

Parallel Determination of Total Thyroxine and Thyrotropin Concentrations in Diagnosis of Primary Hypothyroidism in the Dog

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Abstract:

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The objective of the work was to verify the validity of parallel determination of total thyroxine (tT₄) and thyrotropin (cTSH) concentrations in the diagnosis of primary hypothyroidism in the dog. The concentrations of total thyroxine and thyrotropin were determined by chemiluminescence immunoassay in the serum of a total of 117 dogs: 40 negative controls, 66 dogs with euthyroid sick syndrome and 11 patients with primary hypothyroidism. The average tT₄ concentration in the group of healthy dogs was 25.8 ± 5.48 nmol/l and the average cTSH concentration was 0.17 ± 0.13 ng/ml. In the group of patients with primary hypothyroidism the tT₄ concentrations were significantly decreased in 10 out of 11 cases (10.12 ± 2.01 nmol/l), while the cTSH concentrations were significantly increased (2.9 ± 2.04 ng/ml). In one patient with primary hypothyroidism, only the tT₄ concentration was decreased to 9.0 nmol/l, but the cTSH concentration was found to be within the reference range (0.04 ng/ml). The average tT₄ concentration in the group of patients with euthyroid sick syndrome was 11.7 ± 3.16 nmol/l, while the average cTSH concentration in the same group of patients was 0.14 ± 0.12 ng/ml. The concentrations of the two hormones mentioned above were compared between the different groups using the non-parametric Mann-Whitney test. The concentration of tT₄ was significantly higher ($p < 0.01$) in the group of healthy dogs compared to the dogs with primary hypothyroidism and the dogs with euthyroid sick syndrome. The concentration of cTSH was significantly higher ($p < 0.01$) in the dogs with primary hypothyroidism compared to the healthy dogs and the dogs with euthyroid sick syndrome. The results of this work show that parallel determination of tT₄ and cTSH represents a highly sensitive and specific method which is suitable for differentiating between primary hypothyroidism and other diseases that significantly reduce tT₄ concentrations.

Canine TSH, tT₄, euthyroid sick syndrome

Reduced thyroid function – hypothyroidism – is a disease which may show variable etiology and pathogenesis. Hypothyroidism is always manifested with a lack of thyroid hormones in the organism (Feldman 1996; Němec and Zamrazil 1997). Hypothyroidism in the dog is one of the most frequent diseases of the endocrine system. In 95% of cases primary hypothyroidism is found. The most frequent cause is autoimmune reaction. Hypothyroidism occurs as a result of lymphocyte infiltration into the tissue of the thyroid gland and its subsequent destruction (Rijnberk 1996; Svoboda et al. 1998).

Secondary (pituitary) hypothyroidism occurs in less than 5% of cases (Feldman 1996; Day and Shaw 1999). Secondary hypothyroidism may be caused by an anterior pituitary tumour. It also occurs after hypophysectomy or as a result of suppression of the function of thyrotropic cells caused by therapy with corticosteroids.

The clinical symptoms of the disease are variable and practically the whole organism may be affected (Feldman 1996; Svoboda et al. 1998). The non-specific signs include apathy, increased fatigue and reluctance to exercise. Besides that, the following systems are most

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frequently affected: the skin (symmetric non-pruritic alopecia), the cardiovascular system (bradycardia), the gastrointestinal system (chronic constipation), the nervous system (lethargy, vestibular ataxia, paralysis of the facial nerve) and the reproductive system (irregular sexual cycle, loss of libido).

Exact diagnosis of the disease is very difficult. The TSH stimulation test is most specific. Using this test it is possible to differentiate between low total thyroxine concentrations caused by medication (e.g. corticosteroids and sulphonamides) or certain chronic diseases and primary hypothyroidism (Belshaw and Rijnberk 1979). However, this test cannot be used in practice under local conditions. There are two reasons: (1) the unavailability of bovine TSH on the European market, and (2) relatively frequent anaphylactic reactions after its administration.

For the reasons mentioned above, in some European countries parallel determination of tT₄ and cTSH concentrations (Dixon et al. 1996; Peterson et al. 1997; Kooistra et al. 2000) is used for the diagnosis of primary hypothyroidism in the dog. However, some studies suggest that up to one quarter of the dogs with primary hypothyroidism show cTSH concentrations within the reference range (Jensen et al. 1996; Peterson et al. 1997; Ramsey et al. 1997; Scott-Moncrieff et al. 1998; Dixon and Mooney 1999; Kooistra et al. 2000). So far no studies have been published under local conditions evaluating the diagnostic value of this approach. This is most probably due mainly to the unavailability on the local market of the species-specific kit containing monoclonal antibodies against canine TSH.

The present work contains the results obtained from parallel determination of tT₄ and cTSH in three groups: (1) healthy dogs, (2) dogs with primary hypothyroidism, and (3) dogs with euthyroid sick syndrome.

Materials and Methods

The investigation was carried out in 117 dogs. Of this number, 40 dogs were clinically healthy, 11 suffered from hypothyroidism, and 66 had euthyroid sick syndrome. The group of healthy dogs included individuals that did not show any deviations from the physiological standard detectable by physical and basic laboratory examinations (blood count, biochemical examination of blood, physical and biochemical examination of urine). The age of the dogs in this group varied between 7 months and 12 years. The group included dogs of various breeds, the most frequent being German shepherds (n = 18) and beagles (n = 8).

The group of dogs with euthyroid sick syndrome included patients under long-term corticosteroid treatment (n = 10) and with the following diseases: hypersensitivity and pyoderma of various etiology (n = 11), hyperadrenocorticism (n = 10), peripheral neuropathy (n = 10), congestive heart failure (n = 10), lymphosarcoma (n = 6), chronic renal failure (n = 5) and diabetic ketoacidosis (n = 4). Serum samples were collected in this group at the stage of developed symptoms prior to starting the therapy.

The patients with hypothyroidism were selected on the basis of clinical symptoms and laboratory findings (including hormone profiles) and on the basis of positive response to treatment with L-thyroxin. For each dog in the group the following examinations were carried out: blood count, biochemical examination of blood, physical and biochemical examination of urine, and determination of tT₄ and cTSH concentrations. Blood samples were always collected in the morning around 11 a.m. after twelve hours of fast. Concentrations below 13.0 nmol/l were considered to be a significant decrease in tT₄ (Svoboda et al. 1998). Concentrations above 1.1 ng/ml were considered to be a significant increase in tT₄ (Kooistra et al. 2000). During the period of testing no dog received substitute treatment with L-thyroxin. In cases of patients that had already started to receive medication with thyroid hormones, an agreement to interrupt the treatment for a period of one month was made with the owners. The TSH stimulation test was not carried out owing to the unavailability of bovine TSH. In total, 36 dogs with suspected hypothyroidism were examined. In 25 dogs the diagnosis was not confirmed and another disease was found.

Skin biopsy was carried out in appropriate cases of patients with dermatological disorders. In the patients with lymphosarcoma, fine needle aspiration biopsy of lymph nodes and ultrasonography were carried out. In the patients with cardiovascular symptoms, radiography of the chest was carried out in two aspects, together with electrocardiography, peripheral blood pressure measurement by oscillometry, and ultrasonography of the heart. In the patients with Cushing's syndrome, dynamic functional tests were carried out in order to confirm the diagnosis (the basal C/C ratio in urine was determined together with the C/C ratio after suppression with a high dose of dexamethasone). Ultrasonography of the abdominal cavity was also carried out. Neurological examination was carried out in the patients with neurological symptoms. Cerebrospinal fluid was also examined where appropriate.

An automatic Cobas Mira S analyser was used for the biochemical examinations. Thyroxine and thyrotropin were determined in the serum by chemiluminiscent immunoassay. TSH concentrations in the serum were determined according to the instructions of the kit manufacturer using homologous solid-phase two-site chemiluminiscent immunoassay (Immulite® canine TSH, Diagnostic Products Corporation (DPC), Los Angeles, USA). The intra-assay coefficients of variation were 9.1%, 7.7% and 3.2% for TSH values of 0.11 ng/ml, 0.26 ng/ml and 3.4 ng/ml, respectively. The interassay coefficients of variation were 22%, 15% and 11% for TSH values of 0.09 ng/ml, 0.2 ng/ml and 3.5 ng/ml, respectively. The lowest detectable TSH concentration was 0.03 ng/ml. The TSH values determined by canine-specific Immulite® assay after repeated serial dilution in 4 samples showed good linearity throughout the calibration range, which was up to 12 ng/ml. Cross-reactivity with FSH and LH was negligible.

Serial tT₄ concentrations were determined by chemiluminiscent enzyme assay (Immulite®, Total T₄, Diagnostic Products Corporation (DPC), Los Angeles, USA) according to the instructions of the manufacturer. The intra-assay coefficients of variation were 8.4%, 6.7% and 6.3% for thyroxine values of 48.9 nmol/l, 114.54 nmol/l and 167.34 nmol/l, respectively. The lowest detectable thyroxine concentration was 5 nmol/l. The total thyroxine values determined by Immulite®, Total T₄ after repeated serial dilution in 3 samples showed good linearity throughout the calibration range.

The results of the separate determinations of the two hormones were compared between the groups using the non-parametric Mann-Whitney test. UNISTAT 5.1 software was used for this evaluation.

Results

In total, 117 parallel determinations of tT₄ and cTSH concentrations were carried out. In the group of healthy dogs, the tT₄ concentration ranged between 18.0 and 39.0 nmol/l. The average concentration of tT₄ was 25.8 ± 5.48 nmol/l and the median value was 25.5 nmol/l. The concentration of cTSH in the healthy dogs varied between 0.03 and 0.69 ng/ml. The average concentration was 0.17 ± 0.13 ng/ml and the median value was 0.13 ng/ml. The concentrations of both hormones in the different groups are shown in Figures 1 and 2. As regards the breeds, significantly higher tT₄ concentrations were noted in small breeds of dogs compared to medium-sized and large breeds. There were no differences in cTSH concentrations between the breeds, nor were there any statistically significant differences in the concentrations of the two hormones between the sexes.

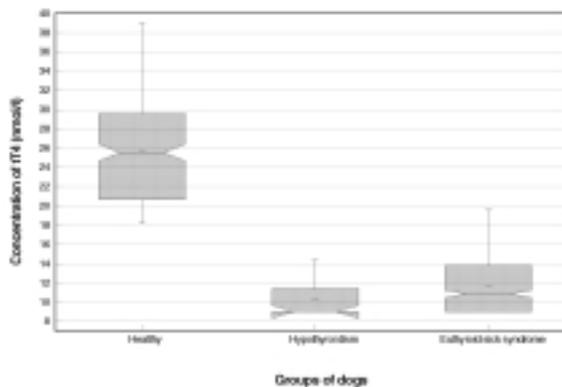


Fig. 1. Concentration of tT₄ in dogs.

In the group of patients with euthyroid sick syndrome, the concentration of tT₄ ranged between 9.0 and 19.6 nmol/l. The average tT₄ concentration was 11.7 ± 3.16 nmol/l and the median value was 10.8 nmol/l. The concentrations of cTSH varied between 0.03 and 0.68 ng/ml. The average value was 0.14 ± 0.12 ng/ml and the median value was 0.11 ng/ml. The differences in the concentrations of the two hormones in each subgroup of patients with euthyroid sick syndrome are shown in Table 1.

In the patients with primary hypothyroidism the concentrations of tT₄ ranged between 9.0 and 14.4 nmol/l. The average value was 10.12 ± 2.01 nmol/l and the median value was 9.0 nmol/l. The concentrations of cTSH were significantly elevated in ten cases out of eleven, ranging between 1.0 and 7.7 ng/ml. The average concentration was 2.9 ± 2.04 ng/ml, with a median value of 2.17 ng/ml. In one patient the TSH concentration was within the reference range of 0.04 ng/ml. The scope of the clinical and laboratory findings in the patients with primary hypothyroidism was as follows: apathy (n = 9), reduced stress tolerance (n = 8),

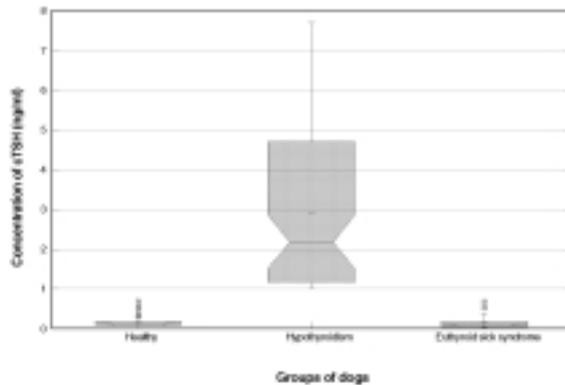


Fig. 2. Concentration of TSH in dogs.

hormones were compared between the different groups using the Mann-Whitney test, with the following results: (1) very significantly higher ($p < 0.01$) tT_4 concentrations in the group of healthy dogs compared to the dogs with primary hypothyroidism and euthyroid sick syndrome; (2) very significantly higher ($p < 0.01$) cTSH concentrations in the dogs with primary hypothyroidism compared to the healthy dogs and the dogs with euthyroid sick syndrome. No significant differences in tT_4 concentrations were found between the dogs with primary hypothyroidism and euthyroid sick syndrome. Nor did the cTSH concentrations show any significant differences between the healthy dogs and the dogs with euthyroid sick syndrome.

Discussion

Primary hypothyroidism is a disease whose diagnosis depends largely on the synthesis of clinical symptom findings, laboratory examinations and results of hormone production analysis. The diagnosis usually poses no problems in dogs with typical clinical symptoms and laboratory findings (Feldmann 1996). However, in cases where only some of the symptoms are manifested the diagnosis is difficult.

Although the TSH stimulation test (Belshaw and Rijnberk 1979) certainly remains the „gold standard“ in the diagnosis of primary hypothyroidism in veterinary medicine, other methods are frequently used owing to the commercial unavailability of bovine TSH. The other diagnostic methods are as follows: (1) determination of antibodies against thyroglobulin (Tg) or antibodies against tetraiodothyronine – T_4 and triiodothyronine – T_3 ; (2) thyroid biopsy; (3) parallel determination of T_4 and cTSH. Determination of antibodies is neither very sensitive nor specific. Antibodies against thyroglobulin occur only in 39 to 59% of dogs with primary hypothyroidism, but have been found in up to 14% of dogs with other cutaneous disorders and up to 43% of dogs with other endocrine disorders (Vollset and Larsen 1987). The main disadvantages of thyroid biopsy are as follows: (1) its invasiveness, i.e. the need for surgical intervention under general anaesthesia; (2) the result may not always correspond to the actual functional status of the thyroid gland (Feldman 1996). Of the methods mentioned above, parallel determination of tT_4 and cTSH concentrations is considered the best after the TSH stimulation test (Dixon et al. 1996; Peterson et al. 1997; Ramsey et al. 1997; Scott-Moncrieff et al. 1998; Dixon and Mooney 1999).

In this work we monitored tT_4 and cTSH concentrations in three groups: healthy dogs, dogs with euthyroid sick syndrome and dogs with primary hypothyroidism. We used the cTSH kit manufactured by Diagnostic Products Corporation. In the healthy dogs in our study

obesity ($n = 5$), cutaneous manifestations ($n = 9$) – symmetric alopecia ($n = 4$), affected hair ($n = 4$), hyperpigmentation ($n = 3$). Three patients manifested cardiovascular symptoms. Neurological symptoms were found in a single patient only. Sexual disorders were detected in four patients. The laboratory findings included mild aregenerative anaemia in 8 patients, hypercholesterolaemia in 9 patients and hypertriglyceridaemia in 6 patients.

The concentrations of the two

Table 1
Concentrations tT_4 and cTSH in the patients with euthyroid sick syndrome

Patients with euthyroid sick syndrome	median of tT_4 (nmol/l)	mean value of tT_4 \pm stand. deviation	median of cTSH (ng/ml)	mean value of cTSH \pm stand. deviation
Cushing's syndrome (n = 10)	9.00	10.37 \pm 2.22	0.05	0.08 \pm 0.05
Diabetes mellitus – ketoacidosis (n = 4)	9.00	10.70 \pm 2.90	0.05	0.08 \pm 0.07
Lymphosarcoma (n = 6)	15.70	15.00 \pm 4.40	0.10	0.13 \pm 0.12
Corticosteroid therapy (n = 10)	9.80	10.60 \pm 1.69	0.15	0.20 \pm 0.08
Chronic renal failure (n = 5)	9.30	9.66 \pm 0.72	0.09	0.13 \pm 0.10
Hypersensitivity. pyoderma (n = 10)	13.75	13.49 \pm 2.04	0.09	0.11 \pm 0.07
Congestive heart failure (n = 10)	9.00	9.90 \pm 1.50	0.09	0.13 \pm 0.10
Peripheral neuropathy	15.40	13.90 \pm 3.40	0.15	0.20 \pm 0.19

the concentration of cTSH ranged between 0.03 and 0.69 ng/ml. Kooistra et al. (2000) reported a reference range of 0.03 to 0.6 ng/ml for the same kit. Ramsey (1997) reported a reference range for cTSH concentrations between 0 and 0.41 ng/ml. Jensen et al. (1996) reported even lower reference range values in healthy dogs (0.06 – 0.34 ng/ml).

In the group of dogs with primary hypothyroidism, in ten cases out of eleven we observed a significant increase in the cTSH concentrations, which ranged between 1.0 and 7.7 ng/ml. In one patient the concentration of cTSH was within the reference range. Kooistra et al. (2000) using the same kit demonstrated cTSH concentrations in the range of 1.1 to 5.7 ng/ml in beagles with iatrogenic primary hypothyroidism. Ramsey et al. (1997) reported in their work that in six out of nine cases of dogs with confirmed primary hypothyroidism, the concentrations of cTSH found were above the upper limit of the reference range. Peterson et al. (1997) found in dogs with primary hypothyroidism (n = 54) cTSH concentrations above the upper limit of the reference range in 41 cases. Scott-Moncrieff et al. (1998) reported cTSH concentrations above the upper limit of the reference range in 10 cases out of 16.

Another group of patients in which we monitored tT_4 and cTSH concentrations was patients with euthyroid sick syndrome. Low concentrations of tT_4 are found in dogs with systemic diseases, hyperadrenocorticism, diabetes mellitus (diabetic ketoacidosis) and some cutaneous and neurological diseases. In some cases the condition in such patients can mimic primary hypothyroidism. Diagnosis may be difficult if a TSH stimulation test or cTSH determination is not possible. There is a direct correlation between the severity of the disease and the degree of reduction of total thyroxine concentration (Feldman 1996). We included in our study patients with developed manifestation of the disease prior to the start of treatment. In the patients with euthyroid sick syndrome, the concentrations of cTSH in our study lay within the reference range in all cases. Ramsey et al. (1997) also observed the concentrations of cTSH in patients with euthyroid sick syndrome. Of sixteen dogs with this condition they demonstrated cTSH concentrations exceeding the upper limit of the

reference range in five cases. Feldman (1996) also reported that in some patients with euthyroid sick syndrome the concentrations of cTSH may occur above the upper limit of the reference range.

It can be concluded from the above results that cTSH determination specificity is high. Peterson et al. (1997) reported a value of specificity of 0.93 and Scott-Moncrieff et al. (1998) a value of 0.88. On the other hand, the sensitivity of cTSH determination is rather low. Peterson et al. (1997) reported a value of sensitivity of 0.76 and Scott-Moncrieff et al. (1998) a value of 0.63. Furthermore, Peterson et al. (1997) and Scott-Moncrieff et al. (1998) also reported that the determination of tT_4 concentration showed higher sensitivity and low specificity in comparison with cTSH. The results from our study were in agreement with this finding. Both sensitivity and specificity are significantly increased by combined determination of the two hormones (Scott-Moncrieff et al. 1997).

Therefore, parallel determination of tT_4 and cTSH can be considered a sensitive and specific diagnostic method. Using this method it is possible to differentiate particularly well between primary hypothyroidism and other diseases that are also accompanied by significant decreases in tT_4 concentration and that in some cases can mimic this condition. If the TSH stimulation test is not available, this method can be used as an alternative.

Využití paralelního stanovení koncentrace celkového tyroxinu a koncentrace tyreotropního hormonu v diagnostice primární hypotyreózy u psa

Cílem práce bylo ověření validity paralelního stanovení koncentrace celkového tyroxinu (tT_4) a koncentrace tyreotropního hormonu (cTSH) v diagnostice primární hypotyreózy u psa. Koncentrace celkového tyroxinu a koncentrace tyreotropního hormonu byla stanovena s využitím chemiluminiscenční imunoanalýzy v séru celkem u 117 psů: 40 negativních standardů, 66 jedinců s „eutyroidním sick syndromem“ a 11 pacientů s primární hypotyreózou. Ve skupině zdravých psů byla průměrná koncentrace tT_4 $25,8 \pm 5,48$ nmol/l; průměrná koncentrace cTSH měla hodnotu $0,17 \pm 0,13$ ng/ml. U skupiny pacientů s primární hypotyreózou byly v 10 případech z jedenácti koncentrace tT_4 signifikantně snižené ($10,12 \pm 2,01$ nmol/l), koncentrace cTSH byly signifikantně zvýšené ($2,9 \pm 2,04$ ng/ml). U jednoho pacienta s primární hypotyreózou byla snižena pouze koncentrace tT_4 9,0 nmol/l, ale koncentrace cTSH se pohybovala v rámci referenčního rozmezí 0,04 ng/ml. U skupiny pacientů s „eutyroidním sick syndromem“ byla průměrná koncentrace tT_4 $11,7 \pm 3,16$ nmol/l, průměrná koncentrace cTSH měla hodnotu $0,14 \pm 0,12$ ng/ml. Koncentrace obou uvedených hormonů byly mezi jednotlivými skupinami vzájemně porovnány neparametrickým Mann-Whitneyovým testem. Ukázalo se, že koncentrace tT_4 byla u skupiny zdravých psů statisticky vysoce významně vyšší ($p < 0,01$) než u psů s primární hypotyreózou a než u psů s „eutyroidním sick syndromem“. Koncentrace cTSH byla statisticky vysoce významně vyšší ($p < 0,01$) u psů s primární hypotyreózou ve srovnání se psy zdravými a s „eutyroidním sick syndromem“. Výsledky práce prokazují, že paralelní stanovení koncentrace tT_4 a cTSH je metodou s vysokou senzitivitou i specifitou, která je vhodná k odlišení primární hypotyreózy od ostatních onemocnění, která signifikantně snižují koncentrace tT_4 .

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