DIAGNOSIS OF CANINE HYPOTHYROIDISM: CASE-BASED APPROACH
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CASE 1
Signalment: 8 yr old, CM, English bulldog; History: Presented for annual exam. Low activity and obesity despite limited feedings only problems noted.; Physical examination: Obese; Laboratory data: Complete CBC, profile, urinalysis done. Abnormalities were: WBC 17.5 x 10^3/µl (6.0-17.0); Neutrophils: 14.6 x 10^3/µl (3.0-11.5); Lymphocytes 0.7 x 10^3/µl (1.0-4.8); Monocytes 1.7 x 10^3/µl (0.2-1.4)

The approach to a dog with no known non-thyroidal illness (e.g. renal disease, neurological disease, neoplasia, etc.) vs. a dog with non-thyroidal illness is a bit different. In dogs with no known non-thyroidal illness the diagnosis is more straightforward. Starting with a measurement of total T4 alone is reasonable and economical. If total T4 is normal, it is highly unlikely that the dog is hypothyroid. Since non-thyroidal factors such as drugs and illness affect T4, if the T4 is below normal, the dog may or may not be hypothyroid, and further testing is required.

CASE SUMMARY: Serum T4 concentration = 28 nmol/L (reference range 20-50 nmol/L). Based on minimal clinical signs and normal serum T4 concentration, diagnosis of hypothyroidism ruled out.

CASE 2
Signalment: 8 yr old, CM, English bulldog; History: Presented for decreased activity, obesity despite being on a weight-loss program, thinning haircoat, heat-seeking behavior.; Physical examination: Obese; partial, bilaterally symmetrical alopecia; Laboratory data: Complete CBC, profile, urinalysis done. Abnormalities were: RBC x 10^6/µl 5.0 (5.5-8.5); Hemoglobin 11.2 g/dL (12-18)); PCV 33 % (37-55); Lymphocytes 0.5 x 10^3/µl (1.0-4.8); Cholesterol 470 mg/dL (130-370)

The clinical findings suggestive of hypothyroidism are much stronger in this dog than in Case 1. Starting with measurement of serum T4 is still a good first choice, but be prepared to do further testing if the serum T4 concentration is below normal.

Free T4 (fT4) is the portion of total T4 not bound to protein and represents about 0.1% of total T4. Since the pituitary-thyroid axis functions to maintain free, not total, T4 within a certain range, fT4 is affected less by non-thyroidal factors and measurement of fT4 is a better test of thyroid function. Accordingly, fT4 is a more sensitive and more specific test for diagnosis of hypothyroidism, but it is also not as good a stand-alone test as once believed (see below). It can be the initial test for the diagnosis of hypothyroidism or can be used in dogs that have been found to have low total T4 concentrations.

Free T4 should always be measured by the equilibrium dialysis method. The other technique for measuring fT4, analogue RIA, is not reliable and provides no additional diagnostic value over measurement of total T4. Equilibrium dialysis is also the only RIA for measuring fT4 that is unaffected by the presence of autoantibodies.

Primary thyroidal failure is believed to be the cause of canine hypothyroidism in 99% of cases. Accordingly, negative feedback of thyroid hormones on the pituitary would be lost and TSH should increase. However, an elevated serum TSH occurs in only 63-85% of hypothyroid dogs, and if measurement of canine TSH were used alone for diagnosis of hypothyroidism, up to 37% of cases would be missed. Serum TSH can also be elevated in approximately 10% of euthyroid dogs with non-thyroidal illness. Therefore, measurement of TSH is best used not as a sole test but in conjunction with T4 or, ideally, fT4. Use of the combination will aid in identifying false-positive and false-negative results seen with assessment of TSH alone.

Measurement of baseline serum T3 is of little value in differentiating hypothyroid from normal dogs. There is no apparent difference in serum T3 concentrations between these groups.

CASE SUMMARY: Serum T4 concentration was 13 nmol/L (borderline range: 12-19; reference range 20-50 nmol/L). Measurement of serum fT4 concentration (by equilibrium dialysis) and serum TSH concentration were requested. The serum fT4 concentration was 8 pmol/L (reference range 15-45 pmol/L, 10-14 pmol/L borderline) and serum TSH concentration was 0.25 ng/ml (normal <0.5 ng/ml).
The interpretation of the case now becomes a clinical dilemma. The question is whether this is a hypothyroid dog with a normal TSH or whether this is a euthyroid sick dog whose TSH has remained normal while the fT4 is falsely lowered. The danger of falsely diagnosing a dog with hypothyroidism are threefold: 1. If clinical signs are incorrectly attributed to hypothyroidism, then the true diagnosis will be delayed or never sought. 2. Thyroxine is a catabolic hormone. Administering a catabolic hormone to an ill patient may be detrimental. 3. The patient will needlessly be treated with thyroid hormone for the rest of its life. On the other hand, the danger of not treating hypothyroidism is that the clinical signs will progress. However, in a case such as this one, the clinical signs are relatively mild and benign and progression is typically insidious, i.e. not treating for a month will most likely not be detrimental in the long-term.

At this point there are 2 choices: 1. Retest in 4-8 weeks. 2. Start trial therapy for hypothyroidism. If choosing option 2, make sure that you have OBJECTIVE measures of the endpoint determined beforehand, i.e. normalization of serum cholesterol concentration and return to normal weight. Regrowth of hair is not a good endpoint to choose; the haircoat of euthyroid dogs will improve in response to thyroid supplementation. Be prepared to stop giving the thyroxine if the clinical signs do not improve given adequate post-pill levels and time. (You must measure post-pill levels to determine if the trial is successful or not.)

**CASE 3**

**Signalment:** 8 yr old, CM, English bulldog; **History:** Presented for lethargy, weight gain and obesity despite a poor appetite, bilaterally symmetrical alopecia (non-pruritic) that has been progressive over the past year, heat-seeking behavior; **Physical examination:** Obese; partial, bilaterally symmetrical alopecia; **Laboratory data:** Complete CBC, profile, urinalysis done. Abnormalities were: RBC x 10^6/µl 5.0 (5.5-8.5); Hemoglobin 11.2 g/dL (12-18)); PCV 33% (37-55); Lymphocytes 0.5 x 10^3/µl (1.0-4.8); Cholesterol 470 mg/dL (130-370)

In a case that seems to be textbook for hypothyroidism, starting with measurement of serum T4 concentration is reasonable. If no other abnormalities are found other than those that can be explained by hypothyroidism and the serum T4 concentration is very low, a presumptive diagnosis of hypothyroidism can be made. It would be ideal to measure a fT4 concentration by dialysis for confirmation, but it may be unnecessary. Measurement of serum TSH concentration is not worth the money in this situation. Given that the sensitivity of measuring serum fT4 concentration is much higher than that of serum TSH concentration measurement, in a case such as this if serum fT4 concentration were low but serum TSH concentration was normal, I would believe the serum fT4 concentration and start treatment for hypothyroidism.

**CASE SUMMARY:** Serum T4 concentration was measured and was non-detectable. Due to financial considerations, fT4 concentration was not measured. Therapy with L-thyroxine was instituted. Post-pill testing was done to ensure adequate serum T4 concentration was achieved. Within 3-4 months clinical signs had resolved.

**CASE 4**

**Signalment:** 8 yr old, CM, English bulldog; **History:** Originally presented to his veterinarian for a geriatric screen and then was referred to the Auburn University Small Animal Clinic for evaluation of an incidental finding of proteinuria. On a urinalysis, a 2+ proteinuria was noted with a specific gravity of 1.014. A urine protein/creatinine ratio (UP/C) was determined to quantify the protein loss and was found to be 5.8 (normal <0.5); The dog had received regular veterinary care at a private veterinary clinic. He lived in Alabama with no history of travel out of state. His vaccinations were up-to-date, and he was receiving Interceptor for heartworm prevention. He spent his days outdoors, but was a house dog at night. The owners reported no problems, and had seen no evidence of coughing, sneezing, vomiting, diarrhea, polydipsia, polyuria or weight loss. The dog’s activity had decreased slowly over the past year and was attributed to aging. His appetite was normal.; **Physical examination:** On PE, the dog was noted to be obese, and he had moderate to severe dental tartar and gingivitis. Chest auscultation and abdominal palpation were within normal limits.; **Laboratory data:** Complete CBC, profile, urinalysis done. Abnormalities were: WBC 17.5 x 10^3/µl (6.0-17.0); Neutrophils: 14.6 x 10^3/µl (3.0-11.5); Lymphocytes 0.7
Due to the magnitude of the UP/C, a possible diagnosis of immune-complex glomerulonephritis (ICGN) was made. ICGN can be idiopathic or secondary to chronic immune stimulation. As the dental disease could be a source of antigens, a dental procedure was recommended and was performed. One month post-dental, the UP/C was essentially unchanged at 7.2 and blood pressure remained normal.

Further diagnostics were initiated to find possible underlying disease processes that could initiate immune stimulation. Three-view chest radiographs were obtained to rule out neoplasia (primary or metastatic) as well as other pulmonic diseases, and they were within normal limits. Abdominal ultrasound was normal. An occult heartworm test was negative. Serology for *Ehrlichia canis*, *Bartonella* and Lyme’s disease was negative. PCR for *Bartonella spp.* and *Ehrlichia spp.* was negative. Urine culture yielded no growth.

At re-evaluation approximately 4 weeks later, after the results of all the tests had been obtained, the UP/C was 8.6, systolic blood pressure was moderately elevated at 190 mm Hg and the cholesterol was moderately elevated (412 mg/dl). In order to determine the pathology underlying the proteinuria (e.g. glomerulonephritis vs. amyloidosis vs. structural glomerulopathy) and whether the disease process was reversible, an ultrasound-guided renal biopsy was performed. The histopathological diagnosis was glomerulonephritis. Enalapril was prescribed (0.5 mg/kg daily) to decrease proteinuria and blood pressure.

On subsequent rechecks the dog was doing well. Systolic blood pressure was 140-150 mm Hg and the UP/C was approximately 4.3. However, persistent hypercholesterolemia (persistent), obesity and poor hair regrowth after abdominal ultrasound were noted, and a diagnosis of hypothyroidism was considered.

Given the complexity of the case, I would start with measurement of serum total T4, fT4, and TSH concentrations. The effect of non-thyroidal illness on testing for hypothyroidism is quite significant. Two hundred twenty-three dogs with normal thyroidal function but with non-thyroidal illness were divided into those with mild, moderate and severe disease. Mildly ill dogs were considered to have clinical signs of disease but could be treated as outpatients, moderately ill dogs were sick enough to generally require hospitalization and more aggressive treatment and severely ill dogs required intensive care and advanced treatment. Interesting results were obtained.  

<table>
<thead>
<tr>
<th>Disease severity</th>
<th>Total T4</th>
<th>T3</th>
<th>Free T4</th>
<th>TSH</th>
</tr>
</thead>
<tbody>
<tr>
<td>All dogs</td>
<td>31</td>
<td>16</td>
<td>22</td>
<td>8</td>
</tr>
<tr>
<td>Mild disease</td>
<td>8</td>
<td>3</td>
<td>8</td>
<td>11</td>
</tr>
<tr>
<td>Moderate disease</td>
<td>28</td>
<td>18</td>
<td>17</td>
<td>6</td>
</tr>
<tr>
<td>Severe disease</td>
<td>60</td>
<td>27</td>
<td>44</td>
<td>8</td>
</tr>
</tbody>
</table>

Of the 69 dogs with low T4, 45% also had a low fT4 whereas only 8.7% also had a high TSH. Only 1.8% of sick dogs had a low T4 and fT4 in combination with a high TSH.  

Similar results have been obtained from other studies as well. In 49 dogs with a variety of illnesses, T4 was low in 22% but TSH was elevated in only 12%. In 127 dogs with “severe disease” that caused the owners to euthanize their dog, 65% had at least one abnormal value. Overall, 59% had decreased T4, 32% had decreased fT4 and 8% had increased TSH. Forty percent had only a decreased T4, 5% had only a decreased fT4, 5% had increased TSH, 43% had decreased T4 and fT4, and 7% had decreased T4 and increased TSH. No dog had decreased fT4 and increased TSH.

Possibly, in order affect thyroid hormones, a non-thyroidal illness must cause metabolic or systemic problems. For example, moderate to severe arthritis had no effect on thyroid testing. However, even transient systemic illness can have effects on thyroid testing and the alterations may be prolonged. Two studies have been done in which endotoxin was administered to dogs to cause transient illness. After 1 dose, 25% of dogs had decreased T4. fT4 was normal, but TSH was not measured. In dogs given endotoxin every 12 hours for 8 doses, T4 decreased during treatment. Interestingly, T4 then returned to
normal but decreased again into the hypothyroid range at days 2-16 after administration ceased. TSH and fT₄ were not affected.

Therefore, in sick dogs, the first choice for diagnosis of hypothyroidism would be a combination of TSH, fT₄ and T₄. 2nd choice is a combination of TSH and fT₄ and third choice is a combination of TSH and T₄. If the results are conflicting (some parameters are suggestive of hypothyroidism while others are not), the ideal would be to resolve the non-thyroidal illness, if possible, and retest the dog at that time. Alternatively, a TSH stimulation test could be done with recombinant human TSH (rhTSH), if available (Thyrogen®, Genzyme Corp.). The exact recommendations are unknown. Previous information stated that a dose of 50 mcg rhTSH should be used IV for dogs < 29 kg and 100 mcg IV for dogs > 29 kg, and serum T₄ concentration measured before injection and 4 hr post. In previous information, in normal dogs, serum T₄ concentration should increase by 20 nmol/L or to at least 40 nmol/L (to convert to mcg/dl, divide value in nmol/L by 12.87). If either one or both of the criteria is met, the dog is not hypothyroid. If neither criteria is met, the dog is hypothyroid (S. Daminet, personal communication). Studies are ongoing to determine if this protocol is optimal.

A vial of Thyrogen® contains 1100 mcg of rhTSH. Once reconstituted, the vial should be divided into 50 mcg aliquots and frozen in syringes at -20°C. At this temperature, the rhTSH is stable for at least 8 weeks.

If resolution of the other disease is not possible or testing with rhTSH is not available, the diagnosis of hypothyroidism poses a clinical dilemma as in Case 2. It is up to the clinician to decide how high their index of suspicion is for hypothyroidism, e.g. what clinical signs are present that could be attributed to hypothyroidism alone and not to the other disease process. The same drawbacks to treating or not treating exist as before but not treating could have more devastating consequences if some of the severe clinical signs are caused by the hypothyroidism, e.g. neuropathy. It may be best to treat the dog for hypothyroidism while still looking for other possible etiologies of the clinical signs.

**Case Summary:** A total serum T₄, fT₄ and TSH concentrations were measured. Serum T₄ concentration was 13 nmol/L (normal 20-55 nmol/L; borderline 12-19 nmol/L), serum fT₄ was 11 pmol/L (normal 15-45 pmol/L, borderline 10-14 pmol/L) and the TSH was 0.04 ng/ml (normal <0.5 ng/ml). Due to the effect that non-thyroidal illness can have on thyroid function testing, the dog was judged to be most likely euthyroid based on a normal TSH and minimal clinical signs. A recheck was recommended in 4-6 weeks.

The dog improved on treatment. Blood pressure remained normal on enalapril, the UP/C stabilized at approximately 3.2 and cholesterol remained very mildly elevated (380-400 mg/dl) as well. Two months after stabilization, the thyroid panel was repeated. Total T₄ was still below normal (16 nmol/L), but the fT₄ (18 pmol/L) and TSH (0.02 ng/ml) were within normal. Hypothyroidism was ruled out.

**Case 5**
**Signalment:** 8 yr old, CM, English bulldog
**History:** Presented for geriatric examination. Doing well at home.
**Physical examination:** Normal (for a bulldog 😃)
**Laboratory data:** Complete geriatric profile done. All within normal limits except ALP = 254 IU/L (normal 10-95) and T₄ = 12 nmol/L (normal 20-50).

What to do now with this case? I believe in geriatric screening in some scenarios - thyroid testing in dogs is not one of them. One thing to consider with random testing (i.e. not testing based on the presence of clinical signs), what is the predictive value of a test? Sensitivity and specificity look at a test from the viewpoint of the patient. Sensitivity is the chance that an animal with the disease will test positive and specificity is the chance that an animal that doesn't have the disease will test negative. Sensitivity and specificity are NOT affected by the prevalence of the disease in a population tested. PPV and NPV look more at a test from the viewpoint of the test. Positive predictive value (PPV) tells you how likely it is that an individual with a positive test result actually has the disease. Negative predictive value (NPV) tells you the likelihood that an animal with a negative test result doesn't have the disease.

Let’s look at the following chart regarding using fT4ed for testing for hypothyroidism. The chart assumes that there is a 50% prevalence in the population, i.e. 50% of tested dogs are truly hypothyroid. Since the prevalence is 50%, in a population of 200 dogs, 100 are hypothyroid (left hand column) and 100 are normal (right hand column). In the chart, it is the left column that is used to calculate sensitivity (that's
why it says sensitivity below that column) and the right hand column that is used to calculate specificity.
Calculations:

<table>
<thead>
<tr>
<th>Calculation</th>
<th>Result</th>
</tr>
</thead>
<tbody>
<tr>
<td>Prevalence: 100/200 = 50%</td>
<td></td>
</tr>
<tr>
<td>Sensitivity = True positive / (True positive + false negative) = 98/100 = 98%</td>
<td></td>
</tr>
<tr>
<td>Spec = True negative / (True negative + false positive) = 93/100 = 93%</td>
<td></td>
</tr>
<tr>
<td>PPV = True positive / (True positive + false positive) = 98/105 = 93%</td>
<td></td>
</tr>
<tr>
<td>NPV = True negative / (True negative + false negative) = 93/95 = 98%</td>
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</table>

So in this population of 200 dogs, an animal with a low ft4ed is truly hypothyroid about 93% of the time and an animal with a normal ft4ed is normal about 98% of the time. That's a pretty darn good test.

However, these values for PPV and NPV are dependent upon the prevalence of the disease in the population in question. The higher the prevalence of a disease in a population being tested, the higher the PPV and NPV. In dogs that have actual clinical signs of a disease being tested for, it is likely that the prevalence is relatively high - if there are clinical signs of a disease, then there is a higher likelihood of that disease being present than in a random population of dogs. Although hypothyroidism is one of the 2 most common endocrine diseases, in the dog population as a whole it is not THAT common. Let's just say for sake of argument that it is 5% (which is WAY too high). Now we test the next 200 dogs for hypothyroidism that walk through our door. If the prevalence of the disease in general is 5%, then chances are 5% of the next 200 dogs are hypothyroid. Based on those percentages, we could construct a 2x2 chart (below). Now the calculations are:

<table>
<thead>
<tr>
<th>Calculation</th>
<th>Result</th>
</tr>
</thead>
<tbody>
<tr>
<td>Prevalence: 10/200 = 5%</td>
<td></td>
</tr>
<tr>
<td>Sensitivity = True positive / (True positive + false negative) = 10/10 = 100%</td>
<td></td>
</tr>
<tr>
<td>Spec = True negative / (True negative + false positive) = 177/190 = 93%</td>
<td></td>
</tr>
<tr>
<td>PPV = True positive / (True positive + false positive) = 10/23 = 43%</td>
<td></td>
</tr>
<tr>
<td>NPV = True negative / (True negative + false negative) = 177/177 = 100%</td>
<td></td>
</tr>
</tbody>
</table>
So when you randomly test dogs or cats for a disease without carefully selecting the population, i.e.
those with clinical signs of the disease, you HUGELY decrease the PPV of the test. In our example, the
prevalence was 5% and that is high for many of the disease we deal with, so PPV would be even lower.
In addition, T4 is not that easy to interpret and depends on so many things - age being one of them!
In a recent paper, Purina kept Labs from birth until death and looked at the effect of lifetime food
restriction on variables. They also looked at effect of age. The figure below shows the mean total T4
from the paper in one group (but the other group was statistically similar):

So there appears to be an effect of age that no laboratory takes into consideration in their reference
intervals. In addition, MANY things besides age can make T4 go down.

**Case Summary:** I would not recommend further treatment of this dog without clinical signs, regardless of
the T4 measurement.

**Measurement of Thyroid Auto-Antibodies**

Except in the evaluation of breeding dogs, measurement of thyroid auto-antibodies does not add
much to evaluation of dogs for possible hypothyroidism. If a hypothyroid dog has auto-antibodies then it
can be determined that the underlying etiology is lymphocytic thyroiditis as compared to idiopathic
hypothyroidism. However, the management of the hypothyroidism does not differ.

In general, the clinical and prognostic significance of autoantibodies is unknown. If autoantibodies
are suspected, measure fT4 for the best assessment of function. If the fT4 concentration is normal,
thyroid function is normal at that time but the patient should be re-evaluated periodically (e.g. q. 3 mths) for development of hypothyroidism. If fT4 is low, the dog is likely hypothyroid. One study followed 234 dogs with normal T4 and TSH levels and elevated anti-thyroglobulin antibodies (TGAA) for 1 year. Only 19% developed clinical signs of hypothyroidism or consistent laboratory values. Another 57% remained TGAA positive without signs or laboratory evidence of hypothyroidism, 8% went from positive to borderline results and 15% became TGAA negative. The final outcome of all the dogs is unknown (i.e. how many would become hypothyroid if followed for more than one year), but it can be said that not all dogs with autoantibodies will become hypothyroid, as at least 15% do not.

References available from author upon request.