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## ODREĐIVANJE ANTITELA NA TIREOIDNU PEROKSIDAZU – DALJA ISKUSTVA

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**Sažetak: Uvod** Autoantitela na tireocitnu peroksidazu su vrlo značajan dijagnostički parametar. Uglavnom se nalaze kod bolesnika sa autoimunim tireoidnim oboljenjima (Hashimoto tireoiditis, Spontanom hipotireozom, Bazedovljevom bolešću) **Cilj:** Analiza rezultata metode koju koristimo u kliničkoj praksi. **Materijal i metodi rada:** Određivanje autoantitela na tireocitnu peroksidazu vršili smo metodom Monobind, USA. Do sada smo izvršili preko 2000 određivanja. Metoda se sastoji od hemiluminescentnog imunotesta, koristi se tireoidna peroksidaza obeležena biotinom i plastične epruvetice obložene streptavidinom. Kalibracija je urađena internacionalnim standardom 66/387 za tireoidne mikrozome. **Rezultati:** Za poslednjih 8 godina uradili smo preko 2000 određivanja kod više stotina osoba. Kod njih pedesetak ispitivani parametar je rađen kao “follow-up” studija. **Zaključak:** Prikazali smo rezultate određivanja antitela na tireocitnu peroksidazu. Metoda koju smo koristili daje vrlo pouzdane rezultate. U toku “follow-up” studije rezultati po nekad nisu bili konstantni kod istog ispitanika. **Ključne reči:** Autoantitela, Tireoidni mikrozomi, Hipotireoza, Tireocitna peroksidaza

### UVOD

Tireocitna peroksidaza (TPO) je enzim koji ima ulogu u biosintezi tireoidnih hormona (katalizuje oksidaciju jodida na tirozilske ostatke u tireoglobulinu). Nalazi se na apikalnoj površini tireoita [1]. U toku destruktivnih procesa štitaste žlezde on kao autoantigen inicira nastanak antitela (TPOAb) [2]. Ova autoantitela koriste se u praksi kao marker autoimunih bolesti tireoideje [3]. Mogu da se nađu i kod nekih drugih autoimunih (netireoidnih) stanja [4]. Pre korišćenja analize antitela na tireoidnu peroksidazu, za određivanje tireoidnih autoantitela korišćeno je testiranje antitela na tireoglobulin, a kasnije na tireoidne mikrozome. Poslednjih godina je razjašnjeno da je antigena supstanca u mikrozmima ustvari, tireoidna peroksidaza. Autor je prvi put objavio svoja iskustva 1985. godine [5], a publikovao u inostranom časopisu 1998. godine [6]. Poslednji put je objavljen u ovom časopisu 2017. godine [7]. Tada smo koristili nekoliko metoda za određivanje TPOAb sledećih proizvođača: firme Thermo, firme Roche, Cobas, firme Beckman Coulter i firme Monobind - USA. U ovom radu prikazani su samo rezultati ovog poslednjeg proizvođača, na velikom broju uzoraka, na više ispitanih bolesnika, na zdravim osobama (kontrolna grupa) i u jednom delu u vidu „follow-up“ studije.

### CILJ RADA

Pokušali smo da odaberemo metodu koja nam najviše odgovara: da može da se radi i u malom broju uzoraka, na jeftinom mernom uređaju, da daje reproducibilne rezultate, da ima „normalne vrednosti“ kao i druge metode (neke od korišćenih metoda imaju referentne vrednosti do 9 IU/ml, većina ostalih ima između 35 i 60). Kod pacijenata, a i kontrolne grupe zdravih, pratili smo reproducibilnost u toku dužeg vremenskog intervala.

### BOLESNICI I METODE

Za određivanje autoantitela na tireoidnu peroksidazu koristili smo Hemiluminescentni imunotest, Monobind mikroplate. Granična vrednost je 40. Monobind Inc. - Lake Forest, CA 92630 USA. Merenje uzoraka smo obavljali na Lumi Stat uređaju. Uređaj je dizajniran da može da se koristi i za mali broj uzoraka.

Metodu smo počeli pre 8 godina - uradili smo preko 2000 uzoraka. Kod pedesetak bolesnika vršili smo i sistematsko praćenje (follow-up studija), na-3-4 meseca, u toku 3-6 godina.

### REZULTATI

U poslednjih 8 godina, od kada radimo određivanje TPOAb metodom firme Monobind, uradili smo 2040 uzoraka. Obradili smo veliku kontrolnu grupu zdravih osoba ili bolesnika sa netireoidnim oboljenjima. Vrednosti smo prikazali na grafikonu 1.

## DETERMINATION OF ANTIBODIES TO THYROID PEROXIDASE - FURTHER EXPERIENCES

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SPECIALIST PRACTICE "DR. PAUNKOVIĆ"

**ABSTRACT: Introduction:** Thyroid peroxidase autoantibodies are a very important diagnostic parameter. They are mainly found in patients with autoimmune thyroid diseases (Hashimoto's thyroiditis, spontaneous hypothyroidism, Bazedov's disease). **Objective:** Analysis of the results of the method we use in clinical practice. **Material and working methods:** Autoantibodies to thyrocytic peroxidase were determined using the Monobind method, USA. So far we have made over 2000 determinations. The method consists of a chemiluminescent immunoassay, using thyroid peroxidase labeled with biotin and plastic tubes coated with streptavidin. Calibration was done with international standard 66/387 for thyroid microsomes. **Results:** In the last 8 years, we have done over 2000 determinations with hundreds of people. In about fifty of them the examined parameter was done as a "follow-up" study. **Conclusion:** We presented the results of determination of antibodies to thyrocytic peroxidase. The method we used gives very reliable results. During the "follow-up" study, the results were sometimes not constant in the same subject.

**Key words:** Autoantibodies, Thyroid microsomes, Hypothyroidism, Thyroid peroxidase

### INTRODUCTION

Thyroid peroxidase (TPO) is an enzyme that plays a role in the biosynthesis of thyroid hormones (it catalyzes the oxidation of iodide to tyrosyl residues in thyroglobulin). It is located on the apical surface of the thyroid [1]. During the destructive processes of the thyroid gland, it initiates the formation of antibodies (TPOAb) as an autoantigen [2]. These autoantibodies are used in practice as a marker of autoimmune thyroid diseases [3]. They can also be found in some other autoimmune (non-thyroid) conditions [4]. Before using the analysis of antibodies to thyroid peroxidase, testing of antibodies to thyroglobulin and later to thyroid microsomes was used to determine thyroid autoantibodies. In recent years, it has been clarified that the antigenic substance in microsomes is actually thyroid peroxidase. The author published his experiences for the first time in 1985 [5] and published them in a foreign magazine in 1998 [6]. It was last published in this journal in 2017 [7] At that time, we used several methods for determining TPOAb from the following manufacturers: Thermo, Roche, Cobas, Beckman Coulter, and Monobind, USA. In this paper, only the results of this last manufacturer are presented, on a large number of samples, on several examined patients, on

healthy people (control group) and in one part in the form of a "follow-up" study.

### PURPOSE OF THE WORK

We tried to choose the method that suits us best: one that can be used in a small number of samples, on an inexpensive measuring device, one that gives reproducible results, one that has "normal values" like other methods (some of the methods used have reference values up to 9 IU/ml, most of the others have between 35 and 60). In patients and in a healthy control group, we monitored reproducibility over a longer period of time.

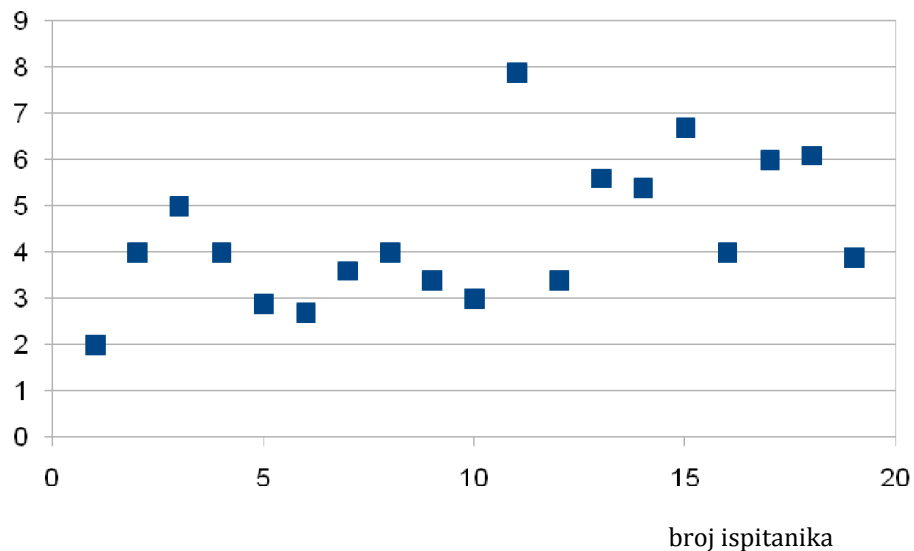
### PATIENTS AND METHODS

Chemiluminescent immunoassay was used to determine autoantibodies to thyroid peroxidase. Monobind microplates Limit value is 40. Monobind Inc.- Lake Forest, CA 92630 USA. The samples were measured on a Lumi Stat device. The device is designed to be used for a small number of samples.

We started the method 8 years ago, we have done over 2000 samples. In about fifty patients, we also carried out systematic monitoring (follow-up study), at 3-4 months, during 3-6 years.

**Grafikon 1.** TPOAb kod kontrolne grupe zdravih osoba, ili bolesnika sa netireoidnim oboljenjima.

TPOAb IU/ml

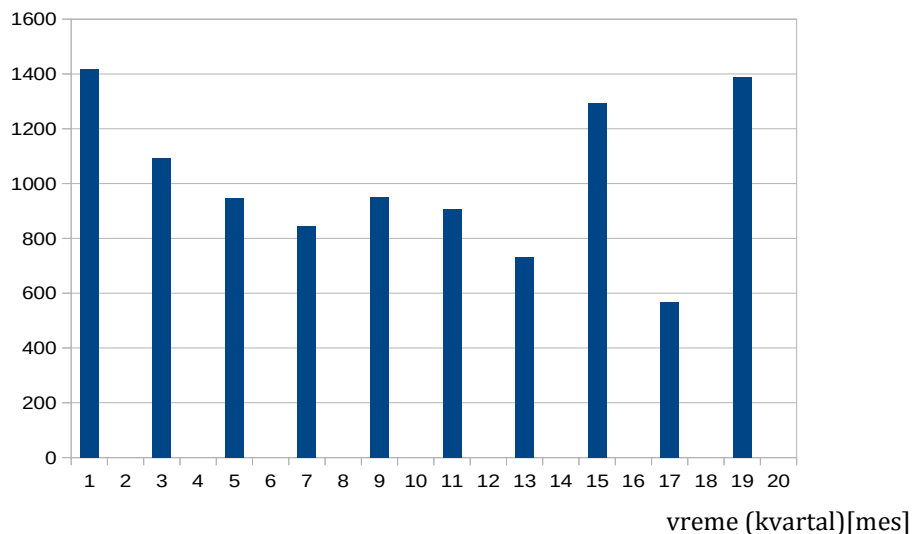


Grupa bolesnika sa autoimunim tireoiditisom (Hashimoto), sastojala se od 323 pacijenta sa hipotireozom, i 156 koji su još uvek bili u eutireoidnij fazi, mada smo većini od njih ordinirali supstituciju l-tiroksinom, pogotovo ako su vrednosti antitela na tireoidnu peroksidazu bile jako visoke- preko 1000. IU/ml.

Poslednjih nekoliko godina smo obradili rezultate koje smo dobili kod istih pacijenata u toku praćenja bolesti (follow-up). Hteli smo da proverimo zapažanja koja smo stekli prethodnom studijom [7] - naime da rezultati nisu dovoljno konstantni kod bolesnika koji su na supstiticionoj terapiji tiroksinom. Ovo zapažanje smo ilustrovali na grafikonima 2 i 3.

**Grafikon 2.** Praćenje koncentracije antitela na tireocitnu peroksidazu od 2016.-2021. godine kod bolesnice P.B.

TPOAb IU/ml



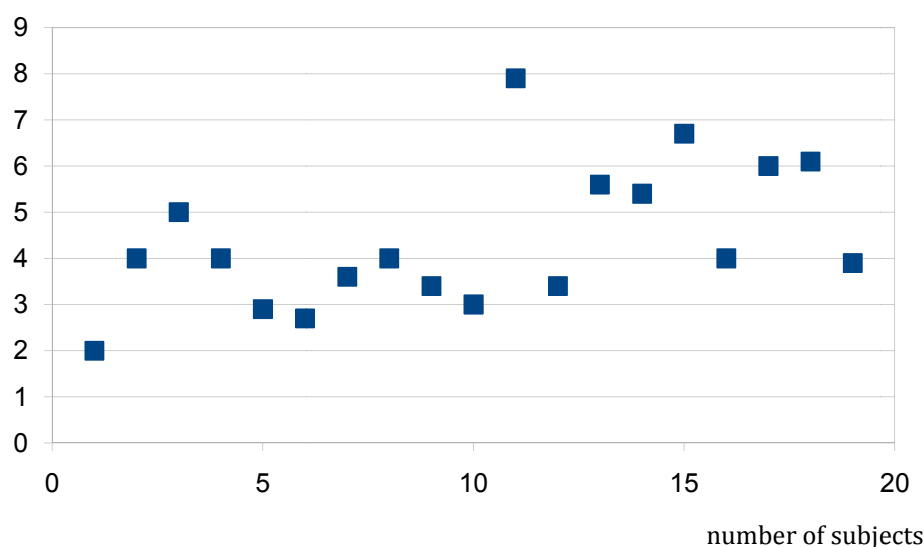
## RESULTS

In the last 8 years, since we have been doing TPOAb determination using the Monobind method, we have done 2040 samples. We

treated a large control group of healthy individuals or patients with non-thyroid diseases. We have shown the values in graph 1.

**Graph 1.** TPOAb in a control group of healthy individuals or patients with non-thyroid diseases.

TPOAb IU/ml



The group of patients with autoimmune thyroiditis (Hashimoto's), consisted of 323 patients with hypothyroidism, and 156 who were still in the euthyroid phase, although we prescribed l-thyroxine replacement for most of them, especially if the values of antibodies to thyroid peroxidase were very high.

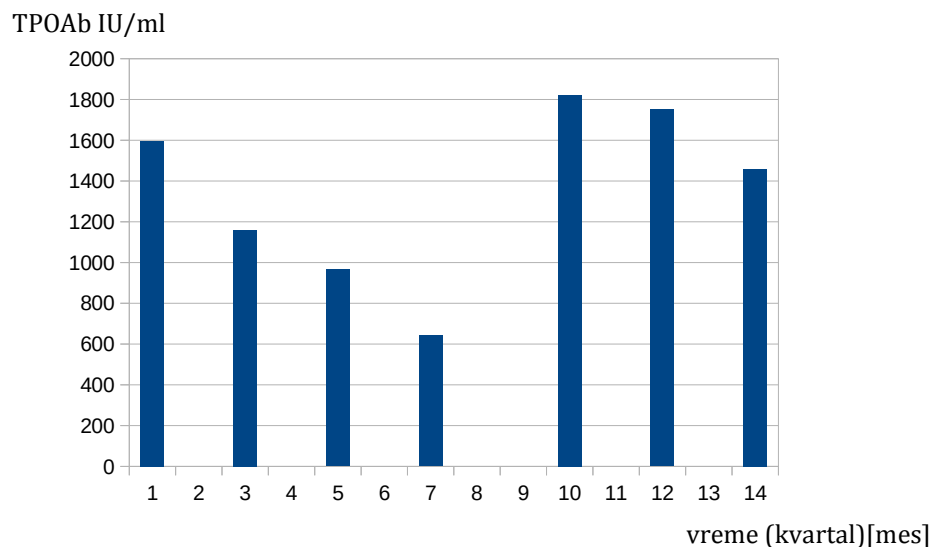
In the last couple of years, we processed the results we obtained from the same patients during follow-up. We wanted to verify the observations we made in a previous study (7), namely that the results are not sufficiently constant in patients on thyroxine substitution therapy. We have illustrated this observation in graphs 2 and 3.

## DISCUSSION

Thyroid peroxidase antibodies (TPOAb) are a parameter used in clinical work to evaluate chronic thyroid diseases. It is especially useful in the first described autoimmune disease - Hashimoto's thyroiditis. The height of this parameter probably depends on the degree of destruction of the thyroid tissue. Unlike the second parameter of thyroid autoimmunity -

thyrotropin receptor antibodies (TRAb), which returns to normal with the passing of immunogenic hyperthyroidism, TPOAb is almost always constant. That's why in these papers we examined what could influence the level of these antibodies. We considered the technical details first. Different kits for testing this parameter do not always have the same "normal values", for example, the Beckman Coulter company has reference values up to 9 IU/ml, unlike other methods that have upper limits of normal from 35-60 (7). It is likely that other epitopes were used for immunization in the production of autoantibodies. A practical tip is to always use the same method if you want to monitor the parameter in the same patient. This is especially important if one wants to use some drugs or supplements (selenium) to influence the course of the disease (8,9). In this work, during the "follow-up" studies, we noticed that about 30% of patients who were only on thyroxine replacement therapy, and do not take other drugs that could eventually affect this parameter (TPOAb), do not have a constant level (graph 2 and 3).

**Grafikon 3.** Praćenje koncentracije antitela na tireocitnu peroksidazu od 2016.–2019. godine kod bolesnice S.C.



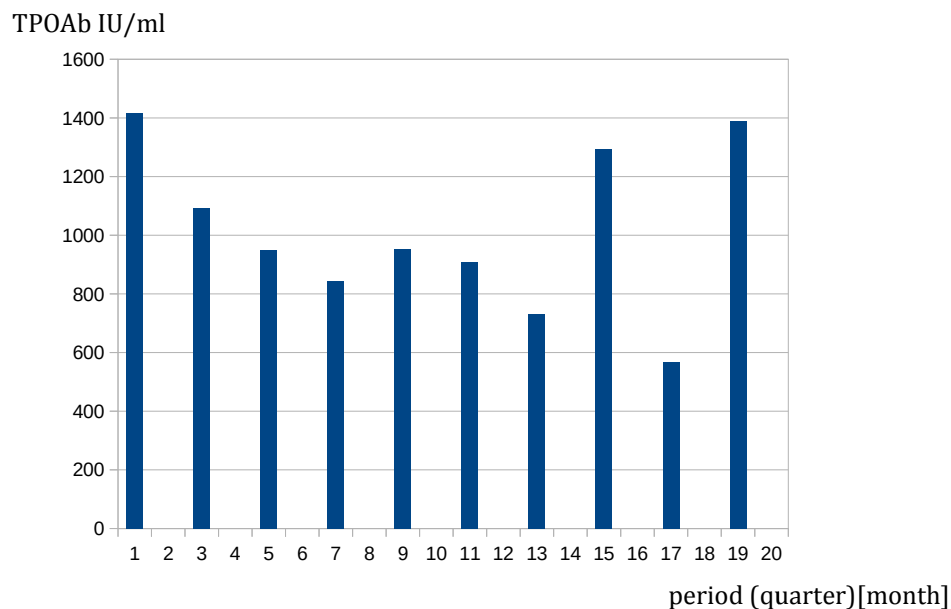
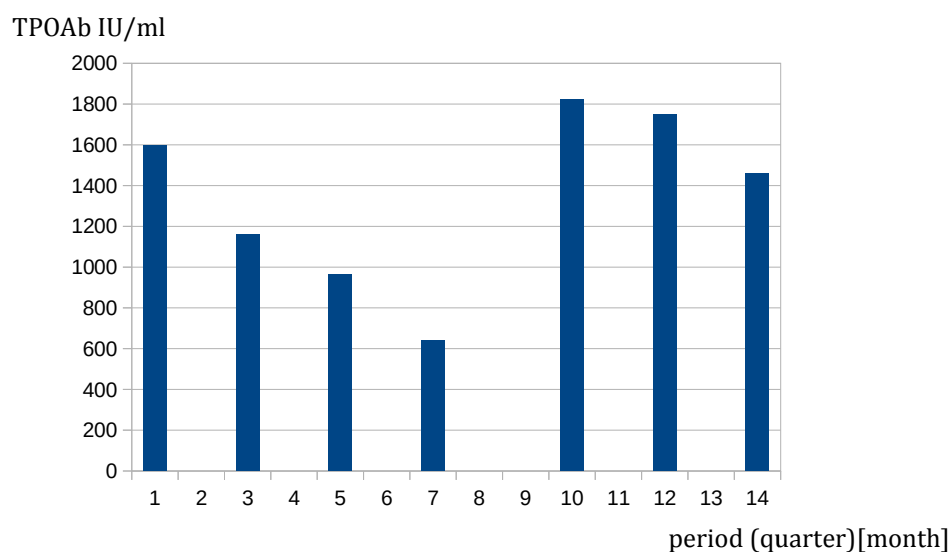
### DISKUSIJA

Antitela na tireocitnu peroksidazu (TPOAb) su parametar koji se u kliničkom radu koristi za procenu hroničnih tireoidnih oboljenja. Posebno je koristan u prvo opisanoj autoimunoj bolesti – Hashimoto tireoiditisu. Visina ovog parametra verovatno zavisi od stepena destrukcije tireoidnog tkiva. Za razliku od drugog parametra tireoidnog autoimuniteta – antitela na tireotropinske receptore (TRAb), koji se sa prolaskom imunogene hipertireoze vraća u normalu, TPOAb je gotovo uvek konstantan. Zato smo u ovim radovima ispitivali šta bi moglo da utiče na visinu ovih antitela. Razmotrili smo prvo tehničke detalje. Različiti kompleti za testiranje ovog parametra nemaju uvek iste „normalne vrednosti“, npr. firma Beckman Coulter, ima referentne vrednosti do 9 IU/ml, za razliku od drugih metoda koje imaju gornje granice normale od 35-60 [7]. Verovatno su drugi epitopi korišćeni za imunizaciju kod produkcije autoantitela. Praktičan savet je da se uvek radi istom metodom ako se želi praćenje

parametra kod istog bolesnika. Ovo je posebno značajno ako se želi nekim lekovima ili suplementima (selen) da utiče na tok bolesti [8,9]. U ovom radu, u toku „follow-up“ studija, primetili smo da oko 30% pacijenata koji su bili samo na supstitucionoj terapiji tiroksinom, koji ne uzimaju druge lekove koji bi mogli eventualno da utiču na ovaj parametar (TPOAb) nemaju konstantan nivo (grafikon 2 i 3).

### ZAKLJUČAK

Prikazali smo rezultate određivanja antitela na tireocitnu peroksidazu. Metoda koju smo koristili daje pouzdane podatke. Sam parametar u rutinskom radu nije pouzdan – u toku kontinuiranog praćenja njegove vrednosti povremeno se javljaju oscilacije za koje nismo našli objašnjenje. Da li se povremeno javlja pojačanje destruktivnih procesa? U ispitivanjima koja će se nastaviti pokušaćemo da nađemo odgovor na to pitanje.

**Graph 2.** Monitoring of thyrocytic peroxidase antibody concentrations from 2016 to 2021 in patient P.B.**Graph 3.** Monitoring of thyrocytic peroxidase antibody concentrations from 2016 –2019 in patient S.C.**CONCLUSION**

We presented the results of determination of antibodies to thyrocytic peroxidase. The method we used provides reliable data. The parameter itself is not reliable in routine work - during the

continuous monitoring of its value, oscillations occasionally occur for which we have not found an explanation. Is there an occasional increase in destructive processes? In further work, we will try to find an answer to that question.

**LITERATURA:**

1. McLachlan SM, Rapoport B. The molecular biology of thyroid peroxidase: Cloning, expression, and role autoantigen in autoimmune thyroid disease. *Endocrine Rev* 1992; 13:192-206.
2. Portmann L, Hamada N, Heinrich G, DeGroot LJ. Antithyroid peroxidase antibody in patients with autoimmune thyroid disease: possible identity with anti-microsomal antibody. *J Clin Endocrinol Metab* 1985; 61:1001-1003.
3. Czarnocka B, Ruf J, Ferrand M, Carayon P, Lissitzky S. Purification of the human thyroid peroxidase and its identification as the microsomal antigen involved in autoimmune thyroid diseases. *FEBS Letters* 1985;190:147-152.
4. Korkij W, Soltani K, Simjee S, Marcincin PG, Chuang TY: Tissue-specific autoantibodies and autoimmune disorders in vitiligo and alopecia areata: a retrospective study. *J Cutan Pathol* 1984; 11: 522-30.
5. Paunković N, Pavlović O, Paunović R.: Dokazivanje antitireoglobulinskih i antimikrozoinskih antitela u hroničnom limfocitarnom tireoiditisu. Zbornik radova, V jugoslovenski simpozijum o štitastoj žlezdi, Zlatibor, 1985, 167-171.
6. Paunkovic N, Paunkovic J. The significance of TSH receptor antibodies and thyroid microsomal antibodies in Graves' disease. *Thyroidol Clin Exp* 1998; 10:13-17.
7. Paunković N, Paunković Dž. Određivanje antitela na tireocitnu peroksidazu – metodološki aspekti i priprema za kliničku primenu. *Tim. Med. glas.* 2017;42(4): 206-208.
8. Duntas L.H, Manzou E, Koutras DA. Effects of a six month treatment with selenomethionine in patients with autoimmune thyroiditis. *European J of Endocrinology* 2003; 148: 389-393.
9. Gartner R, Gasnier BSH, et al. Selenium supplementation in patients with autoimmune thyroiditis decrease thyroid peroxidase antibodies concentrations. *JCEM* 2002; 87: 1687-1691.

**LITERATURE:**

1. McLachlan SM, Rapoport B. The molecular biology of thyroid peroxidase: Cloning, expression, and role autoantigen in autoimmune thyroid disease. *Endocrine Rev* 1992; 13:192-206.
2. Portmann L, Hamada N, Heinrich G, DeGroot LJ. Antithyroid peroxidase antibody in patients with autoimmune thyroid disease: possible identity with anti-microsomal antibody. *J Clin Endocrinol Metab* 1985; 61:1001-1003.
3. Czarnocka B, Ruf J, Ferrand M, Carayon P, Lissitzky S. Purification of the human thyroid peroxidase and its identification as the microsomal antigen involved in autoimmune thyroid diseases. *FEBS Letters* 1985;190:147-152.
4. Korkij W, Soltani K, Simjee S, Marcincin PG, Chuang TY: Tissue-specific autoantibodies and autoimmune disorders in vitiligo and alopecia areata: a retrospective study. *J Cutan Pathol* 1984; 11: 522-30.
5. Paunković N, Pavlović O, Paunović R.: Dokazivanje antitireoglobulinskih i antimikrozomskih antitela u hroničnom limfocitarnom tireoiditisu. Zbornik radova, V jugoslovenski simpozijum o štitastoj žlezdi, Zlatibor, 1985, 167-171.
6. Paunkovic N, Paunkovic J. The significance of TSH receptor antibodies and thyroid microsomal antibodies in Graves' disease. *Thyroidol Clin Exp* 1998; 10:13-17.
7. Paunković N, Paunković DŽ. Određivanje antitela na tireocitnu peroksidazu – metodološki aspekti i priprema za kliničku primenu. *Tim. Med. glas.* 2017;42(4): 206-208.
8. Duntas L.H, Manzou E, Koutras DA. Effects of a six month treatment with selenomethionine in patients with autoimmune thyroiditis. *European J of Endocrinology* 2003; 148: 389-393.
9. Gartner R., Gasnier BSH., et al. Selenium supplementation in patients with autoimmune thyroiditis decrease thyroid peroxidase antibodies concentrations. *JCEM* 2002; 87: 1687-1691.