

The prevalence of autoantibodies to: myosin, troponin, tropomyosin and myoglobin in patients with circulating triiodothyronine and thyroxine autoantibodies (THAA)

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Abstract

OBJECTIVE: Anti-thyroglobulin, anti-thyroid-peroxidase and anti-TSH receptor antibodies have been observed with high frequency in autoimmune thyroid diseases. Thyroid hormone auto-antibodies (THAA): anti-thyroxine (T₄) and anti-triiodothyronine (T₃), conversely, have been reported rarely. In both hyperthyroidism and hypothyroidism, patients suffer from muscle weakness and function disorders. The aim of our study was the evaluation of the occurrence rate of autoantibodies targeting muscle proteins in a group of 24 patients with circulating anti-T₃ and/or anti-T₄ autoantibodies. The control group consisted of 41 healthy blood donors.

METHODS: In polyethylene tubes coated with muscle antigens: actin, myosin, myoglobin, troponin and tropomyosin solid-phase radioimmunoassay was performed to detect autoantibodies. A reaction with ¹²⁵I-labelled staphylococcus protein A was used for the detection of antibodies bound to the antigens on the tubes.

RESULTS: We found a high occurrence of antibodies to muscle proteins in patients with THAA. Anti-myoglobin autoantibodies were most frequent (54.2% of subjects), the binding index values was very high and exceeded normal values two to four fold. Anti-myosin autoantibodies were detected in 50% of subjects; anti-troponin autoantibodies in 33.3%, and anti-tropomyosin autoantibody in 3 patients (12.5%). Differences between the patients and the controls were statistically significant. The antibody binding index to actin was low and statistically insignificant.

CONCLUSIONS: Our study indicates that muscle protein antibodies, especially to myoglobin, myosin and troponin, are very frequently present in patients with autoimmune thyroid disease and circulating anti-T₃ and anti-T₄ autoantibodies, as well as in most cases of chronic thyroiditis with clinical symptoms of hypothyroidisms.

Abbreviations

THAA	- thyroid hormone autoantibody
T ₃	- triiodothyronine
T ₄	- thyroxine
ATPO	- anti-thyroid peroxidase antibody
ATG	- anti-thyroglobulin antibodies
TSH	- thyroid stimulating hormone
MHC	- myosin heavy chains

INTRODUCTION

Antithyroid autoantibodies, particularly antibodies to thyroglobulin, thyroid-peroxidase and TSH receptor are frequently observed in patients with autoimmune thyroid disease. On the other hand, thyroid hormone autoantibodies (THAA) against thyroxine (T₄) and triiodothyronine (T₃) have been reported extremely rarely. These antibodies have been identified in various, mainly autoimmune thyroid diseases [32,34,13,26,45,31,29,14,15], rarely in viral infections and Sjogren's syndrome [30,35]. Graves' disease and Hashimoto's thyroiditis are often associated with other autoimmune diseases and thus the autoimmune process is not restricted to specific thyroid antigens. Anti-pituitary and anti-tubulin antibodies, as well as many other autoantibodies have been identified within this group of patients [3].

Thyroid hormones affect muscle activity. Triiodothyronine induces the transcription of alpha-myosin heavy chains (MHC) and represses beta-MHC transcription improving the contraction of the cardiac muscle; thus, thyroid hormones exert an ino- and chronotropic effect on the heart. In addition, they increase the number of adrenergic receptors in the cardiac muscle and skeletal muscles and also intensify the motor activity of the alimentary tract.

Hyperthyroidism often leads to cardiomyopathy; in elderly patients cardiac rhythm distortions and atrial fibrillation are frequently diagnosed. Skeletal muscles are particularly affected in severe thyrotoxicosis untreated for long periods of time and results in muscle weakness, tremor, rapid fatigue, problems with walking and especially climbing stairs. The patients complain that their knees are wool-like, and they are unable to raise their legs while lying on their back, due to severe muscle weakness. Furthermore, patients suffer from weight loss coupled with muscular atrophy particularly in the shoulder girdle, pelvis and proximal lower limb muscles. Sometimes the clinical picture resembles myasthenia gravis and is even described as "thyroid myasthenia"

In hypothyroidism, muscle stiffness, sluggish movements, slow speech and myotonic manifestations are often observed. Muscle relaxation time is markedly delayed, especially when evoking Achilles tendon reflexes; heart muscle relaxation is also prolonged as is shown by ultrasound studies. Additionally, weakness, adynamia and skeletal muscle pain occur. In patients with hypothyroidism an elevation of creatine phosphokinase levels a sensitive marker of muscle damage is common.

Myosin is the basic protein of muscle cells; it constitutes 40% of all proteins in a myocyte and is a component of thick myofilaments.

Actin is one of the most common cellular proteins. Apart from cooperating with myosin in the mechanism of muscle contraction, it is the main component of the cytoskeleton. Furthermore, it plays an essential role in intracellular processes.

Troponin is a complex of three polypeptide chains, labelled as TnC, TnI and TnT, present in skeletal and cardiac muscle, but not smooth muscle. TnC binds calcium ions, TnI binds actin and TnT binds tropomyosin.

Tropomyosin participates in regulating muscle contraction; it is a helical dimeric rod, with molecular weight 70kDa. Along with troponin it makes up the tropomyosin protein complex, which is localised very near actin filaments and supports the creation of the sarcomere. Hence, troponin, tropomyosin and actin constitute the thin myofilaments of striated muscles.

Myoglobin is a protein present mainly in muscular tissue, which has the ability to bind molecular oxygen and serves as an intracellular storage site for oxygen. As the amount of oxygen in a tissue drops, oxymyoglobin releases its bound oxygen, which is then used in metabolic processes. Myoglobin is released from damaged muscular tissue, making it a sensitive marker of muscle damage.

THE AIM OF THE STUDY

Autoimmunisation has a generalised character in autoimmune thyroid diseases. Muscular fatigue is a frequent symptom in this group of patients and it has not been explained whether the autoimmunisation process involves muscle antigens as well. Our research strived to detect autoantibodies to contractile muscle proteins: myosin, actin, tropomyosin troponin and myoglobin. So far, autoantibodies to muscle antigens in patients with circulating anti-T₃ and anti-T₄ autoantibodies have not been examined.

MATERIALS AND METHODS

The study group

The study involved 24 patients with diagnosed presence of either anti-thyroxine, anti-triiodothyronine antibodies or both. The patients were aged 18–72, 46.9±14.7 years on average. The group consisted of 21 women aged 18–72 (87.5%) and 3 men aged 40–63 (12.5%). In 11 cases with THAA Hashimoto's disease was recognised (45.8%), and in six patients Graves' disease (25.0%). Two patients had nontoxic parenchymatous goitre (8.3%), and one patient displayed toxic nodular goitre (4.2%). Four of the patients with Hashimoto's thyroiditis (16.6%), simultaneously suffered from other immunological diseases; in these patients systemic lupus, Wilson's disease, atrophic gastritis and premature ovarian failure (POF) were identified.

For the detection of anti-T₄ and anti-T₃ autoantibodies serum samples were incubated with ¹²⁵I labelled T₄ and T₃. After a 90 minute incubation at 37 °C, 1ml of 18% polyethylene glycol (PEG) was added to precipitate the immune complexes. After centrifugation for 30 min at 3000 revolutions per minute and decanting the supernatant, impulses were counted using a scintillation gamma-counter (LKB Wallac). The number of counts in the examined serum was divided by the mean of the overall number of counts in control subjects. The resulting index constituted the result of the study. The normal range for healthy individuals does not exceed the non-specific binding (NSB). In our study group the presence of anti-thyroxine autoantibodies was found in 5 patients (20.8%). 14 patients displayed anti-triiodothyronine autoantibodies (58.4%), while the presence of both anti-T₃ and anti-T₄ autoantibodies was stated in 5 patients (20.8%).

For the determination of thyroid autoantibodies to thyroglobulin and thyroid-peroxidase, a RSR Ltd. test kit was employed with the use of highly purified thyroid-peroxidase and thyroglobulin antigens. TSH receptor antibodies were determined using a Brahms test kit. The presence of ATG antibodies was observed in all the examined patients, whereas the presence of ATPO in 22 patients and TSH receptor antibodies were detected in 8 patients (33.3%).

The total T₄ and T₃ was determined by employing radioimmunoassays (immunoprecipitation with the use of PEG, a solid-phase and following ethanol protein precipitation). The determination of FT₄ and FT₃ was performed by a solid-phase radioimmunoassay, while TSH by the IRMA assay.

Thyroid ultrasonography was performed with a 7.5-MHz linear probe using the Aloka SSD1100 instrument. Decreased echogenicity was detected in 91.7% of subjects and the average thyroid volume was 38.1ml. In 32.1% of subjects solid nodules were detected, whereas in 25% of subjects cystic changes and calcifications occurred.

The control group consisted of 41 healthy blood donors without thyroid diseases.

The study was approved by the Ethics Committee of the University and the patients gave their informed consent to participate.

Reagents

Contractile muscle proteins were purchased from SIGMA-Aldrich: actin isolated from bovine muscle, troponin from rabbit muscle, myosin, tropomyosin and myoglobin from bovine skeletal muscle. Staphylococcus-Protein A was acquired from Pharmacia (Uppsala, Sweden). ¹²⁵Iodine for protein iodination, manufactured by NEN, as well as polyethylene tubes size 5×1 cm were purchased from Polatom, Swierk.

Methods

The antibodies were measured by solid-phase radioimmunoassay in polyethylene tubes coated with muscle antigens, according to the method described previously

[31]. The polyethylene tubes 50×10 mm were coated with muscle proteins in 0.05 mol/l phosphate buffer pH 7.4 containing 0.1% sodium azide. To each tube, 0.5 ml of the solution was added, antigen concentration was 2 µg/ml. After 18–20 hours of incubation at room temperature the tubes were washed three times with PBS/Tween (phosphate buffered saline/0.5% Tween). The remaining active sites on the tube walls were blocked for 2 hours with 3% skimmed milk (SM) in PBS/Tween, followed by triple washing with PBS/Tween. Afterwards, the contents of the tubes were aspirated and the tubes washed 3 times with 0.5 ml of PBS/Tween. Next, ¹²⁵I-Protein A 50 000 cpm in 0.5 ml of PBS/Tween/0.3% SM per tube was added to detect the retained autoantibodies. After an 18-hour incubation at room temperature the contents of the tubes were decanted, washed three times with PBS/Tween and counted on a gamma counter (Pharmacia, LKB).

The results were expressed as an antibody index; the number of impulses counted per minute (cpm) in patients' sera were divided by the mean cpm of the healthy controls.

Statistics

Data were expressed as means±SD and a statistical comparison was performed. For statistical analysis following tests were applied: the t-Student test for unpaired data and the Mann-Whitney U test for nonparametric values. p<0.05 was chosen as the level of significance.

RESULTS

The study involved 24 patients. Individual results of autoantibodies to muscle antigens in our patients are given in Table 1.

Anti-myoglobin autoantibodies were most frequent; in 13 patients (54.2%) the value of the binding index was very high and exceeded the normal values by two- to fourfold. The mean value of autoantibodies index±SD of anti-myoglobin autoantibodies was 1.7±1.07 in patients with THAA and 0.73±0.38 in the control group (p<0.005) (Figure 1).

Anti-myosin autoantibodies were present in 12 patients (50%). The mean value of the autoantibodies index±SD of anti-myosin autoantibodies was 2.29±0.98 in patients with THAA and 1.24±0.58 in the control group. The difference was statistically significant with p<0.005 (Figure 2).

Anti-troponin autoantibodies were present in 8 patients (33.3%). The mean value of the autoantibodies index±SD of anti-troponin autoantibodies was 1.56±0.79 in patients with THAA and 0.83±0.38 in the control group (p<0.005). (Figure 3)

Only 3 patients (12.5%) displayed abnormal values of anti-tropomyosin antibodies. The mean value of the autoantibodies index±SD of anti-tropomyosin autoantibodies was 1.55±0.69 in patients with THAA and 1.18±0.46 in the control group. The differences were statistically significant with (p<0.005) (Figure 4).

Table 1. Individual results of anti-muscle antigen autoantibodies in patients with circulating anti-T3 and anti-T4 autoantibodies (THAA).

CASE	AGE (years)	SEX F/M	MUSCLE ANTIGEN				
			anti-myoglobin	anti-myosin	anti-troponin	anti-tropomyosin	anti-actin
J.M.	32	F	+	+	+	-	-
K.W.	61	F	+	-	+	-	-
D.G.	41	F	+	-	-	-	-
T.O.	63	M	-	+	-	-	+
I.T.	60	F	-	-	-	-	-
G.D.	57	F	-	-	-	-	-
M.J.	34	F	+	+	-	-	+
U.G.	48	F	+	+	+	+	+
M.B.	26	F	-	-	-	-	-
Z.G.	38	F	+	-	-	-	-
K.P.	61	M	-	-	-	-	-
W.W.	53	F	-	-	-	-	-
T.B.	49	F	+	+	-	-	+
H.Cz.	49	F	-	-	+	-	-
J. Ma.	72	F	-	-	-	-	-
B.M.	44	F	-	-	-	-	-
W.S.	40	M	-	+	+	-	-
Z.Cz.	31	F	-	-	-	-	-
M.A.	37	F	+	+	+	+	-
M.K.	18	F	+	+	+	-	+
T.Ś.	71	F	+	+	-	-	-
D.W.	30	F	+	+	-	-	-
J.S.	46	F	+	+	-	-	-
T.M.	65	F	+	+	+	+	-
Mean	46.9	F (87.5%)	13	12	8	3	5

In the case of the anti-actin binding index lower values were observed. Only in 5 patients did these values exceed normal levels. The mean value of the autoantibodies index \pm SD of anti-actin autoantibodies was 1.29 ± 0.58 in patients with THAA and 1.22 ± 0.56 in the control group. The differences were not statistically significant as in the control group the range of the binding index was high ($p=0.62$) (Figure 5).

To check the specificity of the detected autoantibodies in singular cases with high level of antibodies the sera of the patients were preabsorbed with excess ($5\mu\text{g}/\text{tube}$) of antigens (myosin, troponin, tropomyosin and myoglobin). This resulted in significant decrease of antibody titres to the range of control subjects.

In summary, in 17 of 24 patients with THAA we detected autoantibodies to skeletal muscle antigens, to the one antigen in three cases, to two antigens in six patients,

to three antigens in three cases and to four antigens in four cases. Only sera of seven patients did not react with muscle antigens (Table 1).

In the control group, 5% of healthy subjects displayed the presence of myoglobin, troponin, TPO and TG antibodies; in 12% of subjects antibodies to myosin were detected and in 10% to tropomyosin and actin. Only 1 patient (2%) had positive values of TSH receptor antibodies (Table 2).

The t-Student and Mann-Whitney tests employed for statistical analysis indicated a statistically significant higher level of antibodies to myoglobin, myosin, troponin and tropomyosin ($p<0.0005$) in the study group as compared to the control group. The level of anti-actin antibodies in patients with THAA was not different from the control group.

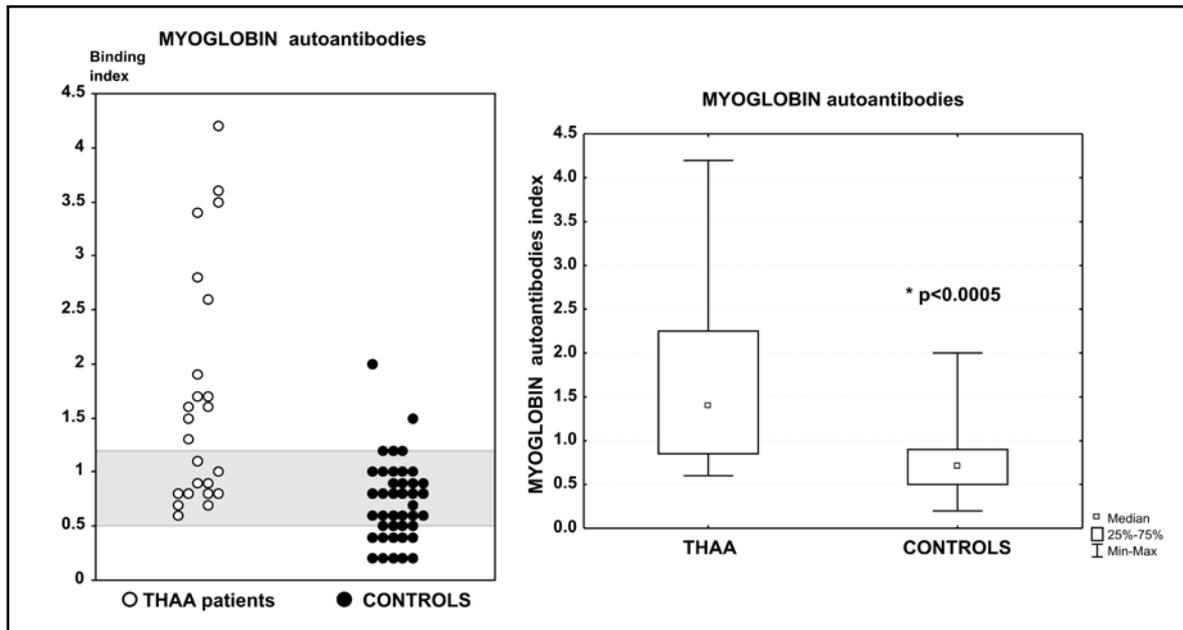


Figure 1. Anti-myoglobin autoantibodies.

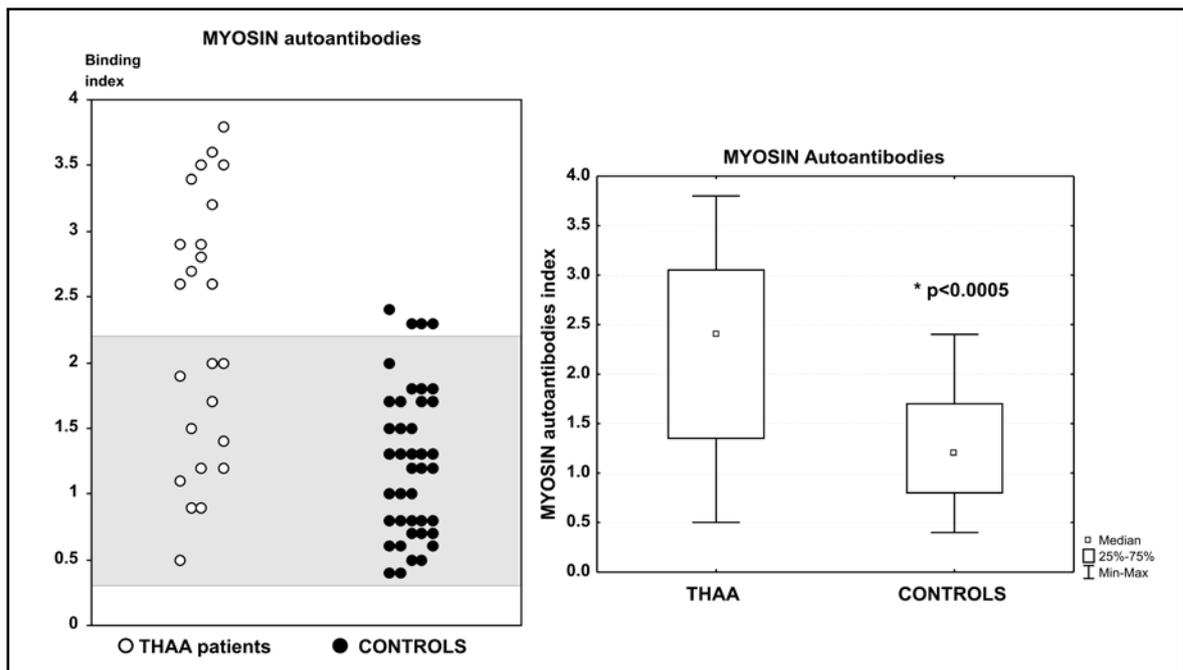


Figure 2. Anti-myosin autoantibodies.

DISCUSSION

The presented data refers to 24 patients with high anti-T₃ and anti-T₄ levels. Our study indicates that in the group of patients with autoimmune thyroid disease and the presence of anti-T₃ and anti-T₄ autoantibodies, the rate of occurrence of antibodies to muscle proteins, especially to myoglobin, myosin and troponin, is very high. With respect to the control group, also a statistically significant difference in the occurrence of anti-tropomyosin autoantibodies has also been demonstrated. No significant difference in the presence of anti-actin

antibodies has been found. Muscle antigen antibodies were discovered in patients irrespective of the diagnosed thyroid disease and the clinical state. However, these antibodies were more frequently present in the group of patients, whose predominant symptoms were fatigue and muscle pain. Still, no correlation between the levels of antibodies and high values of creatine phosphokinase levels was observed.

Skeletal muscle protein antibodies have been described rather infrequently in thyroid diseases, and such studies have typically concentrated on individual antigens. Jasani examined a group of 69 women with Hashimoto's disease

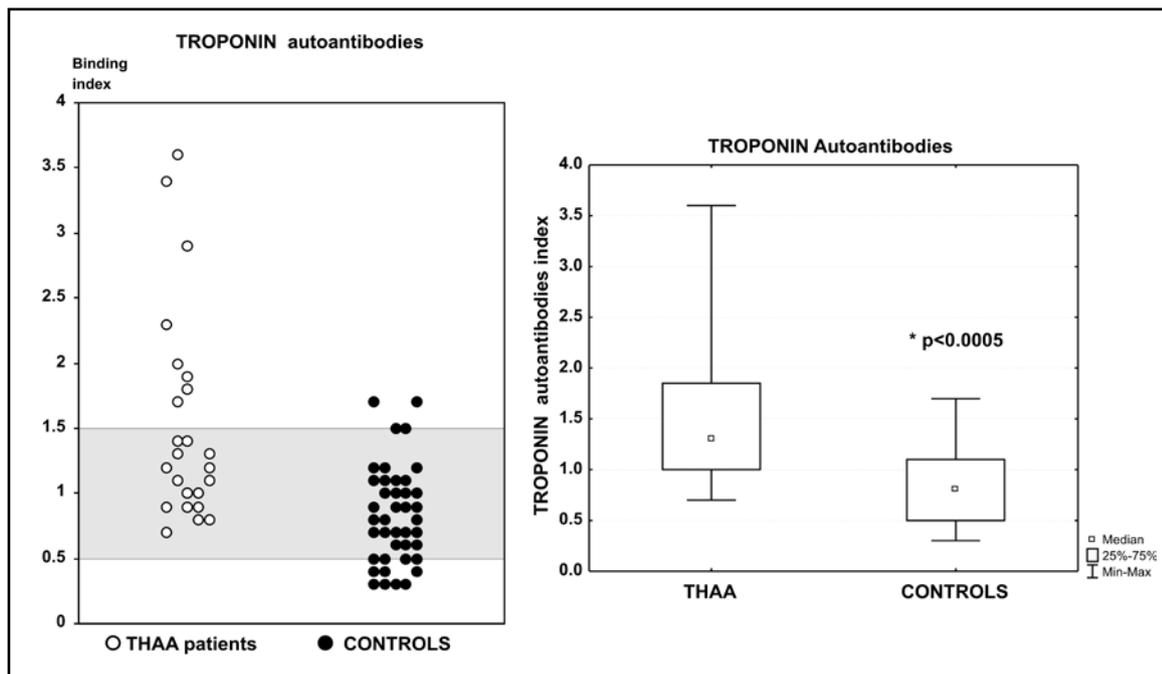


Figure 3. Anti-troponin autoantibodies.

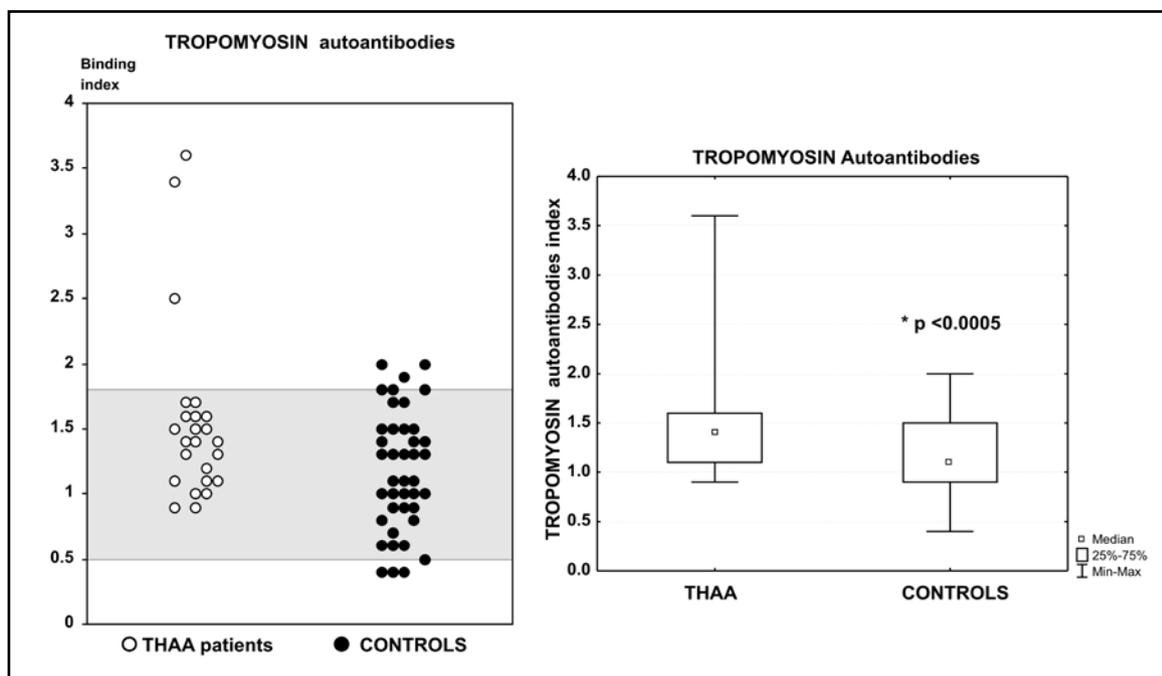


Figure 4. Anti-tropomyosin autoantibodies.

and discovered antibodies to a whole range of antigens, including anti-actin, anti-myosin and anti-tropomyosin antibodies in several cases [16]. The presence of anti-myosin and, in singular cases, anti-actin antibodies was detected by Kadlubowski in patients with Graves' ophthalmopathy [17]. The occurrence of anti-actin and anti-myoglobin antibodies was detected through an immunosorbtion assay in a few percent of healthy subjects as well as patients suffering from multiple myeloma and Waldenström's macroglobulinemia [1]. The occurrence of antibodies to smooth muscle antigens in autoimmune

thyroid disease, both Hashimoto's thyroiditis (6/22) and Graves' disease (12/28) was described by Morita [24].

So far, muscle antigen antibodies have been described mainly in autoimmune and muscle diseases; anti-myosin antibodies have been detected in idiopathic dilated cardiomyopathy [41,42], primary sclerosing cholangitis [9], in Kawasaki disease [8] and in polymyositis [5], whereas, anti-myoglobin antibodies were displayed by patients after cardiac arrest [2]. Anti-actin autoantibodies were observed during chronic aggressive hepatitis [7], autoimmune hepatitis [22,19], celiac disease [11,6], fertility

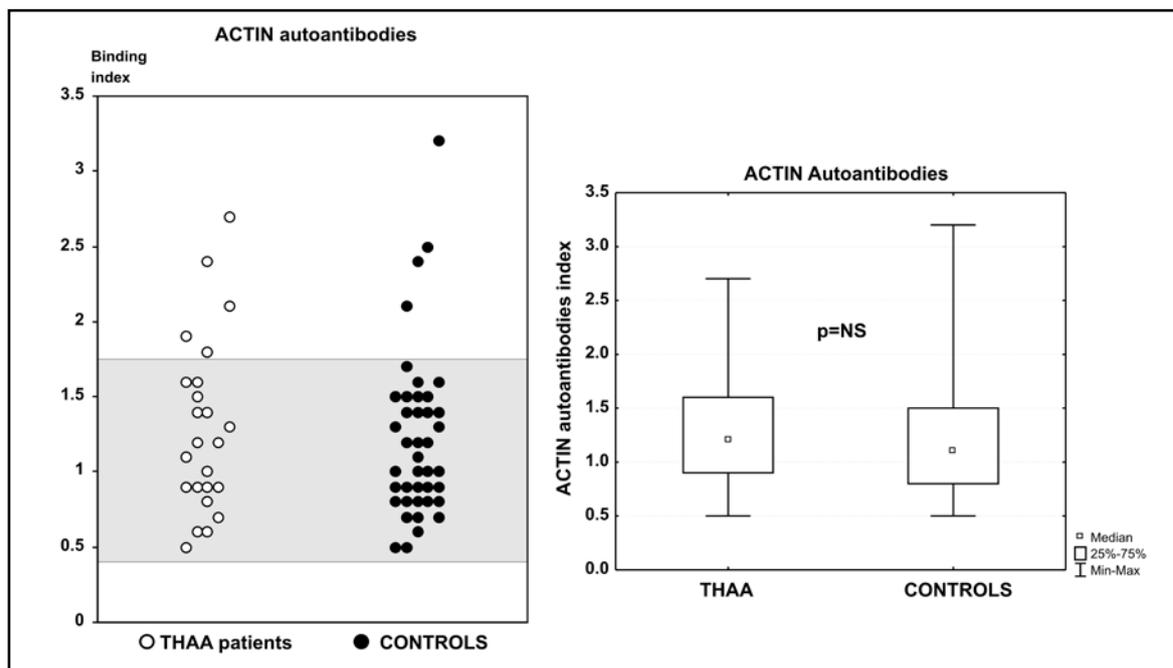


Figure 5. Anti-actin autoantibodies.

disorders [37] and hypertrophic cardiomyopathy [12]. Autoantibodies to tropomyosin occurred in inflammatory bowel disease: ulcerative colitis [33,10] and Crohn's disease as well as colorectal adenocarcinoma [38]. Anti-troponin autoantibodies were present in idiopathic dilated cardiomyopathy [36] and ischemic cardiomyopathy [28]. Additionally, an autoimmune response against muscle proteins was induced in certain infectious diseases like cytomegalovirus infection (CMV) [27] or *Trypanosoma cruzi* [20,21]. Moreover, the presence of anti-muscle antibodies has been described in many patients with myasthenia gravis [23,25,40,44].

In our earlier studies muscle antigen antibodies were detected in the majority of patients with Addison's disease, but in contrast to our study group the occurrence of the tropomyosin antibodies was low and anti-actin antibody was definitely elevated.[4].

It is difficult to determine which factors influence antibody production; however, this process is connected to polyclonal activation of B lymphocytes. A very interesting finding was published by Thrasyvoulides in 2007. A high immunoreactivity to myosin was obtained as a consequence of experimental immunisation with human thyroglobulin [39]. These results are in agreement with our clinical observations, which indicate that muscle antigen antibodies coexist with anti-thyroglobulin antibodies.

The variety of antibodies in our study group indicates a defective immunological surveillance [43]. Long-term observations of our patients with THAA revealed that among the described patients, 3 have died (acute myeloid leukaemia, colorectal carcinoma and ovarian carcinoma). Another patient suffering from colorectal carcinoid

Table 2. The occurrence rate of muscle protein autoantibodies and thyroid antigen antibodies.

Antigen	Control group	Subject group	p-value
Actin	9.8%	20.8%	p=NS
Myosin	12.2%	50%	p<0.005
Tropomyosin	9.8%	12.5%	p<0.005
Troponin	4.9%	33.3%	p<0.005
Myoglobin	4.9%	54.2%	p<0.005
Thyroid antibodies			
TSH receptor	2.4%	33.3%	p<0.005
Thyroglobulin	4.9%	100%	p<0.005
Thyroid peroxidase	4.9%	91.3%	p<0.005

is in poor general state; at present under somatostatin analogue treatment.

In conclusion, our study indicates that antibodies to muscle proteins, especially to myoglobin, myosin and troponin are very common in patients with autoimmune thyroid diseases and anti-T₃ and anti-T₄ autoantibodies. Moreover, detailed clinical observations have shown that most cases of anti-T₃ and anti-T₄ autoantibodies are exhibited by patients suffering from chronic thyroiditis with clinical manifestations of hypothyroidism.

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