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Clinical epidemiology and liver testing

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Veterinarians make diagnostic decisions every day. It can be easy to forget that the diagnostic process is imperfect. Pets rarely develop diseases that can be diagnosed on the basis of one or more pathognomonic findings. In most cases, results of the historical, physical and laboratory examinations lead to the most probable cause of illness.

The majority of diagnoses are made with some degree of uncertainty. The diagnostic process involves removing uncertainty until the veterinarian believes, with a high degree of confidence, that the suspected diagnosis is correct. Uncertainty can be expressed in terms of probability, *i.e.,* the odds of disease are 4 to 1, or the probability of infection is 25 percent. Probability is just as integral to the clinical process for a veterinarian as to a gambler in Las Vegas.

In this article, we review concepts of clinical epidemiology that highlight the probabilities inherent to the diagnostic process, as well as present some population results for liver enzyme testing in our Banfield population. With knowledge of test characteristics, veterinarians can better interpret diagnostic test results and optimize clinical decision-making. In doing so, the client and Pet are both winners.

Sensitivity and specificity

Knowing the sensitivity and specificity of available diagnostic tests helps to determine which particular test may be preferred for a given clinical situation.[1-3] These properties

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Sensitivity: The proportion of Pets with disease that have a positive test result. The mnemonic, **SnNout,** can be used to interpret the finding that when a sign, test or symptom has a high **Sen**sitivity; a **N**egative result rules **out** the diagnosis.

Specificity: The proportion of Pets without disease that have a negative test result. The mnemonic, **SpPin,** can be used to interpret the finding for a sign, test or symptom with a high **Sp**ecificity; a **P**ositive result rules **in** the diagnosis

Positive Predictive Value: A likelihood based on disease prevalence, test sensitivity and specificity that a Pet with a positive test is a true positive, *i.e.*, has the disease.

Negative Predictive Value: A likelihood based on disease prevalence, test sensitivity and specificity that a Pet with a negative test is a true negative, *i.e.*, does not have the disease.

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DataSavant

DataSavant's mission is to:
- Explore the health and well-being of Pet populations
- Evaluate new clinical treatments
- Monitor Pets as sentinels of zoonotic disease in family environments
- Transform Pet medical data into knowledge, *i.e.*, open new windows into Pet health care using the Banfield medical caseload and database.

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are intrinsic to a diagnostic test and reflect the capacity to identify a diseased or non-diseased individual. A "gold standard"–a definitive and valid marker for a disease–must exist to evaluate test sensitivity and specificity. The gold standard serves as a reference for comparison. For example, histopathologic confirmation of a cancer diagnosis serves as the gold standard for cancer screening tests.

Sensitivity is the percentage of individuals with the disease that have a positive test, *i.e.*, the "true" positive rate, while specificity represents the percentage of individuals without the disease that tested negative, *i.e.*, the "true" negative rate. In screening for disease, a test with high sensitivity is most appropriate so that positives are identified and can receive additional testing if needed. A sensitive test will minimize the false negative rate so that the diagnosis (and therefore treatment) will be missed in as few truly diseased individuals as possible. When ruling out a disease, sensitivity is more important than specificity–a negative result has more significance at this point in the diagnostic process. Alternatively, when it is important to confirm a diagnosis or to rule in a disease, a more specific test should be used to minimize the false positive rate. Otherwise, a treatment course or medical management strategy could be undertaken based on an erroneous result.

Unlike sensitivity and specificity, the determination of the predictive value of a test is dependent on the prevalence of disease in the population being tested (the percentage of individuals with existing disease).

Predictive value

Although sensitivity and specificity can be useful in comparing and evaluating tests, knowing the probability that a certain test result is indicative of a truly diseased or non-diseased Pet is even more relevant to the clinical decision-making process. In other words, given a positive or negative result for a specific test, what is the likelihood that the patient does or does not have the disease in question?

Unlike sensitivity and specificity, the determination of the predictive value of a test is dependent on the prevalence of disease in the population being tested (the percentage of individuals with existing disease). Prevalence is also referred to as the pretest probability, *i.e.*, the probability that an individual in a defined population is diseased before any additional diagnostic information is obtained.

The prevalence of disease in a population affects the ability of a specific test to predict whether an individual Pet is truly diseased or not. If a disease is relatively common in a population, the probability is higher that a test will be able to predict a truly diseased individual, *i.e.*, there will be few false positives. Alternatively, if a disease is relatively rare, the ability of the test to identify a truly diseased individual will be diminished and more false positives will be identified.

There is limited literature on sensitivity, specificity and predictive values for liver enzyme activity testing. Canine alanine aminotransferase (ALT) activity testing has been reported to have a sensitivity ranging from 45 percent to 100 percent, depending on the liver pathology (acute or chronic hepatopathy, etc.).[4] The predictive value cited for ALT activity testing in this same paper was 18 percent, *i.e.*, an elevated ALT-activity testing was predictive for liver

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Table 1: Reference Range for Normal Results

Cats < 6 months of age		Dogs < 6 months of age	
ALP	14.0 – 192.0 U/L	ALP	46.0 – 337.0 U/L
ALT	12.0 – 115.0 U/L	ALT	8.0 – 75.0 U/L)
BIL	0.0 – 0.9 mg/dl	BIL	0.0 – 0.9 mg/dl
Cats ≥ 6 months of age		**Dogs ≥ 6 months of age**	
ALP	14.0 – 111.0 U/L	ALP	23.0 – 212.0 U/L
ALT	12.0 – 130.0 U/L	ALT	10.0 – 100.0 U/L
BIL	0.0 – 0.9 mg/dl	BIL	0.0 – 0.9 mg/dl

disease in 1 of 5 patients. Specificity for canine alkaline phosphatase (ALP) ranged from 44 percent to 90 percent depending on the liver disease in question; its predictive value was 21 percent, about the same as ALT activity testing. In general, these tests are not ideal for establishing a definitive diagnosis, and follow-up with more specific tests is essential. Although we don't have all the necessary data to evaluate the sensitivity, specificity and predictive values for liver enzyme testing in the Banfield population, we describe Banfield database results for these tests below, specifically for Pets that are healthy when they present to the veterinarian.

Methods of analysis

We used 2007 Banfield data captured in PetWare® to generate the healthy canine and feline test sample distribution (mean, median and range) by age group (< 6 months, 6 months – 7 years, 8 years and older) for ALP and ALT activities and total bilirubin concentration. We also calculated the proportion of "healthy" Pets by age group that had test elevations for ALP and ALT activities and total bilirubin concentration. A "healthy" Pet was defined as one for whom overall condition in PetWare was *not* defined as "Needs Improvement."

Normal result ranges for ALT, ALP and total bilirubin concentration (BIL) were defined as in *Table 1*.

Results

PetWare records from 382,856 dogs were evaluated for ALP activity, ALT activity or total bilirubin concentration; 166,700 or 43.5 percent of these dogs were healthy as defined for our analysis. A total of 89,598 cats were evaluated for ALP activity, ALT activity or total bilirubin concentration; 34,073 or 38 percent of these cats were healthy as defined for our analysis.

Nearly 21 percent of healthy dogs had one or more elevation in ALP activity, ALT activity or total bilirubin concentration; 15.5 percent of healthy cats had one or more elevation. *Tables 2* and *3* (page 19) provide the proportion of the healthy Pets evaluated that had elevations for ALP activity, ALT

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Table 2: 2007 Banfield Canine Population Proportion with Elevated Liver Test Results—Healthy* Pets

Test	Age group	Elevated healthy*	Total count	Healthy* count	% Elevated in healthy*
ALP	< 6 months	439	56,296	23,461	1.9
	> 6 months	12,998	329,586	143,796	9.0
	Total unique Pets#	13,428	382,599	166,541	8.1
ALT	< 6 months	1,606	56,292	23,467	6.8
	> 6 months	14,586	329,606	143,832	10.1
	Total unique Pets#	16,147	382,604	166,577	9.7
BIL	< 6 months	640	56,285	23,457	2.7
	> 6 months	10,008	329,465	143,735	7.0
	Total unique Pets#	10,647	382,469	166,476	6.4

* Overall condition in PetWare *not* defined as "Needs Improvement"
# Count of unique Pets will be less than sum of counts because one Pet could have multiple tests

Table 3: 2007 Banfield Feline Population Proportion with Elevated Liver Test Results—Healthy* Pets

Test	Age group	Elevated healthy*	Total count	Healthy* count	% Elevated in healthy*
ALP	< 6 months	702	16,378	6,533	10.7
	> 6 months	1,406	73,608	27,617	5.1
	Total unique Pets#	2,100	89,534	34,038	6.2
ALT	< 6 months	384	16,377	6,529	5.9
	> 6 months	1,467	73,635	27,636	5.3
	Total unique Pets#	1,842	89,557	34,050	5.4
BIL	< 6 months	288	16,369	6,528	4.4
	> 6 months	1,622	73,603	27,617	5.9
	Total unique Pets#	1,909	89,521	34,033	5.6

* Overall condition in PetWare *not* defined as "Needs Improvement"
# Count of unique Pets will be less than sum of counts because one Pet could have mutiple tests

activity or total bilirubin concentration individually. For dogs, these proportions range from just under 2 percent for ALP activity in dogs younger than 6 months old to just over 10 percent for ALT activity in dogs older than 6 months. For the cat population, the proportion with elevated ALP activity, ALT activity or total bilirubin concentration ranged from 4.4 percent for ALT activity in cats less than 6 months old to nearly 11 percent for ALP activity for cats younger than 6 months old. *Tables 4* and *5* (page 20) display the distribution of these liver test results for healthy cats and dogs

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Table 4: 2007 Canine Population Liver Test Results by Age Group—All Healthy* Pet Samples

Test	Age group	Total count	Mean	Median	Range
ALP U/L	< 6 months	24,058	183.2	173.0	10.0 – 1,880.0
	6 mos - 7 years	137,463	95.9	72.0	3.0 – 3,330.0
	>8 years	35,723	203.4	102.0	0.2 – 7,373.0
	All ages	197,244	126.0	84.0	0.2 – 7,373.0
ALT U/L	< 6 months	24,043	44.2	38.0	0.9 – 3,491.0
	6 mos - 7 years	137,515	56.9	46.0	0.1 – 2,000.0
	> 8 years	35,706	72.9	53.0	0.1 – 2,677.0
	All ages	197,264	58.3	46.0	0.1 – 3,491.0
BIL mg/dl	< 6 months	24,067	0.2	0.1	0.0 – 11.0
	6 mos- 7 years	137,761	0.3	0.2	0.0 – 20.0
	> 8 years	35,831	0.3	0.2	0.0 – 20.0
	All ages	197,659	0.3	0.2	0.0 – 20.0

*Overall condition in PetWare *not* defined as "Needs Improvement"

Table 5: 2007 Feline Population Liver Test Results by Age Group—All Healthy* Pet Samples

Test	Age Group	Total count	Mean	Median	Range
ALP U/L	<6 months	6,741	126.4	115.0	10.0 – 1950.0
	6 mos - 7 years	21,912	55.2	46.0	5.0 – 13.50
	>8 Years	8,851	55.1	46.0	2.0 – 1,547.0
	All Ages	37,504	68.0	51.0	2.0 – 1,950
ALT U/L	<6 months	6,757	71.4	64.0	1.0 – 785.0
	6 mos -7 years	22,012	69.9	62.0	5.0 – 2,815.0
	> 8 years	8,878	72.3	62.0	1.5 – 2,129.0
	All Ages	37,647	58.1	63.0	1.0 – 2,815.0
BIL mg/dl	<6 months	6,753	0.4	0.3	0.0 – 12.0
	6 mos - 7 years	21,977	0.4	0.3	0.0 – 18.4
	> 8 years	8,864	0.4	0.3	0.0 – 18.8
	All ages	37,594	0.4	0.2	0.0 – 18.8

*Overall condition in PetWare *not* defined as "Needs Improvement"

overall and by age group.

Of the healthy dogs that had elevations in ALP activity and ALT activity, about 8 percent (291/3785) had a bile acids concentration test performed (within 30 days of ALP/ALT activity elevations) to assess liver function. For this canine subset, 39 percent had an elevated bile acids concentration (>25 µmol/L). Of the healthy cats that had elevations in ALP activity and ALT activity,

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about 5 percent (15/316) had a bile acids concentration test to assess liver function within 30 days. For this feline subset, 40 percent had an elevated bile acids result (>20 µmol/L). Bile acids stimulation testing for liver function and other diagnostic tools to identify specific disease pathology are discussed in, "Diagnostic testing for liver disease" (page 34).

Veterinarians can make more informed decisions about the most appropriate diagnostic tests to use for ruling in and ruling out diagnoses when they are equipped with an understanding of sensitivity, specificity and predictive value.

Discussion

Two major points can be identified in summarizing the results from the Banfield database presented in this article. One, liver enzyme activity testing has limited predictive value for definitively diagnosing liver disease in dogs and cats. Two, bile acids concentration results from our healthy population reveal that there may be undetected disease in apparently healthy Pets that present to our hospitals since 40 percent of those healthy Pets with elevated ALT and ALP activities that had bile acids concentration tested were abnormally elevated; less than 10 percent of healthy dogs and cats with elevated ALT and ALP activities had a bile acid concentration test performed within 30 days of the elevated enzyme activities results.

The limitations of our analysis include the potential for misclassification of Pets as healthy based on our analysis definition. Also, the bile acids concentration test used for this analysis was a single sample test rather than the two-sample stimulation test. The single sample has limited sensitivity and specificity. However, the results do underscore the importance of further case work-up with liver function tests, imaging, fine needle aspirate or biopsy when indicated by history, physical examination and clinical signs.

Veterinarians can make more informed decisions about the most appropriate diagnostic tests to use for ruling in and ruling out diagnoses when they are equipped with an understanding of sensitivity, specificity and predictive value. These are additional tools to use to better quantify the likelihood of disease for a particular Pet. Clinical epidemiology is an essential element of medical decision-making processes that enhances the quality of patient care.

References

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