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A Serological Follow-up of Toxocariasis Patients After Chemotherapy Based on the Detection of IgG, IgA, and IgE Antibodies by Enzyme-Linked Immunosorbent Assay

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A serological follow-up study was carried out on 27 children (1–12 years old) with visceral and/or ocular toxocariasis, after treatment with thiabendazole. A total of 159 serum samples were collected in a period ranging from 22–116 months. Enzyme-linked immunosorbent assays (IgG, IgA, and IgE ELISA) were standardized, using excretory–secretory antigens obtained from the second-stage larvae of a *Toxocara canis* culture. The sensitivity found for the IgG, IgA, and IgE ELISA, as determined in visceral toxocariasis patients, was 100%, 47.8%, and 78.3%, respectively. Approximately 84% of the patients presented single or multiple parasitosis, as diagnosed by stool examination, yet such variables did not appear to affect the anti-*Toxocara* immune response. Titers of specific IgE antibody showed a significant decrease during the first year after treatment, followed by a decrease in the IgA titers in the second year, and in the IgG titers from the fourth year onwards. Sera from all patients presented high avidity IgG antibodies, indicating that they were in the chronic phase of the disease. Moreover, 1 year after treatment, the level of leukocytes, eosinophils, and anti-A isohemagglutinin in patients decreased significantly. The present data suggest that IgE antibodies plus eosinophil counts are helpful parameters for patient follow-up after chemotherapy. J. Clin. Lab. Anal. 20:164–172, 2006. © 2006 Wiley-Liss, Inc.

Key words: toxocariasis; serodiagnosis; immunoenzymatic assay; immunoglobulin isotypes; chemotherapy

INTRODUCTION

Toxocariasis is a cosmopolitan zoonosis caused by the nematode parasites *Toxocara canis*, a common canine roundworm, and *Toxocara cati*, frequently found in cats. Humans are accidentally infected by ingestion of embryonated *Toxocara* eggs that hatch in the small intestine and liberate migrating larvae, which may then invade various organs (1). Among the risk factors for human infection are the size of the local animal population, a high prevalence of *Toxocara* species in dogs and cats, soil contamination by infective *Toxocara* eggs, and low socioeconomic conditions (2). *Toxocara* larvae secrete and excrete products that are highly immunogenic, which promotes a Th2 type cellular immune response, leading to the production of inter-

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leukin 4 and 5 and causing IgE-antibody production and eosinophilia (3).

The clinical manifestations of toxocariasis are determined by the size of the *Toxocara* inoculum, frequency of reinfection, organ localization of the larvae, and host response (4). The spectrum of clinical manifestations varies widely, ranging from predominantly asymptomatic cases to those with severe organ injury. Visceral and ocular toxocariasis are the most common clinical forms. Visceral toxocariasis affects primarily 1- to 5-year-old children who may present fever, anemia, hepatomegaly, and pulmonary manifestations (5). On the other hand, ocular toxocariasis occurs in children older than 8 years of age and its clinical manifestations are related to the presence of larvae in the eyes (6). Covert toxocariasis, in turn, has been described as presenting nonspecific symptoms, such as abdominal pain, headache, cough, and limb pains (7). Furthermore, in some instances, neurological manifestations have been associated with the presence of *Toxocara* larvae in the brain (2).

In the absence of parasitological evidence of infection, diagnosis of toxocariasis has relied mainly on immunological methods (1). Thus, the *Toxocara* excreted–secreted antigens (TES), previously described (8), have been applied to different immunological assays (9,10). The TES-based enzyme-linked immunosorbent assay (ELISA) for detection of IgG specific antibodies, in particular, is widely preferred for diagnostic purposes and also for seroepidemiologic surveys (10–12). Moreover, measurement of avidity (functional affinity) of specific IgG antibodies seems to be useful to discriminate between chronic and early phases of the infection, as is the case for other infectious diseases. In other words, high avidity IgG antibodies are associated with the chronic phase and low avidity IgG with freshly acquired toxocariasis (13). There are few follow-up studies of toxocariasis patients after chemotherapy, but it has been reported that specific IgG antibody levels remain elevated for many years (14). In addition, the significance of IgE antibodies in view of toxocariasis diagnosis has seldom been investigated (15,16).

The present work is a follow-up study of toxocariasis patients after chemotherapy with thiabendazole. At this stage, the disease was evaluated through the measurements of specific IgG, IgA, and IgE antibodies along with the determination of IgG avidity in serum samples.

MATERIALS AND METHODS

Subjects

Serum samples from 27 children with toxocariasis (23 with visceral, three with both visceral and ocular, and one with ocular form) ranging from 1–12 years old were evaluated yearly after chemotherapy for a period up to 9 years. The diagnosis was based on clinical, epidemiologic, and laboratory findings. Before treatment, all patients presented anti-*Toxocara* IgG antibodies detected by ELISA, and all of those with visceral toxocariasis presented eosinophilia $\geq 10\%$. The main clinical symptoms were: pulmonary manifestations in 73.9% of the patients, asthma and/or rhinitis in 56.5%, and fever in 39.1%. In patients with ocular toxocariasis, uveitis and retinal detachment of the right eye constituted the main clinical manifestation. Patients were treated with thiabendazole (25 mg/kg/day) for 7 days. In the ocular cases, corticotherapy was associated (prednisone, 1 mg/kg/day).

The control group consisted of 96 clinically healthy individuals, with negative serology for *Toxocara* antibodies, of whom 68 were blood donors and 28 were children from Instituto da Criança do Hospital das Clínicas da Faculdade de Medicina da Universidade de São Paulo (ICr-HC-FMUSP).

Production of TES

Antigen was prepared as described by Rubinsky-Elefant et al. (17), following a modified version of the method published by De Savigny (8). Briefly, *T. canis* eggs were collected from the uterus of female worms obtained from dog necropsies (Centro de Controle de Zoonoses de Sao Paulo, Sao Paulo, Brazil), and in order to embryonate them, they were incubated in 2% formalin for approximately 1 month at 28°C. Embryonated eggs were artificially hatched in serum-free Eagle's medium, and the second-stage larvae were recovered by transferring the suspension on a loose cotton wool plug in a Baermann apparatus. After approximately 4 hr, larvae were collected from the bottom of the apparatus. Cultures containing the larvae were incubated at 37°C, and at weekly intervals, the culture fluid was removed and replaced with fresh medium. To the culture supernatant that constituted the antigen, protease inhibitor was added (5 μL/mL of 200 mM phenylmethyl-sulfonyl-fluoride), concentrated in an Amicon apparatus, dialyzed against distilled water, centrifuged (18,500 g) at 4 °C, for 60 min, and filtered in 0.22 μM Millipore membrane (Millipore, Danvers, MA). The protein content was determined by the Lowry protein assay (18). Three different batches of TES antigens were produced and their reproducibility was evaluated by a 10% sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE), followed by silver staining (19). The antigens were stored in aliquots at –20°C.

Production of *Ascaris suum* Adult Worm Extract (AWE)

Nonspecific antibodies were removed by serum absorption with AWE. This antigenic extract was

obtained based on a previously described method (20) with modifications. In brief, *A. suum* worms recovered from porcine intestine were macerated in distilled water, and 1.5 M NaOH was added to reach a final concentration of 0.15 M. After 2 hr at room temperature, the mixture was neutralized with 6M HCl and centrifuged (18,500 g) at 4°C for 20 min. The pellet was discarded and the protein content of the supernatant was analyzed by the Lowry assay. The extract containing approximately 5 mg/mL protein was delipidized with ether (1/3, v/v). The ether layer was removed and the antigenic extract was filtered in 0.22 μm Millipore membrane and stored in aliquots at −20°C.

Standardization of ELISA

The anti-*Toxocara* IgG, IgA, and IgE antibodies were detected by ELISA, based on the method described by De Savigny et al. (11) with some modifications. Ninety-six well microtitration flat-bottom polystyrene plates (Nunc Polysorp, Rochester, NY) were loaded (100 μl/well) with TES antigen solution (1.9 μg/mL in a 0.1 M carbonate–bicarbonate buffer, pH 9.6). The plates were maintained for 2 hr at 37°C, thereafter, for 18 hr at 4°C in a humid chamber, and then washed three times with 0.01 M phosphate-buffered saline pH 7.2 that contained 0.05% Tween 20 (PBS-T). The blocking solution for IgG- and IgA-ELISA was 200 μl of 1% bovine-serum-albumin in PBS-T (BSA, Sigma, St. Louis, MO), and for IgE-ELISA it was 5% skim-milk (Molico, Nestle, Aracatuba, Sao Paulo, Brazil) in PBS-T. In both assays, the incubation period was carried out for 1 hr at 37°C. After three washing cycles, plates were incubated with serum samples for 40 min at 37°C. All sera were pre-absorbed with AWE (25 μg/mL) in PBS-T for 30 min at 37°C. Serum samples were diluted 1/80 for IgG-ELISA (100 μl/well), 1/50 for IgA-ELISA (100 μl/well), and 1/50 for IgE-ELISA (50 μl/well). In the IgE-ELISA, serum samples were also pre-absorbed with AWE (25 μg/mL) and with RF-Absorbent v/v (Dade Behring, Marburg, Germany) for 18 hr at 4°C. All sera were two-fold serially diluted. After incubation with serum samples, the plates were washed three times and incubated with peroxidase conjugates as follows: 1/16,000 dilution (100 μl/well) of anti-human IgG (Biolab Diagnostic, Brazil, Rio de Janiero, Brazil); 1/5,000 dilution (100 μl/well) of anti-human IgA (Sigma); and 1/500 dilution (50 μl/well) of anti-human IgE (Sigma). After incubation for 40 min at 37°C, the plates were washed three times with PBS-T. As a chromogen substrate (100 μl/well) ortho-phenylenediamine (0.4 mg/mL, OPD-Fast, Sigma) and H_2O_2-urea (0.4 mg/mL), in 0.05 M citrate buffer, were added for 15 min at 37°C. The reaction was stopped with 50 μl of 2 M H_2SO_4. The assay was monitored by including negative and positive serum samples, in addition to a blank (without serum sample). Absorbance at 492 nm was determined in an automatic microplate reader (Titertek Multiskan MCC/340, Lab-system, Helsinki, Finland). The cutoff values were calculated based on the mean absorbance of negative serum controls—± 3 SDs for IgG-ELISA (0.500) and IgA-ELISA (0.200), and ± 2 SDs for IgE-ELISA (0.300).

Avidity of IgG Antibodies by ELISA

Determination of the avidity index (AI) of IgG antibodies was based on the dissociative method using urea as a denaturing agent (21). Sera were diluted serially, two-fold, in PBS-T, and applied in duplicate to the microtiter wells. After incubation for 40 min at 37°C, one well of the doublet was washed with PBS-T three times for 5 min; likewise, the other well was washed with 8 M urea dissolved in PBS-T. The avidity index was calculated by dividing the absorbance of the urea-treated sample by the untreated sample and multiplied by 100. All values higher than 50% were ranked as IgG with high avidity (22).

Complementary Assays

Leukocyte and eosinophil blood count

Blood cell counting was performed in the laboratory of the ICr-HC-FMUSP, using Advia apparatus (Bayer, Tarrytown, NY).

Isohemagglutinin titers

Titers of isohemagglutinin were determined in the Hemocentro de São Paulo, HC-FMUSP, using conventional microplates or tube techniques with human red blood cells.

Fecal parasitologic examinations

Examinations were performed in Laboratório Central-HC-FMUSP by Faust or Hoffman techniques (for helminth and protozoan eggs) and by Rugai with modifications (for *Strongyloides stercoralis* larvae) (23).

This project was approved by the Comitê de Ética em Pesquisa of the Instituto de Medicina Tropical de São Paulo and of the ICr-HC-FMUSP as well as by the Comissão de Ética em Pesquisas com Seres Humanos of the Instituto de Ciências Biomédicas-USP.

Statistical analysis

Student's *t*-test (paired samples) for parametric or Wilcoxon method for nonparametric data were used. The comparison of multiple groups for independent samples was made by Kruskal-Wallis, Dunn's method. Statistically

significant values were set at the $P<0.05\%$ level. (SigmaStat program, Systat Software Inc., Richmond, CA).

RESULTS

Three different batches of TES antigens were produced and evaluated by silver-staining SDS-PAGE gels. The batches proved to be reproducible, showing a predominance of the 105–120, 70, 55, 44, 31–35, and 27 kDa bands (Fig. 1). Serological assays were performed with aliquots from the same TES batch in order to reduce possible variables.

The median values for IgG antibody titers, as detected by ELISA in the follow-up study of 23 patients with visceral toxocariasis after chemotherapy, are shown in Fig. 2. The cutoff value was 0.508, corresponding to the optical density average from 96 serum samples from healthy individuals plus 3 SDs. The results showed a significant decrease in the median values for IgG titers after the fourth year of treatment (Wilcoxon signed rank test, $P<0.05$).

The sensitivity of the ELISA tests for IgA and IgE was 47.8% (cutoff = 0.228) and 78.3% (cutoff = 0.292), respectively. The median values obtained for IgA titers decreased (Wilcoxon test, $P<0.05$) after the second year of treatment (Fig. 3), while the values found for IgE antibodies indicated an earlier decrease (Wilcoxon test, $P<0.05$) in the first year (Fig. 4).

There was only one patient with ocular toxocariasis as a sole form of the disease; the remaining three patients had both ocular and visceral forms. Therefore, the related data could not be statistically evaluated. The follow-up result for the patient having only the ocular manifestation was lower IgG titers (1/80) in the first sampling point as compared to those with visceral toxocariasis. Among patients with both visceral and ocular forms, the level of antibody response varied from low (1/160), medium (1/640), and high (1/10,240) titers of IgG antibodies. Anti-TES IgA antibodies were detected only in the patient with ocular toxocariasis (1/50 titer), while anti-TES IgE antibodies were found in a patient with both ocular and visceral forms (1/800 titer).

Figure 5 shows the mean values for the avidity index of IgG antibodies from patients with visceral toxocariasis after chemotherapy. A high avidity index was observed since the beginning of treatment.

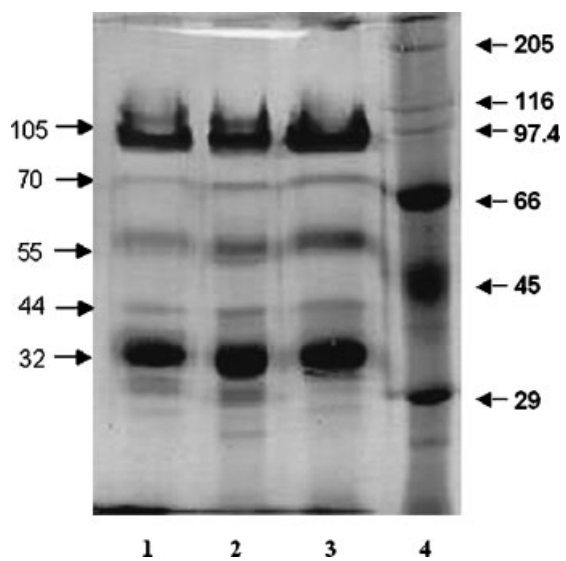

Fig. 1. Reproducibility of *Toxocara* excretory–secretory (TES) antigens in a 10% sodium dodecyl sulfate polyacrylamide gel (SDS-PAGE) with silver staining. Lanes 1–3: Three different batches of TES antigens. Lane 4: Standard marker for molecular weight (kDa). Values on the left indicate the kDa of the major bands of the *Toxocara* excretory–secretory antigens.

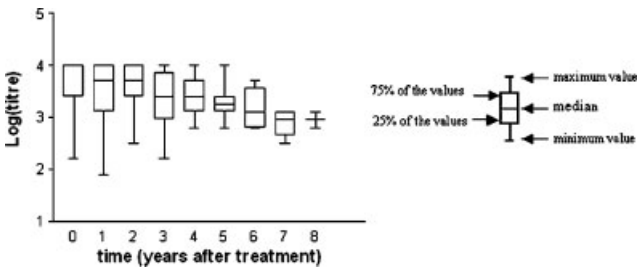

Fig. 2. Median values for IgG antibody titers, detected by ELISA, in the follow-up study of 23 patients with visceral toxocariasis after chemotherapy.

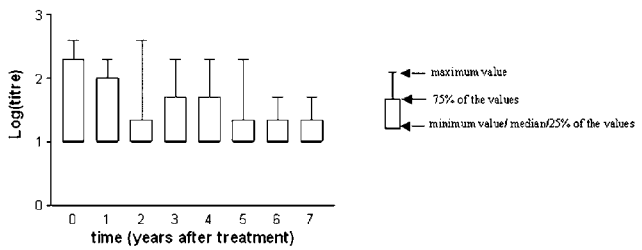

Fig. 3. Median values for IgA antibody titers, detected by ELISA, in the follow-up study of 23 patients with visceral toxocariasis after chemotherapy.

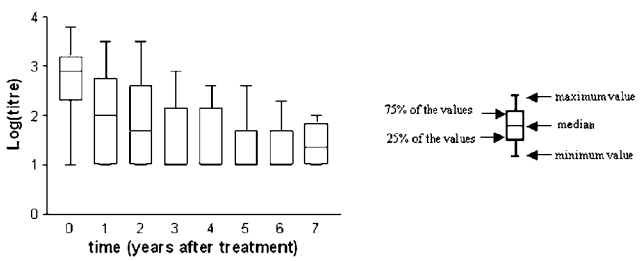

Fig. 4. Median values for IgE antibody titers, detected by ELISA, in the follow-up study of 23 patients with visceral toxocariasis after chemotherapy.

Concerning the complementary assays, there was a decrease in the absolute numbers of leukocytes and eosinophils (Student's *t*-test, $P<0.05$; Wilcoxon, $P<0.05$). In the case of anti-A isohemagglutinin, a decrease in titers was also found one year after chemotherapy (Student's *t*-test, $P<0.05$). On the other hand, the anti-B isohemagglutinin titers remained unchanged (Student's *t*-test, $P>0.05$) throughout the studied period. Figure 6 illustrates the findings for leukocytes and eosinophils, and Fig. 7 illustrates those for anti-A and anti-B isohemagglutinins.

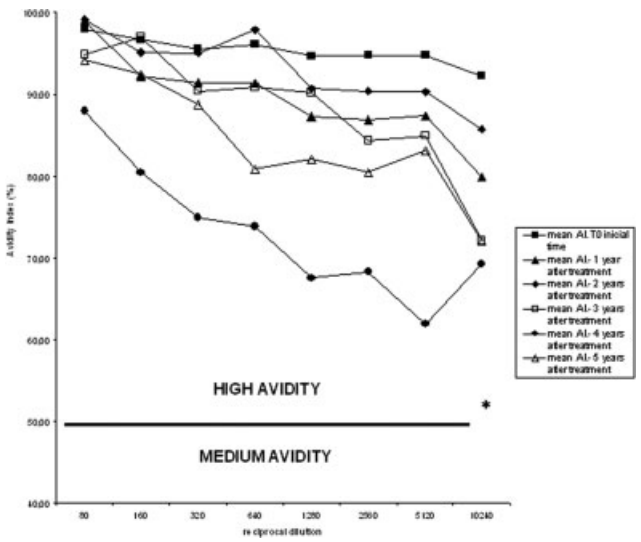

Fig. 5. Mean avidity index (AI) for IgG antibodies, detected by ELISA, in the follow-up study of visceral toxocariasis patients after chemotherapy. *The line separates the high and medium avidity values, according to Hedman and Rousseau (22).

Fecal parasitologic examinations revealed positive results for 84% of the patients, of whom 40% presented one or two different parasite species, and the remaining 44% more than 3 species. *Ascaris lumbricoides* and *Giardia lamblia* were detected in 52% and 48% of the patients, respectively. In order to investigate whether the presence of different intestinal parasites could be interfering in the ELISA results, as well as in the eosinophil counts, patients were divided into three groups: 1) without intestinal parasites, 2) with one or two intestinal parasites, and 3) with three or more intestinal parasites. There was no statistical difference between these groups either in terms of antibody levels or white cell counts (Kruskal-Wallis, $P>0.05$).

DISCUSSION

Human toxocariasis has been widely investigated, but many questions related to diagnosis, trend of immunological and cell profiles during follow-up, and prognosis remain to be elucidated. Undoubtedly, the use of the TES antigens in ELISA tests has contributed to improving epidemiologic surveys as well as clinical and laboratory trials (11). Although being an excellent tool in the above cited circumstances, serologic testing for anti-*Toxocara* antibodies has been of limited use for diagnosis, since antibody levels hardly correlate with clinical outcomes (24).

In this study, the TES antigens preparation has shown the 104–120, 87, 61–65, 44, 31–35. and 27 kDa bands, which are similar to the fractions of 120, 70, 55, and 32 kDa described by Maizels et al. (25). Badley et al. (26)

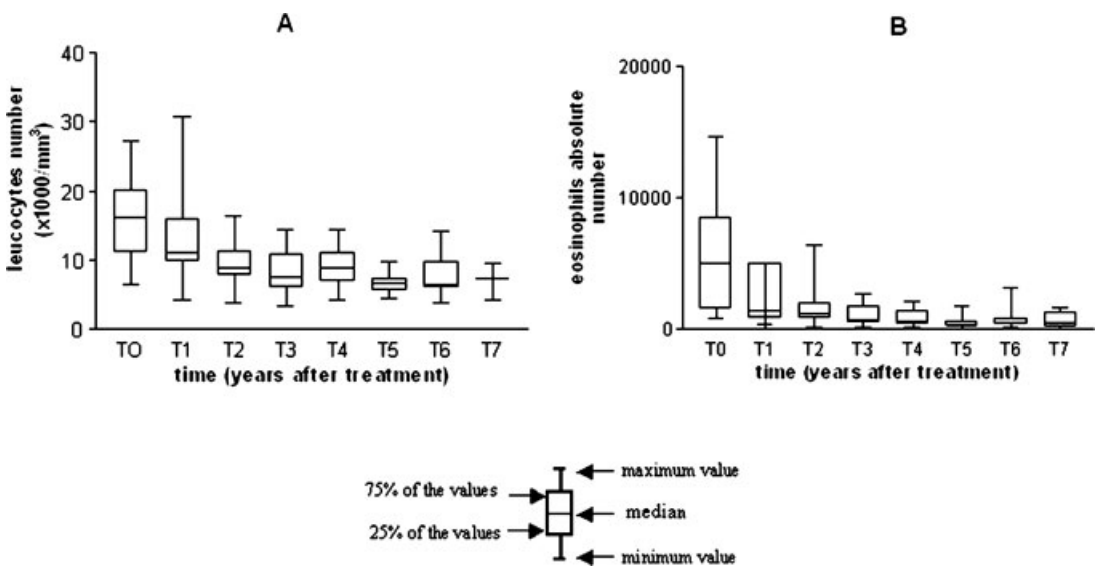

Fig. 6. Median of leukocytes number (**A**) and eosinophils absolute number (**B**) in the follow-up study of 21 patients with visceral toxocariasis after chemotherapy.

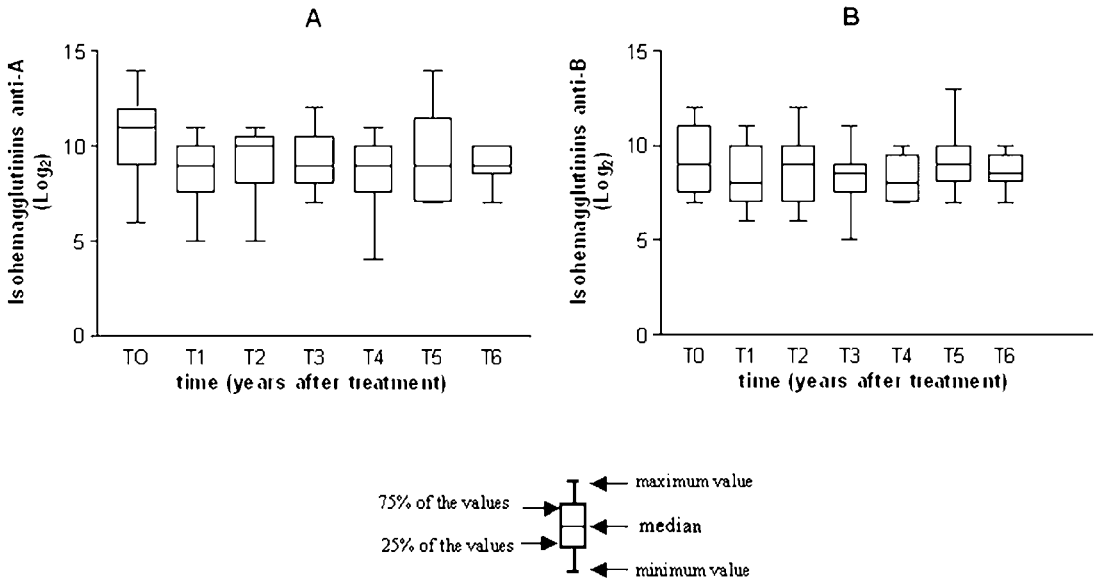

Fig. 7. Median of isohemagglutinin anti-A (**A**) and anti-B titers (**B**) in the follow-up study of patients with visceral toxocariasis after chemotherapy.

found 15 different protein fractions and suggested that differences in the antigen fractions could be explained by technical modifications in TES production or even in methods used for the identification of such fractions. In our case, good reproducibility among different TES batches has been attained. This could be due, at least in part, to the presence of protease-inhibitor PMSF preventing the activity of serine-proteases secreted by *T. canis* larvae (27). In most instances, providing a reliable diagnosis for toxocariasis is a difficult task; biopsies are rarely performed and infection is undetectable in clinically asymptomatic cases. Thus, the sensitivity and specificity of serological tests must be improved.

We took clinical, laboratory, and epidemiologic data into account to classify cases as positive. Gillespie et al. (28) considered clinical signs, symptoms, laboratory data, and eosinophil counts to establish a diagnosis for visceral or ocular toxocariasis. Similarly, Dubinsky et al. (29) used eosinophil counts and clinical symptoms to define cases of toxocariasis.

To define serologic cases as negative by means of the cutoff line, some precautions must be taken. In Switzerland, approximately 5% of the blood donors exhibited anti-*Toxocara* antibodies (30); but in some other countries including the Slovak Republic and the Czech Republic, such antibodies occurred in 15–20% of the healthy population (29). In this study, 5% of the blood donors were seropositive, in a similar fashion to that found in Switzerland. Serum samples from healthy children of the ICr-HC-FMUSP were also included to determine a more reliable cutoff value. The cutoff value obtained for the IgG antibody corresponded to an optical density (OD) of 0.5, resembling those observed by others. Aguila et al. (31) stipulated that serum with optical density over 0.5 (492 nm) should be considered as toxocariasis-positive. On the other hand, De Savigny et al. (11) and Tonz et al. (32) considered 0.5 OD as an absence of infection or a negative result, and Luzna-Lyskov et al. (33) interpreted samples with OD values below 0.6 as non-reagent serum.

In a review of toxocariasis serodiagnosis, Taylor (34) has pointed out some technical handicaps and other factors influencing the diagnostic efficiency: 1) lack of a universal unit for expressing titers, 2) variation in methods between laboratories, 3) disagreement over where to draw the cutoff line, 4) differences among surveyed populations, and 5) interference of unrelated diseases, such as asthma, in the reactivity of anti-*Toxocara* antibodies.

Similarly to the findings of Jacob et al. (5), we have found no correlation between the IgG titers and clinical manifestations. A patient with visceral toxocariasis without clinical manifestations presented IgG titers $\geq$10,240, while another patient with pulmonary manifestations, fever, and abdominal pain showed a titer of 160 at the beginning of the follow-up study. A patient with both visceral and ocular toxocariasis presented an initial IgG titer of 160, and 6 months after treatment presented an increased titer of 640. The patient with the ocular form also presented the same trend: titers elevated from 80–640 between the first- and second-year follow-up. This pattern of enhancing antibody levels could be explained by a possible destruction of the larvae and subsequent antigen release (4).

In this study, IgG antibodies persisted for a long time, and in many patients, this period lasted over 4 years, which is similar to data reported by Cypess et al. (35). Although our patients presented clinical improvement, the serological findings could not be associated with specific conditions leading to cure. Furthermore, Altcheh et al. (36) followed up 54 children with toxocariasis (ocular, visceral, or asymptomatic) for 18 months after treatment and concluded that IgG detection was not useful as an immunological marker for cure.

Toxocara is a parasite with the ability to evade the immune system, which could explain the chronicity and persistence of the infection (37). However, other factors such as infective dose, reinfection, or even larval reactivation should also be considered. Almost all children from the present study reported household contact with dogs, geophagy, and onychophagy, which certainly could contribute to maintain elevated antibody titers for a long time.

The avidity of IgG antibodies as a diagnostic marker for diseases, such as toxoplasmosis, rubella, hanta virus infections, and herpes virus infections, has been widely studied, and it appeared to help in some cases when acute stages were distinguished from chronic stages (38).

All samples hereby tested presented IgG antibodies with high avidity, which could be explained by the fact that the patients were at the chronic stage of the disease. Hubner et al. (13), studying the avidity of IgG antibodies with the use of urea as denaturant agent in 1,376 serum samples of patients with toxocariasis, found 5.1% of low avidity antibodies, reinforcing the idea that most of the patients were at the chronic stage of the infection. The data imply that most infected individuals look for clinical assistance at their chronic rather than acute phase.

In toxocariasis, specific IgM antibodies were reported (14) to occur in both acute and chronic phases, differing from most unrelated infections in which they are transient. Since these antibodies have not been found to be useful for toxocariasis diagnosis, we have now focused on the IgA and IgE isotypes.

There are few studies on the IgA antibody response in parasitic diseases. Matsumara et al. (39) evaluated an IgA antibody response in dogs naturally infected with *T. canis* and could not correlate this antibody with the age of dogs or with the number of worms. IgA levels were found to be higher in dogs at 1 month of age, decreasing thereafter, and remaining at low levels in their adulthood. In puppies, larvae developed into adult worms within 1 month. The intense developmental activity that is typical of this phase of the parasite could explain the presence of high levels of IgA antibodies.

The sensitivity of IgA-ELISA in the present study was low (47.8%). This confirms the findings of other authors who have suggested that the association of both IgA and IgG, or even the association of different antigenic molecules, improves the sensitivity of the assay (40). In order to observe whether the presence of IgG could be interfering in the IgA detection, some serum samples were re-tested after IgG antibody depletion with RF-Absorbent. However, this procedure did not increase the sensitivity of the assay. Matsumara et al. (39) had depleted IgG antibodies using protein A Sepharose, even though the optical densities were extremely low.

Specific IgE antibody response in helminthic diseases can provide relevant information, such as the effect of chemotherapeutic regimens on parasite survival, and be a useful marker in vaccination studies (37). In toxocariasis, Genchi et al. (41) investigated the specific IgE and IgG response in patients with ocular and visceral forms. They pointed out the need for IgG depletion to increase IgE antibody titers, since IgG antibodies seem to mask the presence of IgE in a competitive mechanism with the same epitopes of the parasite. In our study, all serum samples were treated with RF-Absorbent, in order to assure the detection of IgE antibodies.

Among visceral toxocariasis patients, 21.7% of them have not presented specific IgE antibodies, which is similar to the 18.7% of negative subjects reported by Magnaval et al. (15). These authors evaluated specific IgE antibodies in a post-treatment follow-up study and found a significant decrease in their levels. This is in agreement with our own findings, in which IgE levels have significantly decreased after the first year of follow-up.

The efficacy of antihelminthic drugs has long been questioned. In our study with thiabendazole, as well as in that of Magnaval et al. (15) with diethylcarbamazine, it was possible to observe that the treatment's efficacy does correlate with levels of specific IgE antibodies. Magnaval et al. (42) noted that eosinophil counts and anti-*Toxocara* IgE levels were useful markers in a post-treatment follow-up study (except for ocular patients).

In three out of four studied patients with ocular manifestations, IgA and IgE antibodies have not been detected. Since the level of circulating antibodies depends on the number of larvae and the chronicity of the infection, one possibility is that the number of infective larvae was too small in the negative patients (14).

Regarding the complementary assays, the leukocyte and eosinophil blood counts presented a significant decrease in the first year after treatment similar to the IgE decrease. Leukocytosis, however, can not be considered as a diagnostic marker for toxocariasis, because there are other factors that may contribute to cause a leukocyte increase (4).

The present analysis of eosinophil counts was based on the absolute number of eosinophils, according

to Jacob et al. (5), who reported a high sensitivity for this approach, as opposed to basing the analysis on the relative number of eosinophils. All patients with visceral toxocariasis presented eosinophilia, unlike those with ocular toxocariasis of whom only one patient out of four presented eosinophilia. This finding is in agreement to that reported by Luzna-Lyskov et al. (33), who showed the absence of eosinophilia in 81% of the suspected ocular cases.

In general, there was a significant decrease in the eosinophil number, but in many patients the eosinophil numbers were still elevated at the end of our study. In a post-treatment study of 54 children with visceral toxocariasis, ocular toxocariasis, and asymptomatic forms of the disease, Altcheh et al. (36) observed a decrease in eosinophil counts, yet eosinophilia was sustained for a long period as it was in our study.

The majority of patients (84%) presented positive results in the fecal examinations for intestinal parasite infections. However, different parasitic infections should not have interfered in the present results, since the level of specific IgG-ELISA, IgE, or IgA and eosinophil counts were not different between patients with single or multiple intestinal parasite infections.

There is a similarity between toxocariasis-TES epitopes and antigens of the human blood groups that leads to a production of isohemagglutinin. The TES antigen is highly glycosylated, having N-acetylgalactosamine (one of the most abundant sugars), which is an immunodominant antigen of the human blood type A. In this study, anti-A isohemagglutinin titers of visceral toxocariasis patients were higher than anti-B, which was consistent with the results obtained by Glickman et al. (43). Also, the titers significantly decreased one year after treatment.

Despite the advances in the study of toxocariasis, IgG-ELISA has been the most useful test in serodiagnosis and seroepidemiologic surveys. The study has demonstrated that other immunoglobulin class is more helpful for monitoring patient treatment than the IgG antibodies, which tend to last for long periods.

Therefore, treatment evaluation of toxocariasis patients can be performed by monitoring specific IgE antibody levels in addition to eosinophil counts.

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