

DIAGNOSIS DIFFICULTIES IN A CASE WITH HYPERLEUKOCYTOSIS

SORIN IOAN IURIAN¹, LILIANA MOCANU², OFELIA CRIȘTIU³, ANCA VIDRIGHIN⁴

¹Pediatric Clinic Hospital Sibiu, "Lucian Blaga" University of Sibiu, ²Clinical Laboratory, Emergency Clinical Hospital Sibiu, ³Infectious Disease Clinic - Pediatric Clinic Hospital Sibiu, ⁴"Lucian Blaga" University of Sibiu, Polissano Clinic Sibiu

Keywords:
toxocariasis,
hyperleukocytosis,
parasitic coinfection,
child

Abstract: Toxocariasis is a human zoonotic infection caused by ingestion of *Toxocara canis* larvae or *Toxocara cati* larvae. Authors emphasize diagnosis difficulties of a child with hyperleukocytosis. **Material and methods.** Authors present a 3 year-old boy transferred in our clinic for further investigations due to leukemia suspicion. Laboratory investigations: hyperleukocytosis (WBC =104.060/mm³) with 8% eosinophils, normal immunoglobulin serum levels, negative serology for syphilis, for viral infections (Epstein-Barr, Cytomegalovirus, HIV), *Toxoplasma gondii* and *Trichinella spiralis*. Peripheral blood flowcytometry identified high number for CD₄₊-T lymphocytes and 5:1 ratio CD₄₊T cell/CD₈₊T cell; bone marrow aspirate shown eosinophil precursors hyperplasia (17%). Eosinophilia justified additional investigations revealing high serum titers for *Toxocara canis*. Stool exam revealed parasitic co-infection. Imagistic evaluation didn't show abnormalities. Main differential diagnosis included acute and chronic leukemias. Patient received benzimidazole compounds with promptly leukocytosis improvement. **Conclusions.** Authors present a child with parasitic coinfections (toxocariasis, ascariasis, giardiasis, trichuriasis) and hematological anomaly, explaining leukemia suspicion.

Cuvinte cheie:
toxocaroză,
hiperleucocitoză, co-
infestație parazitată,
copil

Rezumat: Toxocaroză este o zoonoză cauzată de ingestia de larve *Toxocara canis* sau *Toxocara cati*. Autorii menționează dificultățile diagnostice la un copil cu hiperleucocitoză. **Material și metode.** Autorii prezintă cazul unui băiat de 3 ani transferat pentru investigații suplimentare în contextul suspiciunii de leucemie acută. Investigațiile de laborator: hiperleucocitoză (leucocite >100.000/mm³) cu eozinofilie (8%), imunoglobuline serice valori normale, serologie negativă pentru lues, Epstein-Barr, Citomegalovirus, HIV, *Toxoplasma*, *Trichinella*. Flowcitometria din sângele periferic: creșterea limfocitelor T CD₄₊ cu raport CD₄₊/CD₈₊=5:1; aspiratul osteo-medular a relevat hiperplazia precursorilor eozinofilelor (17%). Eozinofilia a justificat investigații suplimentare, evidențiind titru crescut pentru *Toxocara canis*. Examenul coproparazitologic a identificat co-infestație parazitată. Investigațiile imagistice nu au arătat modificări patologice. Diagnosticul diferențial a inclus leucemia acută și cronică. Pacientul a primit terapie cu benzimidazoli cu ameliorarea promptă a leucocitozei. **Concluzii.** Autorii prezintă un pacient cu co-infestații parazitare (toxocaroză, ascariadă, giardiază, tricocefaloză) și anomalii hematologice, explicând suspiciunea de leucemie.

INTRODUCTION

I. Toxocariasis.

Toxocariasis is caused by an infection with nematodes parasites (*Toxocara canis* or *Toxocara cati*), the human body being an accidental host. After ingesting the eliminated eggs of the adult parasite, migrating larvae are formed in the human body, a process that can take several months; the larvae can survive several years in the host organism and the body's reaction will limit their development in these organs.

There are 3 clinical variants of disease: larva migrans visceralis, larva migrans ocularis and "covert toxocariasis".

Toxocara canis: life cycle. The adult female of *Toxocara* eliminates 200,000 eggs/ day (the nematode is present in the intestine of young puppies and nursing female dogs). Non-embryonated eggs reach the ground and in wet conditions, they embryonate (the eggs are resistant to extreme variations of pH or frost). Humans and / or dogs ingest embryonated eggs, the larvae develop from eggs at intestinal level, then they pass into the blood/ lymphatic vessels and migrate to various organs. It is

Important to note is that man is the intermediate host and the larvae migrate to organs where they stop their development without becoming adult worms. The conclusion is that *man will not eliminate the eggs of Toxocara in the intestine* (coproparasitologic examination will not reveal parasite eggs). An exception would be the female dog (gestation period) that transmits to the fetus the larvae of the parasite, through the placenta. After the litter, the larvae continue their development, and pass through lungs in the digestive tract and become adult intestinal worms. Afterwards the females reinfest from the puppies and they become together the main source of infestation.

The sources of infection are sand holes from the parks contaminated with *Toxocara* eggs (20-30% of parks soils are contaminated). Infestations were reported after the consumption of snails or raw lamb meat; larva migrans visceralis may be caused by other nematode (*Ascaris suum*, a large roundworm of the pig).(1)

¹Corresponding author: Sorin Iurian, Str. Eschil, Nr. 50, Sibiu, România, E-mail: iurian_sorsab@hotmail.com, Tel: +40723 195 281
Article received on 08.11.2013 and accepted for publication on 10.01.2014
ACTA MEDICA TRANSILVANICA March 2014;2(1):164-166

CLINICAL ASPECTS

Toxocariasis seroprevalence varies from country to country and from region to region (in the USA 4 - 8%, The Netherlands 19%, Germany 2,5%, Slovakia 13%, Czech Republic 5,8 – 36%, Brazil 39%, Spain 0 - 37%, Jordan 9,8%.

Therefore sero prevalence increases with age, children of 1-7 years old have an increased risk of infestation.(2)

The clinical report will be reviewed according to the clinical variant of the disease.

Larva migrans visceralis consists in general signs (fever, fatigue), urticarial lesions, cough, wheezing, abdominal pains, liver enlargement, arthralgia, myocarditis (not a rare feature) (3,4), eosinophilic meningitis (aseptic meningitis), encephalitis (5), epilepsy.(6)

Larva migrans ocularis causes unilateral ocular disease; it is found in children and teenagers; in this disease variant there are lacking of general symptoms or eosinophilia; in addition, the patient can present leukokoria, ocular pains, strabismus, visual impairment (it is necessary the differential with retinoblastoma).(7)

Covert toxocariasis (most of cases are free of symptoms) is more frequent accidentally diagnosed in context of serological evaluations. It manifests as recurrent abdominal pains, cough, wheezing, hepatomegaly, headache, anorexia, growth impairment. It's important to specify that eosinophilia is a rare condition and the anti-Toxocara antibodies titer is low to moderate.

Laboratory diagnosis is based on:

- leukocytosis with eosinophilia (up to 80%);
- hypergammaglobulinemia IgM;
- serological testing (ELISA) in order to identify anti-Toxocara antibodies (90% specificity, 75% sensitivity); serological tests aren't useful in order to distinguish between new infections and old ones; in larva migrans ocularis the serum antibodies levels are low to normal but increased in vitreous humour;
- western-blotting technique (time-consuming) is more sensitive compared to ELISA;
- PCR (polymerase chain reaction) – useful method for parasite identification in the tissue;
- for „covert toxocariasis” diagnosis are important the following symptoms: positive Toxocariasis serology, fatigue, abdominal aches, urticaria un-triggered by allergens and also eosinophilia.

Imaging methods include: chest X-ray (in case of wheezing or coughing) is able to identify eosinophilic pneumonia, pleuritis and/ or cardiomegaly; the echocardiography is useful for myocardial contractility estimation and pericarditis identification; ultrasound abdominal exam can reveal liver granuloma; cerebral CT scan and MRI cerebral are used for central nervous system valuation.

Regarding *surgical procedures*, the tissue biopsy identifies granuloma with eosinophilic and neutrophilic and (sometimes) larva remnants.

Treatment. Most patients cure without therapy. The medical treatment is recommended in case of cerebral, pulmonary or cardiac involvement (larva migrans visceralis): Tiabendazole, Diethylcarbamazine, Albendazole (400 mg/day for 5 days, absorption is facilitated by lipids), Mebendazole (200 mg/day for 3 days). Antiparasitic medication is associated with corticosteroids in order to limit augmentation of inflammation after parasite destruction. In case of larva migrans ocularis, glucocorticoids (without antiparasitic therapy) is recommended and, if needed, surgical intervention. For covert toxocariasis, the treatment depends on the patient's age, severity of symptoms and certainty of diagnosis.

The prevention does not includes just periodical disinfestation of cats and dogs, but also implementation of measures that could limit the free access of animals in children's playgrounds.(8)

The prognosis is favourable for larva migrans visceralis and covert toxocariasis; larva migrans ocularis prognosis depends on the moment when the diagnosis is established (amaurosis risk is increased in case of late diagnosis).

Educational measures refer to observing the rules of hygiene.

CASE REPORT

The authors present a 3-year old male patient who was hospitalized in Contagious Diseases Hospital because he had mucus and blood in stools. The symptoms started 3 days before hospitalisation and the patient had followed a diet without receiving any medication.

Family history: healthy parents, no consanguinity; illiterate mother.

Patient history: the 1st born child in the family (having a 14 months old healthy brother); he was born at 9 months gestational age at Maternity Hospital from Sibiu; he weighted 3.200 kg at birth; APGAR = 10/ 1 minute; the mother has smoked during pregnancy.

Regarding the *personal history:* the patient was hospitalised in the past for lower respiratory tract infection.

Motor and mental skills were suitable according to the age.

Living conditions: insanitary residence, 4 people/1 room.

Concerning *disease history*, the symptoms appeared 3 days before hospitalisation; the patient complained having watery and bloody stools so he was hospitalised in Contagious Disease department. Based on clinical report and investigations, the patient was suspected of acute leukemia (see hyperleukocytosis) and he was transferred at Pediatric Care Hospital .

The necessary investigations were made at Contagious Disease department, as follows:

- complete blood count (CBC) revealed microcytic anemia (haemoglobin level = 11.9 g/dl, MCV =74.2 μm^3), reactive thrombocytosis (437.000 / mm^3), leukocytosis (WBC 104,060 / mm^3) with lymphocyte predominant pattern (82% lymphocytes, 8% neutrophils and 8% eosinophils);
- peripheral blood smear: microcytic and hypochromic erythrocytes; no morphological alteration of leukocytes;
- inflammatory status: normal ranges for "C" reactive protein and ESR;
- iron blood level: normal;
- normal amylasemia, normal calcemia and normal phosphate blood level;
- normal urine test;
- from immunological point of view: antinuclear antibodies, serum complement and immunoglobulin isotypes in normal ranges;
- negative results for strep throat exam, negative results for Rotavirus/Adenovirus test and stool culture.

Clinical report at the admission in Pediatric Care Hospital revealed:

- impaired nutritional status (weight 12 kg, < 3rd percentile), feverless patient;
- skin pallor;
- appendages disorders, pediculosis capitis;

CLINICAL ASPECTS

- no lymph nodes enlargement;
- rickets signs (Harrison's groove, skull bossing);
- the examination of respiratory, cardio-vascular and uro-genital tracts was normal;
- gastro-intestinal tract exam: congestion of pharynx, no hepatomegaly, no splenomegaly;
- Central nervous system: no meningitis clinical signs, normal pupillary response.

The *investigations* performed at Pediatric Hospital revealed:

- CBC: microcytic anemia and leukocytosis ($60.380/\text{mm}^3$) with eosinophilia (absolute eosinophils counted $5,950/\text{mm}^3$);
- the peripheral blood flow cytometry test identified a CD_{45}^+ lymphocyte population (57%) with following features: $\text{CD}_{3}^+ = 59\%$, $\text{CD}_{5}^+ = 71\%$, $\text{CD}_{8}^+ = 13\%$, $\text{CD}_{4}^+ = 64\%$, $\text{CD}_{19}^+ = 16\%$ (B lymphocytes kappa 47% and B lymphocytes lambda 40%); in conclusion proliferate T lymphocyte CD_{4}^+ proliferation;
- bone marrow aspiration emphasized hypercellularity characterised by eosinophil granulocyte hyperplasia (17%, normal range $< 7\%$) and Granulocyte: Erythrocyte ratio = 3:1; no alterations for lymphocyte and erythrocyte precursors; 2% blasts;
- negative inflammatory status;
- microbiological investigations, in addition to the previous ones, they have revealed: negative serology for infectious mononucleosis, lues, cytomegalovirus, *Trichinella spiralis*, *Toxoplasma gondii* and HIV; stool ova and parasites exam identified, using Kato-Miura concentration method, *Ascaris lumbricoides* ova, *Trichiuris trichiura* ova and giardia lamblia cysts;
- ophthalmological examination: no alterations for anterior pole of the eye and ocular fundus;
- imagistic evaluations (chest X-ray, abdominal ultrasound exam) were normal.

Based on inconsistencies between peripheral blood and bone marrow, a series of theories were elaborated:

- peripheral lymphocytosis is probably secondary to peripheral lymphoid organ stimulation;
- peripheral eosinophilia was hidden by almost normal percentage of eosinophils;
- "central" eosinophilia represents an authentic marker for eosinophilia.

First step diagnosis included: hyperleukocytosis, parasitic co-infections, weight deficit, anemia and rickets sequelae.

The major criterion for *differential diagnosis* was lymphocytosis (by definition, absolute lymphocyte count more than $4.000/\text{mm}^3$), monocytosis ($> 950/\text{mm}^3$), granulocytosis ($> 7.000/\text{mm}^3$), eosinophilia ($> 500/\text{mm}^3$), basophilia ($> 150/\text{mm}^3$) and leukemia.

In the case of "central" eosinophilia, authors performed additional tests: total IgE serum level (1098 KUI/l, normal range < 60) and *Toxocara canis* serology (IgG antibodies anti- *Toxocara canis*, ELISA method = 26,89 NTU, normal range < 11), confirming *Toxocariasis*.

The positive diagnosis was parasitic co-infections (*toxocariasis*, *ascariasis*, giardia lamblia infections and *trichuriasis*) in a patient with anemia, rickets sequelae and weight deficit).

Even though the acute versus chronic nature of *Toxocara canis* infection could not be established, antiparasitic therapy was initiated (Albendazole 400 mg/day orally, 4 consecutive days). The absolute leukocytes number decreased ($60.380/\text{mm}^3$) before the therapy started.

DISCUSSIONS

These are few questions that remain to be analyzed:

- *Leukocytosis is secondary to toxocariasis?* As „con” argument the leukocytosis improvement was noticed before initiation of antiparasitic therapy. Among „pro” arguments, studies had confirmed the production of glycosylated proteins by *Toxocara* larvae; these proteins induce a Th2-type CD_{4}^+ cellular immune response characterized by stimulation of interleukin 4 that promotes the switch from B-lymphocytes to immunoglobulin E synthesis; in conclusion, lymphocytosis correlated with $\text{Th}_{2}(\text{CD}_{4})$ predominance and high IgE serum levels are changes that are identified with those of *Toxocariasis*.
- *Was antiparasitic therapy necessary in this case?* Serological tests were not able to differentiate recent and old *toxocariasis*, so the therapy remains disputable and the decision belongs to the clinician.
- *If we consider hyperleukocytosis ($\text{leukocytes count} > 100.000/\text{mm}^3$) as secondary to parasitic co-infections, which parasitic agent is mainly responsible for it?* This question has a merely theoretical approach; high probability for *toxocariasis* it is considered to be involved in hyperleukocytosis. But in medical practice, the question is not justified because antiparasitic therapy is the same.

The case peculiarities: absolute leukocyte number exceeds $100.000/\text{mm}^3$; simultaneous infections with four parasitic species (*toxocara canis*, *ascaris lumbricoides*, *trichiuris trichiura* and giardia lamblia); parasitic co-infections is explained by the lack of a basic sanitation infrastructure and low level for medical knowledge of the population.

REFERENCES

1. Maruyama H, Nawa Y, Noda S, et al. An outbreak of visceral larva migrans due to *Ascaris suum* in Kyushu, Japan. *Lancet* 1996;347(9017):1766-7.
2. Nelson S, Greene T, Ernhart CB. *Toxocara canis* infection in preschool age children: risk factors and the cognitive development of preschool children. *Neurotoxicol Teratol* 1996;18(2):167-74.
3. De Cock C, Lemaitre J, Deuvaert FE. Loeffler endomyocarditis: a clinical presentation as right ventricular tumor. *J Heart Valve Dis*. Nov 1998;7(6):668-71.
4. Herry I, Philippe B, Hennequin C, et al. Acute life-threatening toxocaral tamponade. *Chest* 1997;112(6):1692-3.
5. Tobin EH, Zhang J, Maton B. Meningoencephalitis and visceral larva migrans in a woman with intense exposure to cats. *Infect Dis Clin Pract* 2011;19(3):221-2.
6. Salvador S, Ribeiro R, Winckler MI, Ohlweiler L, Riesgo R. Pediatric neurotoxocariasis with concomitant cerebral, cerebellar, and peripheral nervous system involvement: case report and review of the literature. *J Pediatr (Rio J)* 2010;86(6):531-4.
7. Ocular toxocariasis-United States, 2009-2010. *MMWR Morb Mortal Wkly Rep* 2011;60(22):734-6.
8. Centers for Disease Control and Prevention. Ocular toxocariasis United States, 2009-2010. *MMWR Morb Mortal Wkly Rep* 2011;60(22):734-6.