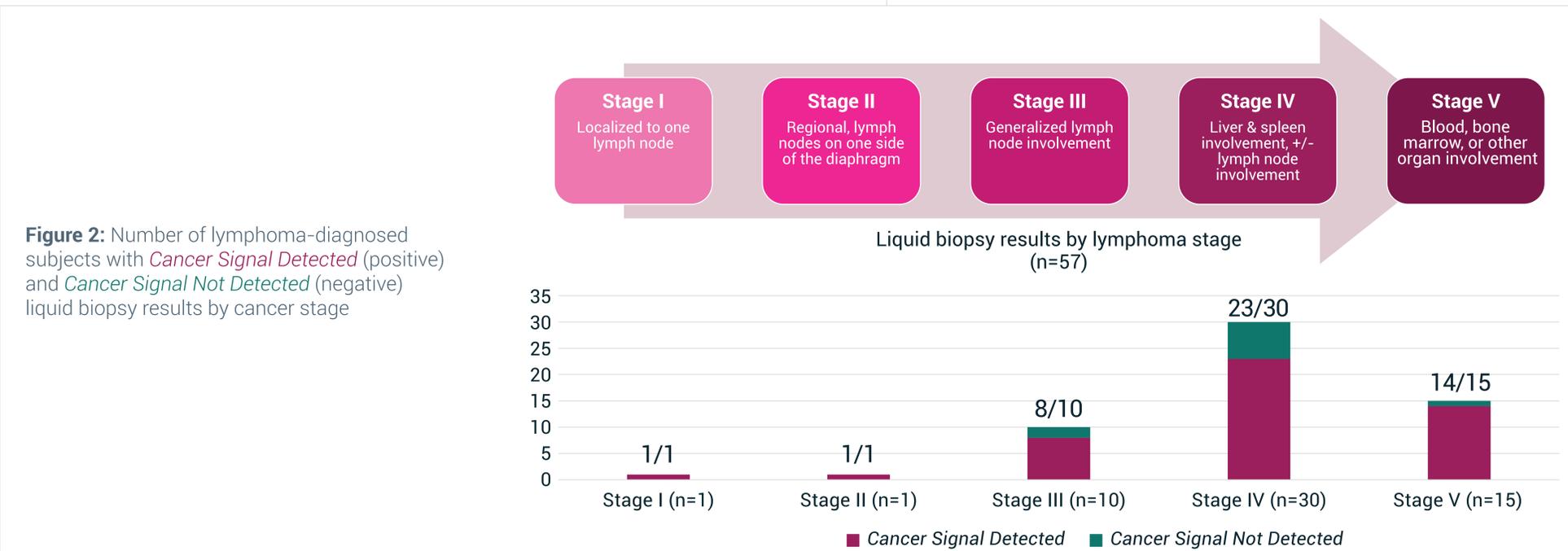
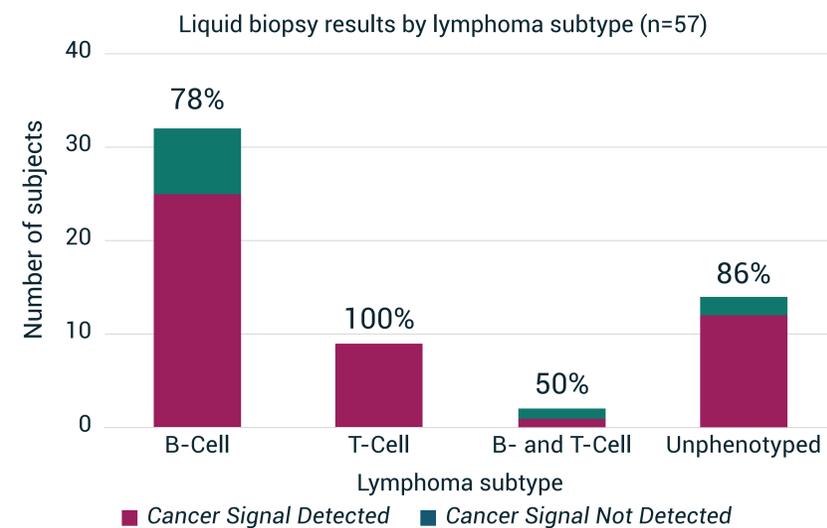
Performance of the liquid biopsy test was also analyzed by cancer stage for the lymphoma-diagnosed subjects. A *Cancer Signal Detected* (positive) result was issued in: 1/1 Stage I cases; 1/1 Stage II; 8/10 Stage III; 23/30 Stage IV; and, 14/15 Stage V. (**Figure 2**)

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

**Table 1:** Demographics of lymphoma-diagnosed subjects

		Demographics of total cohort of cancer-diagnosed subjects with lymphoma (n=57)	Lymphoma-diagnosed subjects with <i>Cancer Signal Detected</i> (n=47)	Lymphoma-diagnosed subjects with <i>Cancer Signal Not Detected</i> (n=10)
Breed	Purebred	28	21	7
	2 Breeds	6	6	0
	3+ Breeds or Unknown	23	20	3
Sex	Male	27	23	4
	Female	30	24	6
Age	Range	2 – 15 years	2-15 years	2-12 years
	Mean (years)	7.35	7.43	7
	Median (years)	7	7	6.5
Weight	Range	10 – 81.5kg	11.8 – 81.5kg	10 – 45.9kg
	Mean	29.2kg	29.2kg	29.4kg
	Median	27kg	26.5kg	29.1kg

**Figure 1:** Number of lymphoma-diagnosed subjects with *Cancer Signal Detected* (positive) and *Cancer Signal Not Detected* (negative) liquid biopsy results by subtype



**Figure 2:** Number of lymphoma-diagnosed subjects with *Cancer Signal Detected* (positive) and *Cancer Signal Not Detected* (negative) liquid biopsy results by cancer stage

## CONCLUSION

A novel, blood-based canine cancer screening test using cell-free DNA was successful at detecting genomic alterations associated with cancer in 82% of dogs with a diagnosis of lymphoma. Additionally, the liquid biopsy test was able to identify patterns of genomic abnormalities that were predictive of lymphoma in 40% of lymphoma-diagnosed cases that received a *Cancer Signal Detected* test result. Detection rate was consistently high across lymphoma subtypes and stages. These results demonstrate that a blood-based liquid biopsy test using cell-free DNA offers a novel, noninvasive approach to the detection and specific identification of lymphoma in dogs.

## KEY POINTS

- 1 Blood-based 'liquid biopsy' testing using cfDNA demonstrated an 82% detection rate for lymphoma in a cohort of 57 lymphoma-diagnosed dogs.
- 2 Specific patterns of genomic abnormalities can be used to predict the presence of lymphoma (vs any other cancer type) among patients with a *Cancer Signal Detected* result.
- 3 The detection rate of liquid biopsy testing in lymphoma-diagnosed dogs was consistently high across lymphoma subtype and stage, and may allow for earlier detection and treatment of the disease.

References:  
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