

## SUMMARY

Toxocariasis is a helminthic zoonosis, caused by the migration and localization of animal nematode larvae in human tissue - mostly *Toxocara canis* in dogs and *Toxocara cati* in cats. The presence of these larvae in organs and tissues causes different pathologic reactions, which are additionally complicated by the reactions of the organism against them and towards permanently produced of them metabolic products. The lack of parasite's eggs and impossibility to identify the larvae in the organism, make the diagnosis difficult, which nowadays is based mainly on serological methods - ELISA and Western blot. The presence of cross reactions with other helminth diseases and long persistence of anti-*Toxocara* IgG antibodies, which are mainly examined in patients, imposes searching of additional diagnostic methods.

That is why we investigate the other isotypes of antibodies - total IgE and specific IgA and anti-*Toxocara* IgG subclass antibodies (IgG1, IgG2, IgG3 and IgG4) to determine their role and significance in *Toxocara* - diagnosis. In addition, we follow up their change in 35 patients in a period of one year after the diagnosis.

In our laboratory conditions, with the application of excretory/secretory antigens from *in vitro* cultivated *T. canis* larvae, we developed serological methods for determination of specific IgG (IgG1-4) subtype antibodies and defined their optimal parameters – antigen concentration - 0.05 µg/ml; sera dilution - 1:100, followed by incubation period of 1 hour in 37<sup>0</sup>C; conjugates dilution - 1:3000 for anti-human IgG1, 1:2000 for IgG2, 1:3500 for IgG3 and 1:3500 for IgG4 and incubation period of 1h/37<sup>0</sup>C. After testing healthy people (blood donors), we determined the diagnostic titer and the specificity of developed methods, which was higher for all reactions of defining IgG1-4 subclasses (95,7% for ELISA IgG2 and IgG4 and 93,5% for ELISA IgG1 and IgG3) from this, determined by ELISA IgG (92%) in the same group.

The presence of specific IgG1 subclass antibodies we have established in 65% of 130 patients with toxocariasis, IgG2 in 43%, IgG3 in 31% and IgG4 in 42% of persons with clinical manifestation and proven serum anti-*Toxocara* IgG antibodies. The

simultaneously presence of the fourth subclasses was found in 7,7% from them. We characterized IgG1-4 subtype antibodies by gender, age and clinical form of the disease (visceral or ocular). Age-related immunity in IgG1, IgG3 and IgG4 subgroups was observed, which was most pronounced in children between 0 to 4 years. Analysis of simultaneously presence of IgG subclasses in patients with toxocariasis revealed a correlation between the existence of specific IgG1 antibodies and the other IgG subtype antibodies - IgG2, IgG3 and IgG4.

The alteration in the presence and the levels of specific IgG subclass antibodies in control examination of *Toxocara* patients 1 year after diagnosis and treatment of the disease revealed retention of specific IgG1 antibodies, IgG3 and IgG4 and negative results for IgG2, which could be used in determination of invasion stage - acute or chronic.

The investigation role of serum anti-*Toxocara* IgA antibodies shown their existence in 26,2% in *Toxocara* patients with highest values in the primary testing. Their depletion in the period between 9 and 12 months, when the specific IgG antibodies still appear, revealed that they are correlated with the early stage of the disease.

Increased levels of total serum IgE antibodies we found in 75% of investigated patients with toxocariasis, such as average concentration was 4 times higher by determined for healthy people. Children with toxocariasis have had significantly higher IgE concentration than the adults. The presence and levels of total IgE antibodies correlated to the presence of specific IgG1 and IgG4 subclass antibodies, which showed development of allergic type immune response against *Toxocara* excretory/secretory antigens. The reduction of total IgE level below the reference limit, which we have established 6 to 9 months after the primary testing, shows that this could be used as a marker for determination disease's activity and could give indirectly information about the efficiency of the treatment.

This new serological methods could be used as additional confirmative tests in *Toxocara* diagnosis. They could find application in determination stage and activity of the disease and could be useful in the follow-up period in patients with this parasitosis.