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Lack of correlation between clinical presentation and clinicopathological abnormalities in canine hypothyroidism

Francesco Lunetta, Med Vet

Gaia Carotenuto, Med Vet

Federico Fracassi*, Med Vet, PhD, Dipl. ECVIM-CA

Sara Corradini, Med Vet, PhD, Dipl. ECVIM-CA

Introduction: The diagnosis of canine hypothyroidism is often challenging and it relies on the combined interpretation of the clinical condition, clinicopathological data and hormonal tests. There are no previous studies in the literature that compare the severity of the clinical condition with the clinicopathological findings of this disease. A previous study involving a group of dogs with experimentally-induced-hypothyroidism showed a progressive reduction, over time, of thyrotropin serum concentration (cTSH)[1]. In the months and years following the induction of the disease these dogs developed overt clinical signs and clinicopathological alterations. It is therefore conceivable that dogs with spontaneous hypothyroidism with normal cTSH serum concentration may over time develop more severe clinical and pathological alterations. Aim of this study was to evaluate the correlation between the clinical severity of hypothyroid dogs and the clinicopathological findings in a population of dogs with spontaneous hypothyroidism.

Materials and Methods: The patients were divided into two groups based on the clinical score (dogs with few clinical signs vs. dogs with many clinical signs) and in two additional groups based on the cTSH serum concentration (within the reference interval vs. above the reference interval). The clinical score, the clinicopathological findings and the specific hormonal tests were then compared between the different groups.

Results: The results of the study did not show any significant difference between the different groups regarding clinicopathological findings, specific hormonal tests and the clinical score.

Discussion: Based on this study, dogs with hypothyroidism and a cTSH serum concentration within the reference range do not exhibit a more severe clinical condition.

Key words: Dog, euthyroid sick syndrome, rhTSH stimulation test.

Dipartimento di Scienze Mediche Veterinarie (DIMEVET), University of Bologna, 40064 Ozzano dell'Emilia (BO), Italy

*Corresponding Author (federico.fracassi@unibo.it)

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INTRODUCTION

Primary hypothyroidism is one of the most common canine endocrinopathies; in 95% of cases it is caused by the absent/reduced production of thyroid hormones secondary to lymphocytic thyroiditis or to idiopathic atrophy[2]. The most common clinical signs are reluctance to physical exercise, lethargy, weight gain and dermatological disorders[3,4]. With regard to blood chemistry findings, the alterations are not very specific; however, in dogs presenting with the typical clinical signs such alterations may support the suspicion of hypothyroidism. The most common clinicopathological alterations are non-regenerative normocytic normochromic anaemia, hypercholesterolemia and increased liver transaminases. The diagnostic protocol for the diagnosis of hypothyroidism should include the combined evaluation of clinical history, direct physical examination and blood chemistry tests, specifically the serum concentrations of cholesterol, triglycerides and transaminase activity, as well as hormonal investigations specific for thyroid function, i.e. total thyroxine (T_4), free thyroxine (fT_4) and canine thyrotropin (cTSH). To date, the gold standard techniques for the diagnosis of hypothyroidism are thyroid scintigraphy[5] and rhTSH[6] stimulation test. However, both techniques are only available in a limited number of reference centres and such tests are also quite expensive. In euthyroid patients with non-thyroid diseases a characteristic decrease in thyroid hormone serum concentration can be observed, the so-called euthyroid sick syndrome or non-thyroidal illness. In these subjects the correct diagnosis is difficult to establish and dogs with euthyroid sick syndrome are often mistakenly diagnosed as hypothyroid. About 70% of hypothyroid dogs have a cTSH serum concentration above the normal reference range while around 30% of dogs do not present such an alteration[7]. Hormonal profiles characterized by low thyroid hormone concentration (T_4 and/or fT_4) associated with normal cTSH concentration are obviously the most difficult to interpret. In such cases it is difficult to discriminate between hypothyroidism and euthyroid sick syndrome. A study in which hypothyroidism was experimentally induced in a group of dogs showed that in the months immediately following the induction such subjects presented a characteristic increase of cTSH plasma concentration. In the subsequent three years, despite not having been treated with levothyroxine, a progressive reduction in cTSH concentration was observed, until reaching values similar to those of euthyroid dogs[1]. It has therefore been hypothesized that in the presence of hypothyroidism the persistent stimulation of pituitary thyroid cells causes - via a negative feedback - a progressive desensitization of TRH pituitary receptors and a consequent decrease in cTSH secretion.

Around 30% of hypothyroid dogs have a cTSH serum concentration within the reference range: in such cases it is difficult to discriminate between hypothyroidism and euthyroid sick syndrome.

In addition, the initial cTSH stimulation apparently promotes the mitosis of pre-existing thyrotropic cells, resulting in a lower differentiation of stem cells and thus leading to the development of non-secretory thyrotropic cells[1].

Consequently, if this were true, hypothyroid subjects with normal cTSH who have been ill for longer, if left untreated, should have a more pronounced clinical and clinicopathological condition compared to hypothyroid dogs with high cTSH concentration.

MATERIALS AND METHODS

This retrospective study included dogs diagnosed with hypothyroidism examined at the University Veterinary Hospital of the Department of Veterinary Medical Sciences of the University of Bologna (DIMEVET) from 2006 to 2018. All the clinicopathological assessments were performed at the CLINLAB laboratory of DIMEVET. The diagnosis of hypothyroidism was made through the evaluation of the clinical history, direct physical examination, basic haematochemical alterations and hormone concentrations and was confirmed if one of the following conditions was met:

- $T_4 < 13$ nmol/L (reference range 13-51 nmol/L) and cTSH > 0.38 ng/ml (reference range 0.03-0.38 ng/ml);
- T_4 post rhTSH < 20 nmol/L or < 1.5 times the baseline T_4;
- Both of the above.

Dogs with manifest concomitant diseases (e.g., neoplasms, severe infections, Cushing's syndrome) that could have influenced the endocrine tests (euthyroid sick syndrome) were excluded from the study population.

The hormonal reference values were those established by the laboratory in which the analyses were performed. Reference values established in the literature were instead used for the rhTSH stimulation test[8].

A specific clinical score was devised in order to classify the severity of the clinical picture; a score was assigned to each clinical alteration found in the clinical history and physical examination. In particular, as specified in Table 1, a score of 1 was assigned to clinical changes compatible with hypothyroidism (lethargy, sensory blunting, asthenia, weight gain, intolerance to cold, body alopecia, haircoat changes, seborrhoea or pyoderma, behavioural changes, voice changes, bradycardia or arrhyth-

Table 1 - Main clinical signs in the group of 68 hypothyroid patients. The clinical signs "tail alopecia", "hyperpigmentation of the nose" and "facial myxoedema" were given a score of 2; the other clinical signs were given a score of 1

Signs/Symptoms	Score	Signs/Symptoms	Score
Lethargy	1	Bradycardia/arrhythmia	1
Sensory blunting	1	Constipation/diarrhoea	1
Asthenia	1	Megaoesophagus	1
Weight gain	1	Otitis externa	1
Cold Intolerance	1	Facial mixedema	2
Body alopecia	1	Peripheral neuropathy/myopathy	1
Tail alopecia	2	Peripheral vestibular syndrome	1
Altered coat / hyperkeratosis of the pads / hyperpigmentation of the body	1	Trigeminal paralysis	1
Hyperpigmentation of the nose	2	Seizures	1
Seborrhoea/ pyoderma	1	Mixedema/coma	1
Altered behaviour	1	Corneal lipid deposits	1
Voice change	1	Reproductive problems	1

mia, constipation or diarrhoea, megaoesophagus, external otitis, peripheral neuropathy or myopathy, peripheral vestibular syndrome, trigeminal paralysis, seizures, mixoedema, coma, corneal lipid deposits and reproductive problems); a score of 2 was instead assigned to clinical signs highly indicative of hypothyroidism (hyperpigmentation of the nose, alopecia of the tail, facial myxoedema). The clinical score consisted in the sum of the scores given to each patient.

The dogs were then divided into two groups:

1) a Low Clinical Score Group (LCS), with scores ≤ 5, i.e. the least symptomatic dogs;
2) a High Clinical Score Group (HCS), with scores > 5, i.e. the most symptomatic dogs.

The rhTSH stimulation test was performed by evaluating T_4 concentrations on a serum sample collected before and 6 hours after (post-stimulation T_4) the intravenous administration of 75 µg of rhTSH (Thyrogen®, Sanofi Genzyme, Cambridge).

All blood-chemical evaluations were performed at the reference laboratory using Siemens ADVIA® 2120 (Siemens Healthcare Diagnostic, Tarrytown, NY, USA), Olympus® AU400 (Olympus/Beckam Coulter, Brea, California, USA) and Beckman Coulter® AU480 (Olympus/Beckman Coulter, Brea, CA, USA) analysers, used in a timed sequence.

T_4 assays were performed at the reference laboratory using a solid phase extraction technique combined with a chemiluminescent enzymatic immunoassay (Immulite 2000®, Siemens Healthcare Diagnostic, Flanders, NJ, USA; Canine Total T_4, Diagnostic Products Corporation, Los Angeles, USA). The intra-assay coefficients of variation were 5.9% and 5.3% at T_4 concentrations of 8.75 and 146.7 nmol/L, respectively, as reported by the manufacturers of the analyser.

A clinical score was devised by assigning a score to each clinical alteration compatible with hypothyroidism; hypothyroid subjects were divided into a high clinical score group (HCS, with more evident and severe clinical signs) and a low clinical score group (LCS, with less evident and less severe clinical signs).

The cTSH serum concentration was performed at the reference laboratory using a solid phase extraction technique combined with a chemiluminescent enzyme immunoassay performed in two sites (Immulite 2000®, Siemens Healthcare Diagnostic, Flanders, NJ, USA and Canine TSH, Diagnostic Products Corporation, Los Angeles, USA). The intra-assay coefficients of variation were 5.0%, 4.7% and 5.7% at cTSH concentrations of 0.2, 2.35 and 6.1 ng/mL, respectively, as reported by the manufacturers of the analyser.

Two groups were created based on the cTSH serum concentration and on the values used by the reference laboratory:

1) cTSH above the reference range, if >0.38 ng/ml;
2) cTSH within the reference range, if 0.03-0.38 ng/ml.

The two groups were then compared in order to identify possible differences in terms of weight or age of the animals, clinical score, baseline haematochemical parameters, i.e. the haematocrit value (Hct), the serum concentration of cholesterol, triglycerides, total calcium and T_4, the serum activity of lactate dehydrogenase (LDH),

alanine aminotransferase (ALT), aspartate aminotransferase (AST) and alkaline phosphatase (ALP).
The same comparisons - with the addition of cTSH serum concentration - were also made dividing the subjects in two groups based on the clinical score (HCS and LCS).
The statistical analysis was performed using GraphPad Prism 5.0 commercial software. Data distribution was evaluated with the D'Agostino and Pearson test, which allows to distinguish the normal distribution variables expressed as a mean ± the standard deviation from the non-normal distribution variables expressed as a median (max-min).
For group comparisons the Student t test was used for parametric data and the Mann-Whitney test for non-parametric data.
The results were considered statistically significant for p values < 0.05.

An assessment was made of whether there were significant differences between hypothyroid patients with cTSH above and those with cTSH within the reference range in terms of signalment, clinical score, haematochemical alterations and hormone tests.

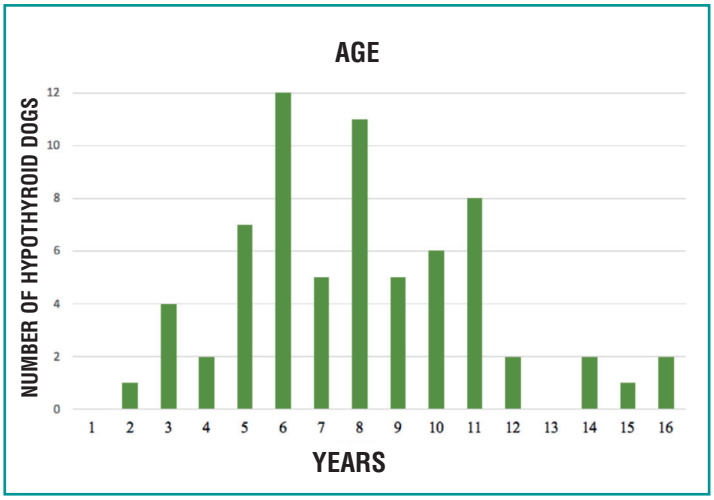

Figure 1 - Histogram of the age distribution of the hypothyroid subjects included in the study.

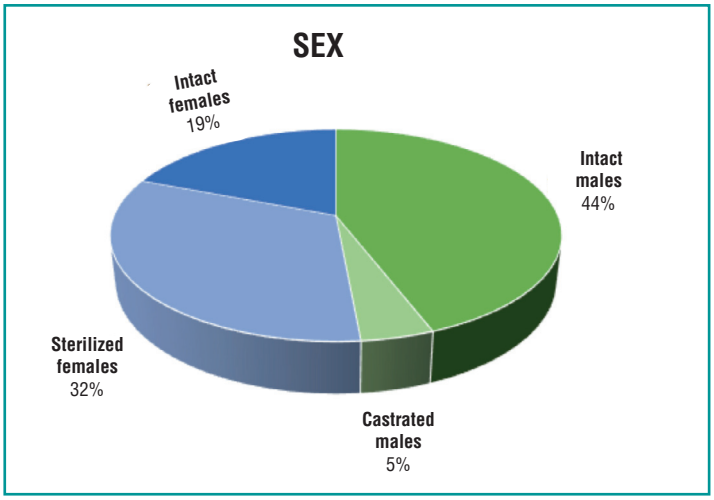

Figure 2 - Sex distribution of the hypothyroid dog population.

RESULTS

Sixty-eight (68) hypothyroid dogs were included in the present study. The mean age was 7.4 years (± 3.2) (Figure 1); 33 dogs (49%) were male (30 intact and 3 castrated) and 35 (51%) were female (13 intact and 22 sterilised) (Figure 2). The mean body weight was 29.1 kg (± 14.15). The dogs belonged to 28 different breeds, i.e. 21 mongrels, 5 German Shepherd, 4 Labrador Retriever, 4 English Setter, 3 Dobermann Pinscher, 3 Maremmano Abruzzese Shepherd Dogs, 3 Italian Short-haired Hound, 2 of each of the breeds Irish Setter, Beagle, Dogue de Bordeaux, Lagotto Romagnolo and 1 Rottweiler, Épagneul Breton, German Pinscher, Hovawart, Belgian Shepherd's Dog, Bull Terrier, Italian Cane Corso, Swiss White Shepherd's Dog, Medium Schnauzer, Argentinean Dogo, English Pointer, Deutscher Boxer, American Pit Bull Terrier, Tibetan Terrier, American Cocker, German Spitz, American Staffordshire Terrier.
Table 2 shows the blood-chemical alterations detected in the group of hypothyroid dogs at the time of diagnosis.
Serum T_4 concentrations were assessed in all 68 dogs; all the subjects presented a decreased concentration of the hormone with a median value of 6.4 nmol/L (6.4-8.8).
The cTSH serum concentration was assessed in all 68 dogs and the median value was of 0.77 ng/ml (0.03-11.9) (Figure 3). Seventy-two percent (72%) of the hypothyroid subjects presented an increased cTSH serum concentration.
Post-stimulation T_4 serum concentrations were assessed in 32/68 dogs (47%); all hypothyroid subjects presented a reduced concentration of the hormone with a median value of 6.4 nmol/L (6.4-16.2 nmol/L).
The clinical score was evaluated in 58/68 hypothyroid dogs; the score was not assessed in 10/68 dogs belonging to the group with increased serum cTSH as the medical records were not properly compiled at the time of diagnosis.
The main clinical signs found in hypothyroid dogs are reported in Table 3.
The mean clinical score for hypothyroid patients was 5.2 (± 2.2). Forty-one percent (41%) (24/58) of the patients presented a high clinical score (> 5) (HCS), while 59% (34/58) presented a low clinical score (≤ 5) (LCS) (Figure 4).
The results of the comparison between dogs with serum cTSH above the reference interval (n=50) vs. those with

Table 2 - Haematochemical test abnormalities in the group of hypothyroid dogs				
Haematobiochemical alterations	**Mean (± SD) o Median (minimum-maximum)**	**Reference range (unit of measurement)**	**Nr. of subjects for which the data was available**	**% of subjects with the abnormal results**
Non-regenerative normocytic normochromic anaemia	37.7% (± 5,6)	37-55%	60/68	48%
Hypercholesterolemia	564 mg/dl (219-1339)	140-350 mg/dl	59/68	95%
Hypertriglyceridemia	195 mg/dl (41-1231)	30-120 mg/dl	33/68	79%
Increased lactate dehydrogenase (LDH)	559 UI/L (± 460)	30-130 UI/L	13/68	100%
Increased aspartate aminotransferase (AST)	41.5 UI/L (20-202)	20-42 UI/L	60/68	48%
Increased alanine aminotransferase (ALT)	64 UI/L (10-491)	20-55 UI/L	61/68	54%
Increased alkaline phosphatase (ALP)	144.5 UI/L (13-1794)	42-180 UI/L	58/68	43%
Hypercalcemia	10.4 mg/dl (± 0.7)	9-11.8 mg/dl	56/68	0%

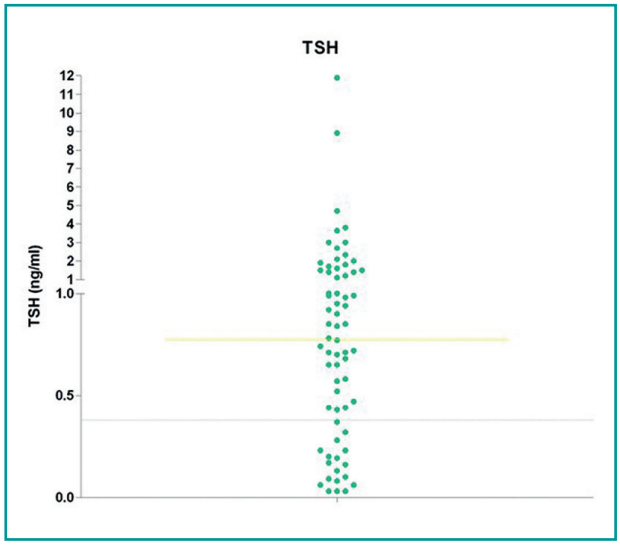

Figure 3 - Dot plot of the cTSH serum concentration measured in the population of 68 hypothyroid dogs. The horizontal yellow line represents the median concentration of the study group. The dashed horizontal line marks the upper limit of the reference range for this parameter.

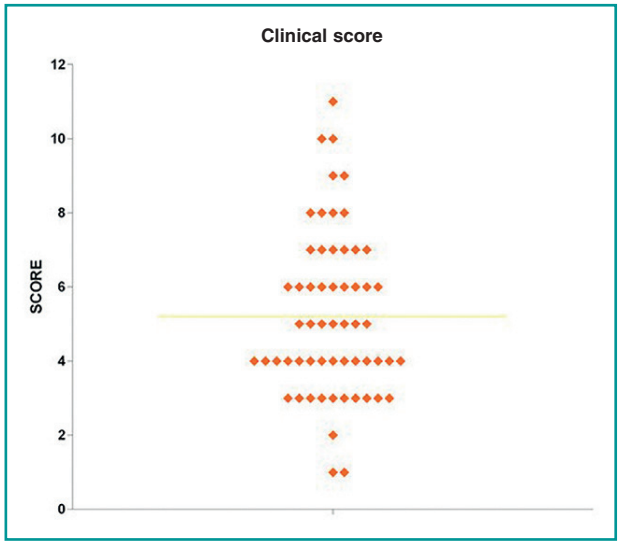

Figure 4 - Dot plot of the clinical score values calculated in the population of 58 hypothyroid dogs. The horizontal yellow line represents the median value of the study group.

cTSH within the reference interval (n=18) are shown in Table 4. No significant difference between the two groups was found.

No significant differences between the two HCS and LCS groups were found in terms of age, weight, haematochemical and hormonal abnormalities (Table 5).

DISCUSSION

With regard to breeds, mongrels made up the biggest group (30%), followed by the German Shepherd (7%), English Setter (6%), Labrador Retriever (5%), Italian Short-haired Hound (4%) and Maremmano Abruzzese Shepherd Dog (4%). This finding partially differs from what found in previous studies, in which a greater predisposition had been found in the Golden Retriever and Doberman Pinscher[7,9,10,11]. The different prevalence of hypothyroid dog breeds found in this study may be due to the different geographic location compared to previous studies.

The prevalence of clinical signs in the hypothyroid dogs was consistent with what is reported in the literature: 84% of patients showed dermatological signs indicative of hypothyroidism, 46% were overweight, 41% with lethargy, 15% with bradycardia and 5% of dogs presented neurological signs attributable to peripheral polyneuropathy[12]. Weakness (48%), peripheral polyneuropathy/my-

Table 3 - Main clinical signs and symptoms found in the group of 58 hypothyroid dogs

Apparatus/System	Signs/Symptoms
Metabolic	• Asthenia and reluctance to exercise (48%) • Weight gain (46%) • Lethargy (41%) • Cold Intolerance (33%) • Facial myxedema (14%) • Mixedema coma (3%)
Dermatocutaneous	• Symmetrical, non-itching alopecic areas on the body (55%) • Diffuse hyperpigmentation of trunk/abdomen, dandruffy skin, hyperkeratosis of plantar foot pads (53%) • Tail alopecia (40%) • Dorsal hyperpigmentation of the nose (9%) • Seborrhoea (2%) • Pyoderma (2%)
Neuromuscular	• Peripheral neuropathy/myopathy (26%) • Sensory blunting (21%) • Altered barking sound (17%) • Peripheral vestibular syndrome (5%) • Seizures (3%) • Behavioural changes (2%) • Decreased spinal reflexes (2%)
Cardiovascular	• Bradycardia (15%) • Arrhythmia (2%)
Reproductive	• Small testicles, prolonged anoestrus, galactorrhoea (12%)
Gastroenteric	• Constipation (5%) • Diarrhoea (2%)

opathy (26%) and reproductive system disorders (12%) were more frequently reported than in previous studies[12,13].

With regard to clinicopathological alterations, 48% of the dogs included in the study presented non-regenerative normocytic normochromic anaemia; the percentage is comparable to what is reported in previous studies[12,14]. Similarly to what reported in the literature, an increased serum concentration of cholesterol, triglycerides and enzymatic activity of AST, ALT and ALP[12,13] was reported.

A T_4 value below the reference range associated with an increased serum cTSH concentration was found in 72% of the patients included in the study: such prevalence is comparable to what is reported in previous studies, where the percentage of hypothyroid dogs with decreased T_4 serum concentration was 76% and 62%, respectively.

No differences were found between the HCS and LCS hypothyroid dogs with regard to age, weight, clinicopathological variables and T_4 and cTSH serum concentrations. A high clinical score does not therefore seem to correlate with haematochemical alterations indicative of hypothyroidism and neither with the results of spe-

No significant differences were found between the groups of dogs with cTSH serum concentrations above and those with serum concentrations within the reference range, as well as between the HCS and LCS dog groups.

Table 4 - Comparison of weight, age, clinical score, haematochemical and hormonal parameters indicative of hypothyroidism between the dog groups with cTSH above the reference range (>0.38 ng/ml) and within the reference range (0.03-0.38 ng/ml). Columns 2 and 3 show the mean (SD) or median (minimum and maximum) values of the various parameters. Column 3 shows the p value

Parameter	Elevated cTSH	Normal cTSH	*p*
Haematocrit	38.4% (± 5.74)	36.1% (± 5.2)	0.165
Cholesterol	552 mg/dl (± 229)	562 mg/dl (± 255)	0.890
Triglycerides	194 mg/dl (41 - 1231)	244 mg/dl (66 - 1032)	0.919
AST	42 UI/L (23 - 202)	37 UI/L (20 - 176)	0.393
ALT	54 UI/L (10 - 491)	69 UI/L (20 - 320)	0.182
ALP	118 UI/L (24 - 1794)	232 UI/L (13 - 1194)	0.452
Calcium	10.4 mg/dl (± 0.59)	10.26 mg/dl (± 0.89)	0.388
T_4	6.4 nmol/L (6.4 - 8.8)	6.4 nmol/L (6.4 - 6.5)	0.534
Clinical score	5.37 (± 2.3)	4.8 (± 1.9)	0.393
Post stimulation T_4	6.4 nmol/L (6.4 - 16.2)	6.4 nmol/L (6.4 - 10.9)	0.709
Weight	30.3 kg (± 13.7)	26.1 kg (± 15.2)	0.202
Age	7.4 years (± 3)	7.6 years (± 3.7)	0.820

Table 5 - Comparison of weight, age, haematochemical and hormonal parameters indicative of hypothyroidism between the high clinical score (HCS) and the low clinical score (LCS) hypothyroid dog groups. Columns 2 and 3 show the mean (SD) or median (minimum and maximum) values of the various parameters. Column 3 shows the p-value

Parameter	HCS hypothyroid dogs	LCS hypothyroid dogs	*p*
Haematocrit	36.4% (± 5.2)	38.1% (± 5.8)	0.260
Cholesterol	554 mg/dl (321 - 748)	565 mg/dl (219 - 1339)	0.538
Triglycerides	195 mg/dl (41 - 859)	218 mg/dl (47 - 1231)	0.674
LDH	647 UI/L (± 685)	523 UI/L (± 310)	0.697
AST	41 UI/L (27 - 133)	43 UI/L (20 - 202)	0.336
ALT	64 UI/L (10 - 491)	67 UI/L (18 - 275)	0.894
ALP	76 UI/L (13 - 551)	179 UI/L (39 - 1478)	0.069
Calcium	10.6 mg/dl (± 0.65)	10.2 mg/dl (± 0.68)	0.102
T_4	6.4 nmol/L (6.4 - 6.5)	6.4 nmol/L (6.4 - 8.8)	0.483
cTSH	0.695 ng/ml (0.06 - 8.9)	0.725 ng/ml (0.03 - 3)	0.608
Post stimulation T_4	6.4 nmol/L (6.4 - 6.5)	6.4 nmol/L (6.4 - 16.2)	0.324
Weight	29.2 kg (± 14.7)	29.1 kg (± 14.9)	0.980
Age	7 years (± 2.8)	7.8 years (± 3.6)	0.347

cific hormonal tests. The detection of clinical signs may still be useful as it may increase the suspicion of hypothyroidism in subjects with haematochemical alterations and hormone concentrations not particularly suggestive of this dysendocrinism.

Although a previous study has shown that hypothyroid dogs with cTSH concentrations within the reference range have probably been ill for longer compared to dogs with increased cTSH[1], the results of this study did not show any significant difference in the severity of the clinical condition between the two groups.

The study did not reveal significant differences in age, weight, haematochemical alterations and hormonal values (T_4) between the groups of hypothyroid dogs with cTSH serum concentrations above or within the reference range. The initial hypothesis, i.e. the presence of an inverse relationship between the extent of clinical symptoms and the cTSH serum concentration was therefore not confirmed.

We can therefore affirm that hypothyroid patients with cTSH concentrations within the reference interval do not present more evident clinicopathological alterations compared to subjects with serum cTSH above the reference interval.

CONCLUSIONS

The present study did not allow to establish a correlation between the extent of clinical symptoms and the results of clinicopathological variables suggestive of hypothyroidism.

KEY POINTS

- The differentiation between hypothyroidism and euthyroid sick syndrome is often difficult in the absence of specific tests such as scintigraphy and rhTSH stimulation tests, which are currently only available in specialized centres.
- Previous scientific studies have shown that hypothyroid dogs not undergoing hormonal treatment tend, over time, to normalize (decrease) cTSH serum levels.
- There is no evidence suggesting that the clinical condition of hypothyroid patients can be related to the cTSH serum concentration and therefore that patients with cTSH within the reference range have been hypothyroid for longer compared to those with cTSH above the reference range.

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