

Usefulness of measuring the concentration of thyroglobulin antibodies in serum of dogs for the assessment of thyroid functioning

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Abstract

The aim of the study was to estimate the usefulness of measuring the thyroglobulin antibodies (TgAb_s) concentration in dogs' serum in order to assess thyroid functioning. The study was performed on 383 dogs. The animals were divided into two groups: group A (n = 308) consisted of dogs with hypothyroidism and group B (n = 75) consisted of dogs with euthyrosis. TgAb_s was determined in both groups. The reaction to the TgAb_s in group A was strongly positive in 32% of dogs, weakly positive in 33% of dogs, and negative in 35% of dogs. The TgAb_s were observed in 32% of the dogs from group B, in which 8% of the animals had strongly positive reaction (++) and 24% - slightly positive (+). The correlation between the concentration of total and free fraction of the thyroxine and the level of the TgAb_s were observed in group A. The tendency to positive reaction to the antibodies (++) in dogs with lower concentrations of total thyroxine and free thyroxine was observed. It was noted that the presence of the TgAb_s was common in dogs with hypothyroidism. However, it could be also found in the animals with euthyrosis.

Key words: dogs, hypothyroidism, thyroglobulin antibodies.

Introduction

Comparison of statistical data of many studies on numerous dog populations indicates that hypothyroidism is the most frequent pathological state of endocrinological origin appearing in this species of animals (6, 15). Hypothyroidism can be classified, depending on the time of its appearance, as inborn or acquired disease, and according to the degree of the intensity of clinical symptoms – as clinically clear or sub-clinical. Primary hypothyroidism is caused by thyroid gland damage. It is estimated that even 95% of all cases of hypothyroidism in adult dogs are the result of the disease of this type. Lymphocytic thyroiditis and idiopathic atrophy of the thyroid are considered to be its main reasons. In cases of lymphocytic thyroiditis, the lymphocyte, plasmatic cell, and macrophage infiltrations in the tissue of the gland were observed. Antibodies against thyroid hormones, thyroglobulin, or microsomal cells of the gland can be found in the serum. Thyroglobulin antibodies (TgAb_s) significantly

prevail in dogs; they are found even in 60% of lymphocytic thyroiditis (3, 11, 15). The process is long, and damage of follicular cells and organ fibrosis are the effect of inflammation development. Defect of T lymphocyte-suppressors constitutes probably the basic reason of this disease. It was proven in humans, that *e.g.* viral thyroid infection may cause excessive production of γ -interferon by thyroid lymphocytes because of suppressor T lymphocytes (Ts) defect (3, 9, 11–13). Because of the influence of γ -interferon, the thyroid follicular cells begin to produce MHC class II antigens and expose them on their surface, whereas normally these antigens are present only on macrophages, some B lymphocytes, neutrophils, dendritic cells, and endothelium. Then, the presentation of the antigen of follicular thyroid cells to T lymphocytes takes place in MHC class II, what results in cellular and humoral response against the thyroid. Auto-antibodies against thyroid hormones (to a smaller degree), against thyroid peroxidase (in small degree), and against thyroglobulin (in significant

majority) are created (3, 9, 11–14, 29). Idiopathic atrophy may be also the cause of the atrophy of thyroid glandular cells, which results in the substitution of thyroid parenchyma with fatty tissue. Infiltration of inflammatory cells is not observed. The cause of this pathological condition still remains unknown. Some authors suggest that it may be the last stage or the effect of lymphocytic thyroiditis (3, 10). Both conditions result in a gradual diminishing of the glandular part of the thyroid, which in consequence may cause hypothyroidism. It is considered that the damage of at least 75% of glandular tissue results in clinical symptoms of hypothyroidism. The frequency of lymphocytic thyroiditis and idiopathic atrophy of the thyroid are similar. Therefore, it is assumed that each of these pathologies is responsible for 50% of the cases of primary hypothyroidism. The thyroid gland function is assessed by measurements of thyroid hormones concentration in serum or the assessment of the thyroid gland response to a induced stimulation (*e.g.* TSH stimulation test). It is believed that the presence of circulating antibodies against thyroid hormones is related to the presence of lymphocytic thyroiditis. Thyroid hormones are haptens and do not stimulate antibodies production *per se*. Thyroglobulin (Tg) excreted from the thyroid gland, as it is visible in lymphocytic thyroiditis, may serve as a protein transporter for thyroid hormones. As a result, an antigen complex protein-hormone is created. Tg contains large sub-units T4 and T3 which may provide antigen sites, which induce antibody formation (3, 11, 27). It has been noted that TgAb_s are present together with the antibodies against T3 and T4 hormones (3, 10, 12, 27). However, more often TgAb_s are present as single antibodies, and this kind of antibodies is most frequently found in lymphocytic thyroid inflammation (5, 7).

The aim of the study was to estimate the usefulness of measuring the concentration of TgAb_s in dogs' serum in order to assess thyroid functioning.

Material and Methods

Animals and study protocol. Three hundred and eighty-three dogs were qualified for the tests. There was a consent of the Local Ethical Commission for Experiments on Animals to perform the tests. The animals were divided into two groups: A and B. Group A consisted of 308 dogs of various breeds and sex, 2–12 years of age, which were affected with hypothyroidism. The diagnosis was confirmed by the analysis of clinical symptoms, results of morphological and biochemical blood tests, and the measurement of the concentration of hormones. Cases of other diseases of similar clinical picture were excluded from the study. Group B consisted of 75 dogs of various breeds, both sex, at the age from 2 to 7 years. The dogs were affected with euthyrosis. In this group, a detailed

medical history, clinical examination, results of morphological and biochemical tests, and concentration of hormones did not reveal any signs of hormonal and other systemic disorders.

The number of females was 150 in group A and 36 in group B, whereas the number of males was 158 in group A and 39 in group B. Among the animals, 60.8% were of four breeds: Golden Retriever 22.1%, Labrador retriever 18.2%, German shepherd 10.4%, crossbreed 10.1%, and 39.2% of dogs were of 29 different breeds.

Blood sampling and testing. The following parameters were estimated in haematological and biochemical tests: number of white blood cells (WBC), number of red blood cells (RBC), haematocrit (Hct, Ht), haemoglobin level (HGB, Hb), activity of alanine aminotransferase (ALT), aspartate aminotransferase (AST), alkaline phosphatase (ALP), and the levels of urea, creatinine, and cholesterol. Haematological tests were conducted using ABX Micros ABC Vet apparatus, and biochemical tests using Konelab Prime 30i analyser. In hormonal analysis, the concentration of total thyroxine (T4), free fraction thyroxine (fT4), and species specific thyrotropin (cTSH) were determined. The tests were conducted using radio-immunological or immune-radiometric methods (RIA or IRMA). The serum for hormonal tests was collected and frozen to -20°C for the period not longer than 7 d (according to the producer's recommendation, the samples of serum may be stored for the period of up to two months). Coat-A-Count Canine T4 (DPC Diagnostic, USA) was used to estimate the hormone concentration. FT4 RIA KIT (Immunotech) was used to estimate the concentration of fT4. Coat-A-Count Canine TSH IRMA (DPC Diagnostic, USA) was used to determine the thyrotropin concentration.

The following reference values were assumed: for T4 range - 20–50 nmol/L, for fT4 range - 8.2–25.4 pmol/L, and for cTSH range - 0–0.5 ng/mL. The reference values were based on the results published by the Orthopedic Foundation for Animal (http://www.offa.org/stats_thyroid.html).

In all dogs qualified for the tests, TgAb_s were detected with the use of Small Rapid ELISA Test-Thyroglobulin D3212-HR01 (EVL, the Netherlands). According to the producer's recommendation, the obtained test results were classified as negative (-), weakly positive (+), and positive (++)

Statistical analysis. The values obtained were analysed calculating the average, standard deviation, and percentage of values. In order to assess the difference between the groups, parametric tests (Student-*t* test) or non-parametric (U-Man-Whitney) were applied for the not connected variables. For the connected variables the parametric Student-*t* test was used if the distribution was found as normal and non-parametric test (Wilcoxon pair order test) was applied in other cases. The level of statistical significance was assessed for $P \leq 0.05$. Comparison of average concentration of T4 and fT4 to the level of TgAb_s was

conducted using ANOVA taking into account post-hoc Scheffe tests. Clinical tests and laboratory blood tests were carried out at the Department of Internal Medicine and Clinic of Diseases of Horses, Dogs and Cats, Wrocław University of Environmental and Life Sciences. The radio-immunological tests, immuno-radiometric tests, and immunological tests were performed at the Isotope Lab of the Department and Clinic of Cardiology, Wrocław Medical University.

Results

No statistically significant difference in the morphological and biochemical indices between groups A and B of dogs, except cholesterol concentration ($P < 0.005$) (Fig. 1) was demonstrated. Because of the volume of the data, the results of all assessments of morphological and biochemical parameters are not published, however, may be provided by the authors if requested.

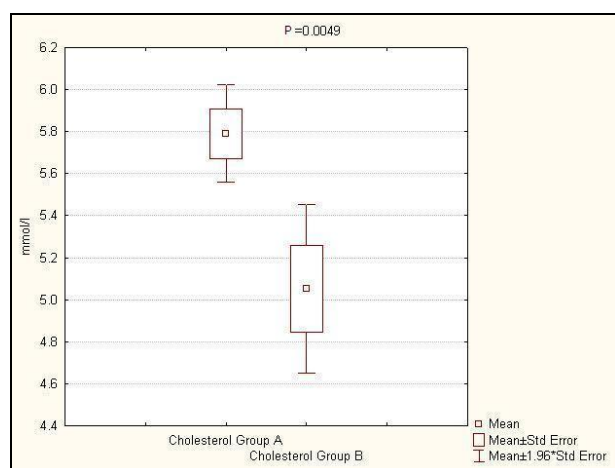


Fig. 1. Comparison of serum concentration of cholesterol in dogs from groups A and B

The concentrations of total T4 ranged from 2.05 nmol/L to 49.67 nmol/L in the group A (dogs with hypothyroidism). Average concentration of the T4 in group A was 25.04 ± 11.04 nmol/L. Concentrations below the lowest values accepted as the reference (20 nmol/L) were observed in 101 (33%) dogs. There were no significant differences in T4 concentrations depending on the dogs' sex. The average T4 level in the group of clinically healthy dogs (group B) was 33.05 ± 7.26 nmol/L. The minimum observed value was 18.9 nmol/L, the maximum value of T4 in this group was 48.5 nmol/L. Comparison of the concentrations of T4 in serum of dogs from groups A and B illustrated in Fig. 2, revealed highly significant differences ($P < 0.001$).

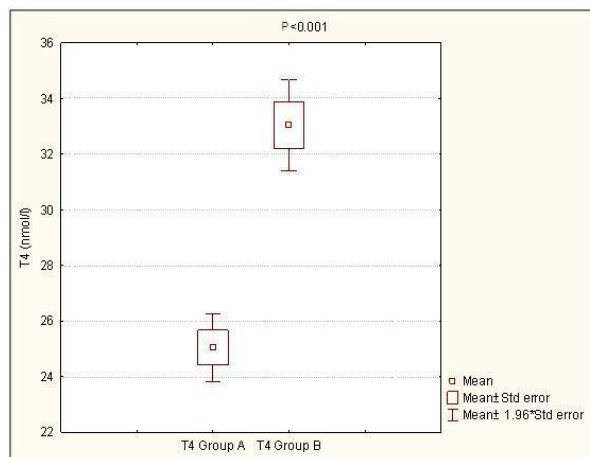


Fig. 2. Comparison of total T4 serum concentration in dogs from groups A and B

The concentration of fT4 in the group of animals with clinical symptoms of hypothyroidism (group A) were on average 3.93 ± 1.83 pmol/L. The lowest observed value of fT4 was 0.30 pmol/L, and the maximum value of fT4 was 7.48 pmol/L. The differences depending on the animals' sex were not observed. In the group of clinically healthy animals, the concentration of fT4 was on average 10.16 ± 2.34 pmol/L. The lowest observed value in this group was 7.5 pmol/L, the highest fT4 concentration was 16.16 pmol/L. Statistically lower values ($P < 0.001$) were observed in the group of dogs with hypothyroidism (group A), as presented in Fig. 3.

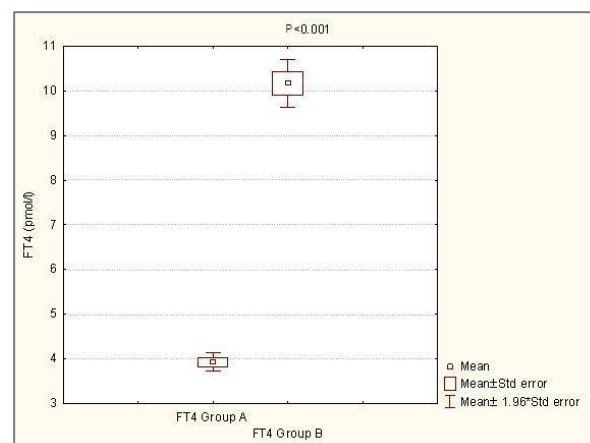


Fig. 3. Comparison of free T4 (fT4) serum concentration in dogs from groups A and B

In the group of sick dogs (group A), no TgAb_s were observed in 107 dogs (35%) and the presence of TgAb_s was observed in 201 dogs, out of which in 98 dogs (32%) test results were strongly positive and in 103 dogs (33%) - weakly positive. In the group of clinically healthy animals (group B), the presence of TgAb_s was observed in 24 (32%) dogs, out of which

six (8%) dogs had strongly positive test results (++) and 18 dogs (24%) - slightly positive (+) (Fig. 4).

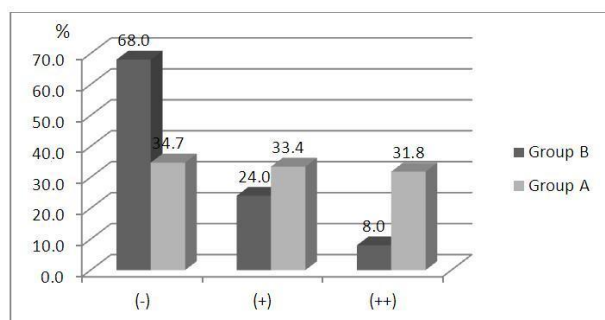


Fig. 4. Comparison of thyroglobulin antibody (TgAb_s) concentration in groups A and B

Comparison of concentrations of T4 and fT4 to the level of TgAb_s was conducted using single factor variance analysis (ANOVA). The hypothesis concerning various averages in all the groups was rejected. Comparison of the concentration in dogs of group A is presented in Figs 5 and 6. Within post-hoc Scheffe tests, the tendency of the presence of a high level of antibodies (++) in dogs with lower concentrations of total T4 ($P < 0.001$) and the presence of a low level of antibodies (+) in dogs with lower concentrations of fT4 ($P < 0.001$) in group of sick dogs (group A) was observed. A similar difference was not observed in the group of dogs with euthyrosis (group B). The dependence between the age of the tested dogs and the presence of TgAb_s in both groups was not observed.

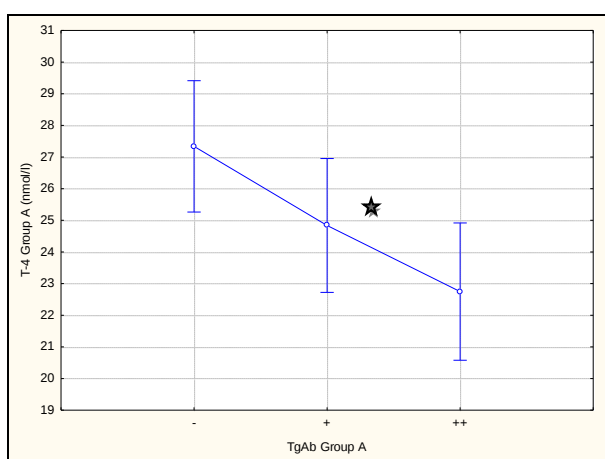


Fig. 5. Comparison of T4 average concentrations to the level of TgAb_s in the group of hypothyroid dogs (group A) (vertical bars mean 0.95 confidence intervals for the average)

★ $P < 0.001$

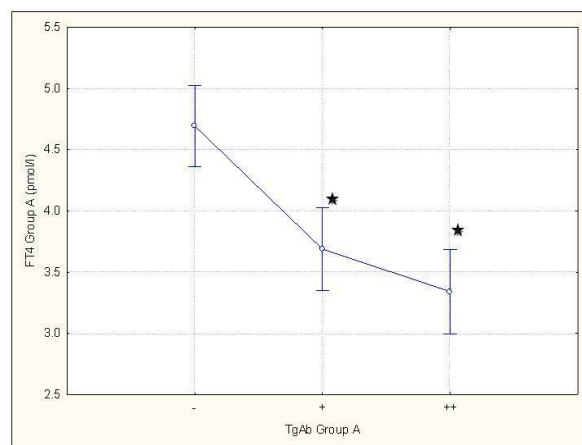


Fig. 6. Comparison of average concentrations of fT4 to the level of TgAb_s in the group of hypothyroid dogs (group A) (vertical bars mean 0.95 confidence intervals for the averages)

★ $P < 0.001$

Discussion

In spite of the fact that hypothyroidism is the most frequent endocrinological disease in dogs, its diagnosis can be problematic. It forces the researchers to look for more sensitive, accurate, and more specific diagnostic methods. Most of the authors agree that a detailed medical history and clinical examination revealing the symptoms typical for the thyroid gland dysfunction constitute the basis for qualification of the patient to detailed hormonal tests (1, 20). A routine blood test consisting most often of morphology and basic organ profiles (assessment of kidney and liver functions) usually do not show the parameter indicating the dysfunction of the organism (8, 15). In our studies, only cholesterol concentrations differed statistically in dogs with hypothyroidism comparing to clinically healthy dogs. According to Nelson (19), determination of total T4 concentration is the most rational and economic procedure, however, it is not very probable that the dogs with normal T4 concentration (the author gives the range of 20-50 nmol/L) would suffer from hypothyroidism. Some authors also recommended more detailed hormonal diagnostics (4, 22). In our studies, the concentrations of total T4 ranged from 2.05 nmol/L (minimum) to 49.67 nmol/L in the dogs with hypothyroidism. The mean T4 concentration in group A was 25.04 nmol/L. The concentrations below basic values assumed as the reference ones (20 nmol/L) were observed in 101 (33%) dogs, i.e. 2/3 dogs of this group did not manifest the fall of thyroxine concentration despite of an existent dysfunction of the thyroid gland. In the group of healthy animals, only 5.3% of the dogs manifested the concentrations lower from the referential ones. The concentration of this hormone is most often indicated as the most susceptible to the influences of the substances and external factors (coexistent diseases, euthyroid sick syndrome, or applied treatment) (15, 16, 19, 28). It seems that the

majority of factors which may cause total thyroxin suppression have a significantly lower impact on the concentration of its free fraction (fT4). Determination of fT4 concentration, measured in pmol/L, requires very sensitive and reliable diagnostic methods. In our study, the concentration of fT4 in animals with clinical symptoms of hypothyroidism was on average 3.93 pmol/L, and in none of the dogs the concentration was higher than the reference values (assumed minimum is 8.2 pmol/L). The lowest values of fT4 were 0.30 pmol/L and the maximum value was 7.8 pmol/L. In the group of healthy animals, the concentration of fT4 was on average 10.16 pmol/L. The lowest value in this group was 7.5 pmol/L, and the highest - 16.16 pmol/L.

Detection of thyroid antibodies in the serum of dogs allows considering hypothyroidism - lymphocytic thyroiditis - as an autoimmune disease. Happ *et al.* (12) presented lymphocytic thyroiditis as a model of autoimmune disease in dogs, and Rajatanavin *et al.* (23) and Czumińska (3) compared this type of thyroid inflammation in dogs to Hashimoto disease present in people. Proving the breed susceptibility in Dobermans, Labradors, and Schnauzers by pointing out the relation with major histocompatibility complex class II (MHCII), which is typical for Hashimoto disease in people, was also important (29).

The assessment of the antibodies specific to thyroglobulin in dogs allows evaluating thyroid functional status in detail and may be the prognosis of possible future dysfunctions of this gland. Usefulness of the selected kind of antibodies in dogs was assessed by many researchers. Skopek *et al.* (26) made an attempt to prove the dependency of lymphocytic thyroid inflammation on the presence of the antibodies directed against thyroid peroxidase (TPO Ab), as it is in human Hashimoto disease. Only 17% of serum samples showed the presence of TPOAB, thus the parameter cannot be used for diagnosing lymphocytic thyroiditis in dogs. The presence of antibodies against thyroid hormones was tested, among others, by Nachreiner *et al.* (18). The authors observed T3 and T4 antibodies in 6.3% of the tested dogs. The frequency of occurrence of TgAb_s in hypothyroidism showed by Nachreiner *et al.* (17) was 37%. Patzl and Mostl (21) obtained a positive result in 55% of dogs with hypothyroidism. Dixon and Mooney (6) observed the TgAb_s in 36% of dogs with hypothyroidism, and Breyer *et al.* (2) found TgAb_s in 58.6 % of dogs with hypothyroidism but also in 6.25% of dogs with euthyreosis. In our studies, TgAb_s in the group of affected dogs were found in 98 (32%) animals and the slightly positive values, (not unequivocal result, repetition is recommended in about 6 months), were found in 103 (33%) dogs. What is interesting, positive results were observed in 24 (32%) healthy dogs, out of which six (8%) dogs had the strongly positive results and 18 (24%) dogs slightly positive. Perspective tests performed by Breyer *et al.* (2) showed that 22.5% of

dogs demonstrating positive test for TgAb_s without clinical symptoms of the thyroid gland dysfunction, after, on average 2.5 years, had the symptoms of hypothyroidism. Similar prospective tests were performed by Graham *et al.* (10). The scientists observed 234 dogs with euthyreosis in which the presence of TgAb_s was detected. After one year observation it turned out that clinical symptoms of hypothyroidism were developed only in 19% of the dogs, 57% of the dogs still indicated high concentration of TgAb_s, 8% of the dogs were slightly positive for the antibodies, and 15% of the dogs did not demonstrate the presence of the antibodies in repeated test. Scott-Moncrieff *et al.* (24, 25) in the test performed in the group of healthy beagles verified if the routine vaccinations of dogs against contagious diseases can influence the TgAb_s concentration. They proved that the last vaccination of puppies against rabies may cause an increase in TgAb_s level. It was not defined, however, whether these antibodies had a harmful effect on the functioning of the thyroid gland. Therefore, it is recommended to take into consideration the time from the last vaccination when interpreting the TgAb_s concentration. In our study, all dogs qualified to the experiment had the defined period which passed from the last vaccination against rabies. Twelve weeks were assumed to be the minimal period. There was an interesting tendency of the presence of TgAb_s in animals with the lowest values of both T4 and fT4 concentrations, which was not observed in healthy animals.

The presence of TgAb_s is common in dog population. It mainly concerns the animals with hypothyroidism, but also the dogs with euthyreosis. Taking into account significantly higher levels of cholesterol in dogs with hypothyroidism, it seems sensible to add this parameter to the basic thyroid panel. Assay of antibodies specific to thyroglobulin has two aspects. Firstly, it allows diagnosing lymphocytic thyroiditis, if it is present together with the lower concentrations of T4 and fT4, and with higher concentration of cTSH. Secondly, it can be a prognostic factor. If it is found in young animals with euthyreosis it can be suspected that hypothyroidism will develop in these animals during the next few years. However, TgAb_s do not have to be always found in dogs with confirmed hypothyroidism, and they can be present in dogs with euthyreosis. Only when the animal has characteristic clinical symptoms, which can be assessed during clinical examination, and the remaining results of laboratory tests confirm the suspicion of the thyroid disease, TgAb_s identification can be the basis for diagnosing hypothyroidism caused by lymphocytic thyroiditis. As a genetic susceptibility to lymphocytic thyroiditis has been described, it seems rational to perform such tests in breeding dogs (2, 18, 21). It also seems to be advisable to propose the owners of dogs used in breeding programmes, especially of breeds considered to be prone to hypothyroidism, to carry out

prophylactic screening tests aiming at selecting animals with the TgAb_s. Such animals, as suspected of developing hypothyroidism in future, should have the tests repeated every 6-12 months in order to observe the dynamics of antibodies concentration growth and possible development of thyroid disease.

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