Endogenous TSH in the diagnosis of hypothyroidism in dogs

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Summary

To determine whether measurement of canine thyrotropin (cTSH) would aid in the diagnosis of hypothyroidism, serum samples of 65 dogs with clinical signs suggestive of hypothyroidism were evaluated. Diagnosis was confirmed in 26 dogs and excluded in 39 dogs based on TSH-stimulation testing. Total thyroxine (T_4) was significantly lower and cTSH significantly higher in hypothyroid dogs compared to euthyroid dogs. Canine TSH was above (> 0.6ng/ml) in 15 (57.7%) and below the upper limit of the reference range in 11 (42.3%) of the hypothyroid dogs. All of the euthyroid dogs had a cTSH < 0.6ng/ml. In all dogs with a cTSH above the upper limit of the reference range hypothyroidism could be confirmed. Therefore, our results show that measurement of cTSH has an excellent specificity (100%) and is a valuable tool in confirming canine hypothyroidism. However, due to the low sensitivity of cTSH assays (60%), it can not be recommended to exclude the disease.

Key words: thyrotropin, cTSH, thyroxine, hypothyroidism, dog

Untersuchungen zur Bedeutung des endogenen TSH in der Diagnostik der Hypothyreose beim Hund

Ziel der vorliegenden Studie war die Evaluation des caninen Thyreotropins (cTSH) in der Diagnostik der Hypothyreose beim Hund. Dazu wurde in Serumproben von 65 Hunden bei denen klinisch der Verdacht einer Hypothyreose bestand, cTSH bestimmt. Als Goldstandard diente der TSH-Stimulationstest unter Verwendung von bovinem TSH. Anhand dieses Tests konnte in 26 Hunden eine Hypothyreose bestätigt und in 39 Hunden ausgeschlossen werden. Das Gesamtthyroxin war bei den hypothyreoten Hunden signifikant tiefer und das cTSH signifikant höher als bei den Euthyreoten. In 15 (57.7%) der hypothyreoten Hunde war das cTSH über (> 0.6ng/ml) und in 11 (42.3%) unter der oberen Grenze des Referenzbereichs. Hingegen hatten alle Euthyreoten ein cTSH < 0.6ng/ml. In allen Hunden mit einem erhöhten cTSH konnte eine Hypothyreose bestätigt werden. Unsere Untersuchungen zeigten also eine hervorragende Spezifität des cTSHs (100%), daher können wir sagen, dass die Bestimmung des cTSH sehr gut geeignet ist, um eine Hypothyreose zu bestätigen. Allerdings kann aufgrund der niedrigen Sensitivität (60%) das cTSH nicht empfohlen werden, um die Erkrankung auszuschliessen.

Schlüsselwörter: Thyreotropin, cTSH, Thyroxin, Hypothyreose, Hund

Introduction

Hypothyroidism is a common endocrine disorder in dogs most often caused by idiopathic atrophy or immune-mediated destruction of the thyroid gland (Feldman and Nelson, 1996). Due to its non-specific clinical presentation the diagnosis can be challenging for the clinician. Although low serum total thyroxine (T_4) concentrations are intuitively suggestive of hypothyroidism, it must be considered that low T_4 levels are frequently encountered in euthyroid dogs with various non-thyroidal diseases and also as a side effect of certain pharmacological agents (Nelson et al., 1991; Miller et al., 1992; Gulikers and Panciera, 2002; Daminet and Ferguson, 2003).

The increase in T_4 after bovine TSH administration has been widely used to estimate the functional status of the thyroid gland. The TSH-stimulation test using bovine TSH is currently considered the gold standard for the discrimination of true hypothyroidism from other conditions with low T_4 concentrations in dogs (Panciera, 1999). Unfortunately bovine TSH is not licensed for use in dogs and is difficult and expensive

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to obtain. Furthermore, the test is associated with considerable inconvenience and costs since it requires hospitalisation of the animal and the collection of at least two blood samples.

Determination of TSH levels in the circulation, which are expected to be increased during states of low functional T_4, has become standard practice for the initial assessment of human patients with suspected thyroid insufficiency (Ladenson, 1996). Within the last few years, diagnostic test kits for measuring canine TSH (cTSH) have become available in veterinary medicine. However, studies from different countries revealed varying sensitivity and specificity of these assays leading to controversial opinions concerning their clinical value as a routine test for the assessment of thyroid function (Peterson et al., 1997; Scott-Moncrieff et al., 1998; Dixon and Mooney, 1999).

The aim of the present study was to evaluate cTSH as a first line laboratory parameter for the diagnosis of hypothyroidism in a population of 65 dogs with suspected hypothyroidism in Switzerland.

Animals, Materials and Methods

Case material and sample collection

The case material comprised a series of 65 dogs referred to the Clinic for Small Animal Internal Medicine of the University of Zurich between March 1996 and July 2002 for investigation of clinical signs consistent with hypothyroidism. Dogs undergoing both a TSH-stimulation test and determination of endogenous TSH were included in the study. TSH-stimulation test was performed by collecting blood samples (jugular venipuncture) immediately before and 6 hours after the intramuscular injection of bovine TSH (Thyrotropic Hormone, Sigma, St. Louis, MO) at a dose of 1 IU for dogs < 25 kg and 2 IU for dogs ≥ 25 kg body weight.

After clot retraction, serum was harvested by centrifugation and transferred to tubes for storage at –20° C for subsequent hormone assay.

Hormone assays

Total T_4 was measured by a commercially available radioimmunoassay (canine DPC radioimmunoassay, Coat-A-Count, Diagnostic Products Corp -DPC-, Los Angeles, CA). Serum cTSH concentrations were determined by a homologous solid-phase, two-site chemiluminescent enzyme immunometric assay (Immulite® canine TSH, DPC, Los Angeles, CA).

Case allocation

Dogs were classified as either euthyroid or hypothyroid based on the results of the TSH-response test. Hypothyroidism was defined as a post-TSH T_4 of less than 1.6 µg/dl or less than 1.5 times the basal concentration. Euthyroid was defined as a post-TSH T_4 > 2.5 µg/dl and at least 1.5 times the basal concentration. In dogs with a post-TSH T_4 between 1.6 and 2.5 and an increase of at least 1.5 times the basal concentration, additional criteria such as recovery from clinical signs without the need for thyroid hormone supplementation were applied.

Statistical analysis

Data were analysed using SPSS (Statistical Package for the Social Science, Software Package for Windows Version 11). Ranges and median values are given. Correlations were tested by Spearman Analysis. The Mann-Whitney-U-Test was used to determine differences between groups. Values of $P < 0.05$ were considered statistically significant.

Results

Case material

Hypothyroidism was confirmed in 26 dogs ranging from 2 to 14 years in age (median 7), comprising 10 female (7 neutered) and 16 male (6 neutered) dogs. Euthyroidism was confirmed in 39 dogs ranging from 1 to 12 years (median 6), comprising 22 female (11 neutered) and 17 male (7 neutered) dogs.
No adverse reactions to bovine TSH administration were recognized in any of the dogs.

Fig. 1

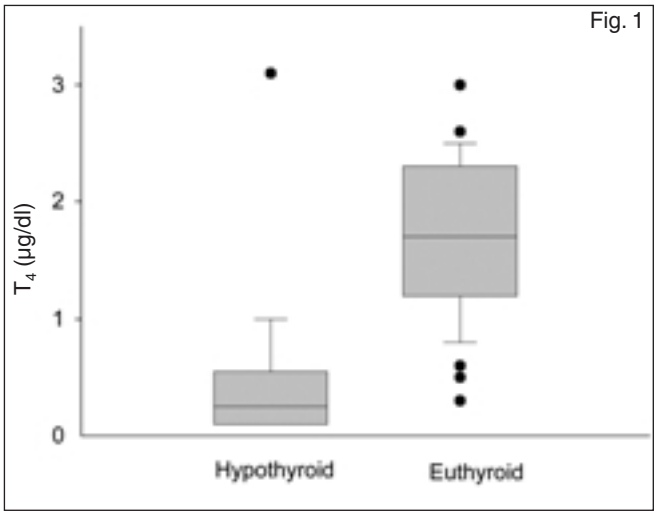

Figure 1: Box plots of serum T_4 concentrations in 26 hypothyroid and 39 euthyroid dogs. The whiskers represent the 25th and 75th percentile range with values outside this range being represented by a dot. Differences between the two groups were statistically significant; (P<0.01).

Fig. 2

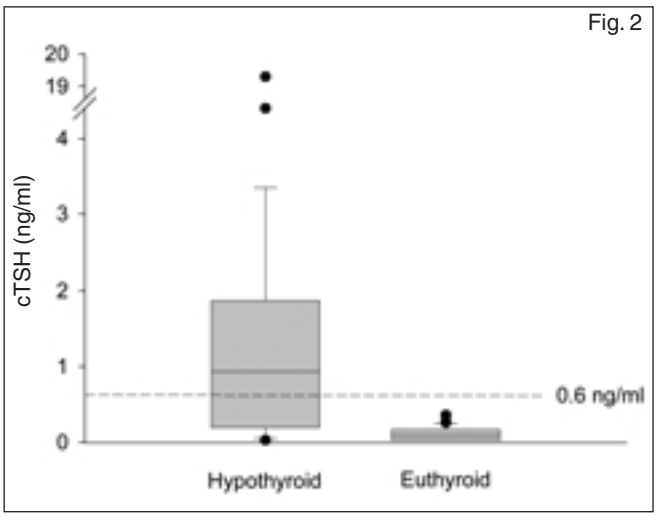

Figure 2: Box plots of serum cTSH in 26 hypothyroid and 39 euthyroid dogs. The upper limit of the reference range (0.06ng/ml) is indicated by a horizontal dashed line. Differences between the two groups were statistically significant; (P<0.01).

Fig. 3

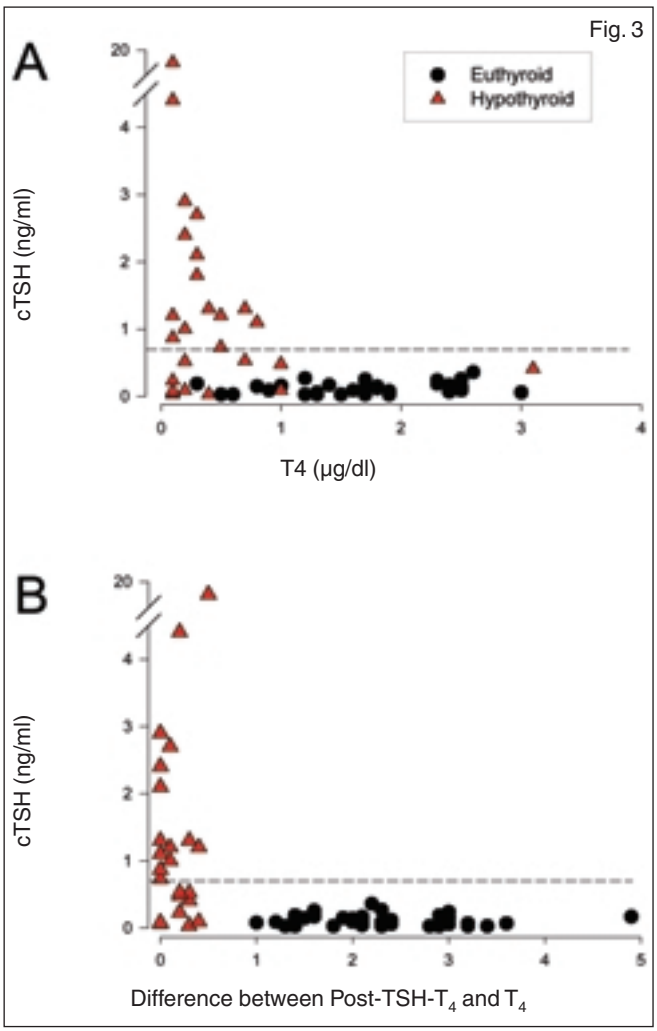

Figure: Scatterplot of serum T_4 and cTSH concentrations (A) in 26 hypothyroid (triangles) and 39 euthyroid dogs (circles). (coefficient of correlation: r= –0.34).
B: Scatterplot of the difference between post-TSH-T_4 and T_4 value and cTSH (B) in 26 hypothyroid and 39 euthyroid dogs (coefficient of correlation: r=0.5). The upper limit of the cTSH reference range (0.06ng/ml) is indicated by a horizontal dashed line.

Total T_4

Median basal T_4 concentration was significantly lower in hypothyroid (median 0.25 µg/dl, range 0.1–3.1) compared with euthyroid dogs (median 1.7 µg/dl, range 0.3–3.0) (Fig. 1).

Of the 26 hypothyroid dogs, 18 (69.2%) had T_4 concentrations <0.5 µg/dl; 5 dogs (19.3%) between 0.5 and 1 µg/dl and 3 dogs (11.5%) ≥1 µg/dl. One of the 39 euthyroid dogs had a T_4 concentration <0.5 µg/dl; 4 dogs (10.3%) between 0.5 and 1 µg/dl; 11 dogs (28.2%) ≥1.0 and ≤1.4 µg/dl and 23 (59%) ≥1.5 µg/dl.

Post-TSH T_4 values ranged from 0.1–3.4 µg/dl (median 0.35) in hypothyroid and from 1.7–7.3 µg/dl (median 4) in euthyroid dogs. Differences between the 2 groups were statistically significant (P <0.01).

One dog, which was referred because of episodical weakness, had a basal T_4 of 0.3 µg/dl and a post-TSH T_4 of 1.7 µg/dl; the signs resolved without supplementation of thyroxine. The low T_4 values of this dog were attributed to the fact that it was a Greyhound, a breed known for low T_4 concentrations, whereas cTSH concentrations seem to be similar in Greyhounds and Non-Greyhound breads (Gaughan and Bruyette, 2001).

cTSH

Median serum cTSH concentration was significantly higher in hypothyroid (median 0.94 ng/ml, range 0.03–19.3) compared with euthyroid dogs (median 0.09 ng/ml, range 0.03–0.36) (Fig. 2), P < 0.01. Canine TSH was above the upper range of the reference limit (> 0.6ng/ml) in 15 hypothyroid dogs (57.7%) and within the reference range in 11 hypothyroid dogs (42.3%), yielding a diagnostic sensitivity of 57.7%. All of the euthyroid dogs had a cTSH < 0.6 ng/ml, diagnostic specificity 100%. Using 0.4 ng/ml as upper limit of the reference range the sensitivity was 73% and the specificity remained unchanged 100%.

Correlation among hormone concentrations

When data for hypothyroid and euthyroid dogs were analysed, a significant negative correlation was found between T_4 and cTSH concentrations (r = –0.34; Fig. 3A) and between the difference of post-TSH T_4 and basal T_4 and the cTSH (r = 0.5, Fig. 3B).

Discussion

Determination of TSH concentration is routinely used in human medicine for the diagnosis of thyroid dys-

function (Ladenson, 1996; Ridgway, 1996; Ladenson et al., 2000). TSH is expected to be high as a consequence of the weakening negative thyroid-pituitary feedback exerted by the failing thyroid gland. In veterinary medicine only limited specificities of TSH for the diagnosis of canine hypothyroidism have been shown (Peterson et al., 1997; Scott-Moncrieff et al., 1998; Dixon and Mooney, 1999). Interestingly, and in contrast to these previous reports, the diagnostic specificity of cTSH determination was excellent in our study. None of the tested euthyroid dogs had elevated cTSH levels even if the upper limit of the reference range was lowered to 0.4 ng/ml. Several reasons may explain the different results between our study and the results of earlier investigations. Firstly, we did not evaluate severely ill dogs or animals with concurrent medications known to affect serum cTSH levels. And secondly, in our study the TSH- stimulation test was performed by intramuscular injection of TSH and the doses used were lower compared to earlier investigations (Peterson et al., 1997; Scott-Moncrieff et al., 1998; Dixon and Mooney, 1999). To our knowledge, the influence of the route of administration and the dose of TSH, has not been investigated systematically. We hypothesize, that the dose of TSH used may affect the diagnostic accuracy of the stimulation test considerably. Higher doses of injected TSH may lead to an overestimation of the functional reserve of the failing thyroid gland and thus yield false negative results in a significant fraction of animals with hypothyroidism and elevated TSH levels. The term of subclinical hypothyroidism has been introduced in human medicine to describe a condition of mild or even absent clinical signs of thyroid dysfunction, normal T_4 concentration but consistently elevated levels of endogenous TSH (Ladenson et al., 2000). A markedly reduced functional reserve of the thyroid gland is suspected in these cases. However, it is important to appreciate that not every mild elevation of TSH is consistent with reduced functional capacity of the thyroid. Other causes of elevated TSH and normal levels of T_4 that must be considered in the differential diagnosis, including recovery from severe non thyroidal illness and the influence of various medications for example long term administration (more than 6 months) of phenobarbital to dogs has consistently decreased T_4 and led to an increased TSH (Gaskill et al., 1999; Muller et al., 2000). In many of these conditions, the TSH elevation is only transient. Increased TSH concentrations are also described as an artefact due to circulating heterophilic antibodies against TSH (Ward et al., 1997; Ismail et al., 2002) or mutations causing inactivation of the TSH receptor (Simanainen et al., 1999). The latter two possibilities have not been described in dogs so far. Recovery from non thyroidal disease and administration of sulphonamide result in increased cTSH levels in otherwise euthyroid dogs (Dixon and Mooney, 1999; Williamson et al., 2002). Thus, it seems prudent to restrict application of the cTSH-assay to animals without history of severe illness or medication known to influence thyroid function. In dogs with an unknown history, mild clinical signs of hypothyroidism and elevated cTSH, the test should be repeated at a later time point after exclusion of confounding conditions. If TSH remains elevated consistently, mild or even subclinical hypothyroidism should be considered.

In our study the sensitivity of cTSH was approximately 60% if a reference limit of cTSH was set at 0.6 ng/ml, as suggested by the manufacturer, and 73% if the upper limit of the reference range was lowered to 0.4 ng/ml. Similar results ranging from 63–87% were observed in other studies evaluating the value of cTSH in diagnosing canine hypothyroidism (Peterson et al., 1997; Scott-Moncrieff et al., 1998; Dixon and Mooney, 1999). Several reasons have been suggested to explain the inappropriately low cTSH concentrations in hypothyroid dogs. Significant circadian and pulsatile variations in hypothalamic TSH secretion has been demonstrated in euthyroid and hypothyroid humans (Greenspan et al., 1986; Adriaanse et al., 1992). This could be confirmed in a model of experimentally induced primary canine hypothyroidism (Kooistra et al., 2000) and in a total of 6 adult dogs with naturally developing hypothyroidism (Bruner et al., 1998). Thus it seems unlikely to be the major reason for the limited sensitivity of cTSH determination. Secondary hypothyroidism may be diagnosed in a few hypothyroid dogs with low TSH. However, pituitary failure is thought to occur only in about 5% of hypothyroid dogs (Feldman and Nelson, 1996) and other signs of endocrine dysfunction suggestive of pituitary disease are expected to lead the diagnosis in these cases. Glucocorticoids have been shown to suppress circulating TSH in both euthyroid and hypothyroid people (Werner and Platman, 1965; Chopra et al., 1975) and should thus be considered as a potential disruptive factor. However, dogs with hyperadrenocorticism and high endogenous glucocorticoid levels did not show altered cTSH concentrations although T_4 concentrations were low (Meij et al., 1997). Controlled studies evaluating the influence of exogenously administered glucocorticoids on cTSH in spontaneously occurring hypothyroid dogs have not yet been performed. Severe illness suppresses cTSH secretion in humans. None of our hypothyroid dogs with very low levels of cTSH showed signs of an underlying disease and none of these dogs had received medication during or up to 4 weeks before presentation; thus these factors seem an unlikely explanation for the low cTSH concentrations observed in our dogs. A commonly proposed explanation for the low sensitivity of cTSH is that the currently used cTSH assays fail to recognize all isoforms of the protein due to significant variations in their re-

spective antigenic sites. Although not definitely proven, this seems a likely reason for a significant proportion of false negative test results in our study.

Conclusions

Determination of cTSH may be an attractive alternative for the diagnosis of canine hypothyroidism. However, considerable experience and knowledge of potential confounding factors is a prerequisite for its use in practice. Due to the limited sensitivity of the currently available TSH assays normal TSH concentrations could not exclude the presence of hypothyroidism in our population. However, development of novel assays with a higher susceptibility to immunologically different cTSH isoforms might further increase the accuracy of cTSH determination.

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Specificità diagnosticata della tireotropina canina nell'ipotiroidismo del cani

Scopo del presente studio è la valutazione della tireotropina canina (cTSH) nella diagnosi dell'ipotiroidismo nel cane. Si è valutato il cTSH nei campioni serologici di 65 cani sospetti di essere clinicamente affetti da ipotiroidismo. Come standard ideale è stato usato il test di stimolazione del TSH usando TSH bovino. Sulla base di questo test si potuto constatare in 26 cani un ipotiroidismo e in 39 escluderlo. Comparativamente agli eutiroidei, nei cani affetti da ipotiroidismo la tirossina totale si presentava più bassa e il cTSH più alto. In 15 cani ipotiroidei (57,7%) il cTSH era maggiore e in 11 (42,3%) minore del limite massimo dei valori normali (> 0,6 ng/ml). Per contro tutti gli eutiroidei avevano un cTSH < 0,6 ng/ml. In tutti i cani con alto cTSH si è potuto confermare un ipotiroidismo. Sulla base della straordinaria specificità del cTSH (100%) dimostrata dalle analisi, si può affermare che la valutazione del cTSH è particolarmente idonea per confermare un ipotiroidismo. Tuttavia, a causa della bassa sensibilità (60%), non si può consigliare il cTSH per escludere la malattia.

Sensibilité diagnostique de la thyréotropine canine dans le diagnostic de l'hypothyroïdie du chien

Le but de la présente étude est l'évaluation de la thyréotropine canine (cTSH) dans le diagnostic de l'hypothyroïdie du chien. La cTSH a été mesurée dans des échantillons de sérums provenant de 65 chiens présentant une suspicion d'hypothyroïdie. Le test de stimulation à la TSH, utilisant une TSH bovine, a été utilisé comme référence. Sur la base de ces analyses, une hypothyroïdie a pu être confirmée chez 26 chiens et exclue chez 39 autres. La thyroxine totale était significativement plus basse chez les chiens hypothyréotes et la cTSH significativement plus haute que chez les euthyréotes. Chez 15 chiens (57,7%) hypothyréotes la cTSH dépassait la valeur de référence (> 0,6 ng/ml) et chez 11 d'entre eux (42,3%) elle se situait au-dessous de la limite supérieure de référence. Tous les chiens euthyréotes avait par contre une cTSH < 0,6 ng/ml. Chez tous les chiens présentant une cTSH augmentée, une hypothyroïdie a pu être confirmée. Nos recherches montrent donc une remarquable sensibilité de la cTSH (100%) ce qui nous permet d'affirmer que sa détermination est très utile pour confirmer une hypothyroïdie. Par contre, au vu de sa faible sensitivité (60%) la cTSH ne peut pas être recommandée pour exclure cette affection.

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