

Case report

Eosinophil cationic protein as a possible marker of active human *Toxocara* infection

Background: Human toxocariasis is a common, worldwide helminthozoonosis that may elicit syndromes including various allergy symptoms. The diagnosis relies upon specific serology. However, this parasitosis is often self-limiting, and many subjects have residual antibodies, thus making differential diagnosis quite difficult when blood eosinophilia, a commonly accepted criterion of active helminthiasis due to tissue-dwelling parasites, is lacking.

Methods and results: We present a patient with chronic irritant cough displaying negative allergologic screening, normal blood eosinophilia, but positive toxocariasis immunodiagnosis. Therefore, this case presented the fortuitous association of an unexplained allergic picture with residual anti-*Toxocara* antibodies. In an attempt to distinguish between active and past toxocaral infection, the subject's level of eosinophil cationic protein (ECP) was assessed and then compared to those of four control groups, namely, healthy volunteers, subjects presenting anti-*Toxocara* residual antibodies, patients with various helminthiasis, and patients with active toxocaral disease. Since the patient's ECP level was found to be sharply elevated, we hypothesized that viable *Toxocara* larvae were still present in the tissues, and the patient was given anthelmintic therapy. At the control checkup, the cough had waned and the ECP level had decreased to below the mean value observed in both healthy subjects and in subjects with past toxocaral infections.

Conclusions: These data suggest, first, that patients presenting unexplained allergic syndromes should be checked for helminthiasis, even if blood eosinophilia is lacking, and, second, in such subjects displaying positive toxocariasis immunodiagnosis, ECP assessment would be a useful marker to distinguish between active and past toxocaral disease.

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Eosinophil cationic protein (ECP), which is released by activated eosinophils, has been proposed for the monitoring of asthma (1, 2), a disease in which elevated levels have been found (3). Regarding helminthiasis, which often elicit blood eosinophilia, high levels of ECP have been reported in patients with untreated schistosomiasis and filariasis (4).

Toxocariasis is a helminthozoonosis found worldwide, and caused by the migration in man of L2 larvae of *Toxocara canis*, an intestinal roundworm of dogs (5). Common toxocariasis, primarily described in France (6, 7), and covert toxocariasis, described in Ireland (8), are the most frequently encountered syndromes in Western countries. In French adults, toxocariasis was characterized by weakness, pruritus, rash, difficult breathing, and abdominal pain, along with moderate blood eosinophilia and increased serum total IgE level. In Irish children, the most frequent clinical findings were fever,

anorexia, headache, abdominal pain, sleep and behavioral disorders, pneumonia, cough, wheeze, and hepatomegaly.

However, most toxocaral infections are clinically unapparent and self-limiting, leaving only residual seropositivities, which can persist for many years (9). Therefore, epidemiologic surveys carried out in various countries have constantly found high positivity rates. For instance, seroprevalence was about 5% in French urban adults (10), and was, by far, higher in rural populations, ranging from 17% among Swedish farmers (11) to 37% in their French counterparts (12).

When investigating patients presenting clinical symptoms consistent with toxocariasis, but with a blood eosinophil count result inside the 0–500 cell/mm³ normal range (13), physicians may be in a quandary when a positive *Toxocara* immunodiagnosis is found. Since all of the aforementioned clinical symptoms are

nonspecific to toxocariasis, and even to any form of helminthiasis, a careful attitude is to think that these subjects have residual anti-*Toxocara* antibodies, and that their clinical picture is due to a different etiology than toxocariasis.

Toxocara larvae are tissue-dwelling parasites, and the blood eosinophil count imperfectly reflects the real production level of these cells, which preferentially accumulate in tissues (14). Therefore, it could be hypothesized that some of these patients could have true toxocaral disease, albeit without blood eosinophilia. To establish such a diagnosis, the level of anti-*Toxocara* antibodies yielded by ELISA and/or that of total serum IgE is of little value. Asymptomatic subjects, especially if exposed to trickle infestations, may exhibit increased values in both these laboratory tests, together with normal blood eosinophil count. Direct diagnosis of toxocariasis, e.g., by pathologic examination of biopsy specimens, is an awkward and insensitive method, and practically never available. Therapeutic testing should be avoided, since it relies upon rather long (21 days) schedules and uses drugs (diethylcarbamazine or mebendazole) which are not easily available, and which sometimes elicit unpleasant side-effects (15). Moreover, it delays the implementation of further etiologic investigation if unsuccessful.

We report hereby the case of a young girl in whom a diagnosis of active toxocariasis eliciting a chronic irritant cough was made on the basis of both a positive specific immunodiagnosis and an elevated titer of ECP, although no blood eosinophilia was present. Anthelmintic therapy was effective. Such a diagnostic decision could be reached only because of previous assessments of ECP mean level in healthy subjects, in asymptomatic subjects with a positive *Toxocara* immunodiagnosis, in patients with various helminthiasis, and in patients with active common toxocariasis.

Material and methods

Case report

In October 1999, an 11-year-old girl attended the Department of Parasitology in the University Hospitals in Toulouse, France, due to a chronic irritant cough that had developed 4 years earlier and had been found to be associated with a toxocariasis-positive immunodiagnosis. Her medical history was unremarkable. An epidemiologic questionnaire detected an evident risk factor, namely, a sand heap close to the patient's home which, although known to be contaminated by pets' feces, was used as a playground by toddlers and other young children.

Since the onset of the cough, the patient had successively visited several general practitioners, who had each diagnosed asthma. Each time, she had been successfully treated by inhaled beclomethasone and salbutamol. As soon as this drug association was discontinued, the cough recurred. For the following 3 years, the girl was successively investigated in the Department of Pediatric Pneumology and Allergology of a University Hospital, and then in a specialized private clinic. No etiologic diagnosis could be made. In particular, the patient was found by both skin and serum testing to be free of any sensitization

to inhalant or food allergens. According to the laboratory data provided by her parents, no blood eosinophilia was observed during these investigations. Since the parents did not wish to treat their daughter permanently with inhaled corticosteroids and β_2 -adrenoceptor stimulants, they consulted another general practitioner in September 1999. By that time, the patient's blood eosinophil count was 310 cells/mm³, and serum total IgE was 102 kIU/l. This physician requested a toxocariasis immunodiagnosis, which was found to be positive by ELISA in a specialized private laboratory.

On the day of the consultation, the young girl displayed a very good general status. Apart from the cough, she did not complain of any other allergy symptom. She had not taken corticosteroids or β_2 -adrenoceptor stimulants for several months. Physical examination, and especially chest auscultation, was normal. Two brief (about 15 s) coughing fits occurred within about 40 min, but the parents reported a greater fit frequency at night. Total and differential blood count (SHT 330TM blood analyzer, TOA Sysmex, Roche Diagnostics, Neuilly-sur-Seine, France) showed 6400 white blood cells/mm³, along with 324 eosinophils/mm³. Serum total IgE level (IMXTM, Abbott Laboratories, Rungis, France) was 89 kIU/l. Detection of specific IgE against 14 inhalant allergens (Matrix AeroTM, Abbott Laboratories, Rungis, France) was negative. Stool examination, including Baermann's method for *S. stercoralis* larvae detection, was negative. Of the immunodiagnoses for autochthonous helminthiasis (ascariasis, echinococcosis, fascioliasis, strongyloidiasis, and toxocariasis), only the one for toxocariasis was found to be positive by Western blot with *T. canis* excretory-secretory antigens (16). The patient's profile was rather suggestive of a fortuitous association of residual anti-*T. canis* antibodies with an allergy problem of unknown origin. However, given the negative result of the various previous allergologic investigations, the patient's ECP level was assessed and then compared to those from several groups of subjects included in an ongoing study of ECP and toxocariasis.

ECP assay

ECP titration was performed with the CAPTM System (Pharmacia, Guyancourt, France). Blood samples were taken according to the manufacturer's instructions, with special attention paid to the clotting temperature (always 20–24°C).

Control groups

In the control groups, hematology, immunology, and parasitology laboratory tests were performed with the same materials and methods as for our patient.

Group 1. A total of 27 apparently healthy subjects (residents or technicians) gave informed consent to be included in the study. They had to have a blood eosinophil count of <500 cells/mm³, and a serum total IgE of <150 kIU/l, as well as to display a negative result for stool examination, serologic checkup for autochthonous helminthiasis, and detection of specific IgE to common inhalant allergens.

Group 2. A total of 23 subjects displayed anti-*Toxocara* residual antibodies. They were fully asymptomatic, or presented clinical symptoms not consistent with toxocariasis, such as acute diarrhea during an outbreak of viral gastroenteritis. Apart from serum total IgE level, the inclusion requirements were identical to those for group 1.

Group 3. A total of 36 patients presented various helminthiasis, namely, one case of cysticercosis, one of fascioliasis, three of

hookworm infection, 19 of strongyloidiasis, three of schistosomiasis, four of loiasis, two of lymphatic filariasis, and three of onchocerciasis.

Group 4. A total of 110 patients exhibited clinical and laboratory features of common toxocariasis.

Statistical tests

The distribution of laboratory results was compared between groups by the Mann-Whitney U-test, and correlation studies used both Spearman's rank test and Pearson's test, as implemented by StatpakTM statistical software (Northwest Analytical, Portland, OR, USA).

Results

Table 1 displays demographic and laboratory results, and Table 2 the comparisons of result distributions. Regarding the mean of eosinophil count results, the difference between group 1 (healthy subjects) and group 2 (patients with anti-*Toxocara* residual antibodies) was quite tiny (about 54 cells/mm³). Although significant ($P=0.03$), it fell into the reading variation of the blood analyzer, and therefore could not be considered.

Whether age could be a confounding factor, explaining the increased ECP level observed in our patient, was assessed by testing the correlation between these two variables in all control groups. The results of Spearman's rank test were not significant. On the other hand, correlation assessment between ECP level and blood eosinophil count yielded interesting results. In group 1, Spearman's rank test was borderline ($r: 0.380$; Z-statistic: 1.96, $P: 0.049$) and not significant in group 2 ($r: 0.403$; Z-statistic: 1.9, $P: 0.056$). Conversely, a high degree of correlation was found between these

two parameters in group 3 ($r: 0.404$; Z-statistic: 2.46, $P: 0.014$), and especially in common toxocariasis cases from group 4 ($r: 0.438$; Z-statistic: 4.87, $P<0.0001$). When a linear regression test was done in group 4, the equation of the regression line appeared to be as follows: $ECP=0.016$ eosinophil count result plus 23. Applied to our patient's laboratory data, this equation gave a predicted ECP value of 28.184 $\mu\text{g/l}$ for an observed result of 28 $\mu\text{g/l}$, thus being consistent with active toxocaral disease, in spite of a blood eosinophilia inside the normal 0–500 cells/mm³ range.

Given these facts, we hypothesized that this young patient still harbored viable tissue-dwelling *Toxocara* larvae that elicited some eosinophil degranulation, but no peripheral eosinophilia. She was given diethylcarbamazine (4 mg/kg body weight daily for 21 days) (15), and the sand heap was removed to avoid any reinfection. A control checkup was done 1 month after the anthelmintic therapy ended. The cough had waned, and the results of the laboratory analysis were as follows: blood eosinophil count, 100 cells/mm³; serum total IgE: 47 kIU/l; ECP: 8 $\mu\text{g/l}$; toxocariasis immunodiagnosis by Western blot: full pattern still present. The patient was clinically checked by her personal physician 6 and 12 months later, and her recovery was confirmed.

Discussion

Firstly, this case report underlines the need to rule out helminthiasis in any patient presenting an unexplained allergy syndrome, even in Western countries.

Regarding toxocariasis, it could be suspected that

Table 1. Demographic values and laboratory results

<i>n</i>	Case (BT ⁵) na ⁴	Case (AT ⁶) na ⁴	Group 1 27	Group 2 23	Group 3 36	Group 4 110
Age (years) ¹	11	na ⁴	28.5	46.1	37.8	45.8
SEM ²	na ⁴	na ⁴	0.96	3.07	3.06	1.8
95% CI ³	na ⁴	na ⁴	26.5–30.4	39.8–52.5	31.6–44.0	42.2–49.4
Blood eosinophil count ¹ (cells/mm ³)	324	100	137.3	191.1	1753.2	1172.3
SEM ²	na ⁴	na ⁴	15.64	10.31	260.64	86.89
95% CI ³	na ⁴	na ⁴	105.1–169.4	169.8–212.6	1224.0–2282.3	1000.1–1344.5
Serum total IgE ¹ (IU/ml)	89	47	41.4	156.4	1103.9	1565.2
SEM ²	na ⁴	na ⁴	6.5	38.34	268.7	326.58
95% CI ³	na ⁴	na ⁴	28.0–54.8	76.9–235.9	558.3–1649.4	917.9–2212.5
ECP ¹ ($\mu\text{g/l}$)	28	8	10.8	11.2	76.6	42.3
SEM ²	na ⁴	na ⁴	1.4	1.3	9.6	3.5
95% CI ³	na ⁴	na ⁴	7.9–13.6	8.5–14.0	57.2–96.0	35.4–49.2
Matrix Aero TM	Negative	Not done	All negative	All negative	na ⁴	na ⁴
Toxocariasis serodiagnosis by Western blot	Positive	Positive	All negative	All positive	na ⁴	All positive

¹Mean value if applicable..

²SEM: standard error of mean.

³CI: confidence interval.

⁴na: not applicable.

⁵BT: before DEC therapy.

⁶AT: after DEC therapy.

Table 2. Comparison of results distributions between control groups (Mann-Whitney U test)

		Group 1	Group 2	Group 3	Group 4
Blood eosinophil count	Group 1	*	$P=0.03$	$P=0.00001$	$P=0.00001$
	Group 2		*	$P=0.00001$	$P=0.00001$
	Group 3			*	$P=0.02$
Serum total IgE	Group 1	*	$P=0.0001$	$P=0.00001$	$P=0.00001$
	Group 2		*	$P=0.0001$	$P=0.00001$
	Group 3			*	NS ¹
ECP	Group 1	*	NS	$P=0.00001$	$P=0.00001$
	Group 2			$P=0.00001$	$P=0.00001$
	Group 3			*	$P=0.0001$

NS: not significant.

intermediate pictures existed between the two polar forms, active and past, but that an accurate diagnostic tool was lacking.

In 23 subjects exhibiting residual anti-*Toxocara* antibodies due to a self-limited infection, the mean value of eosinophil count was practically the same as that of 27 healthy, nonatopic, nonparasitized volunteers. The ECP average value also did not significantly differ between these two groups. The mean serum total IgE level was significantly greater in group 2 than in group 1, thus indicating a slow decrease of circulating

Th₂ cells after spontaneous cure. Such slow kinetics has been previously reported in toxocariasis cases after anthelmintic therapy (17).

If only the eosinophil count and serum total IgE result were considered, the young girl would have been classified as a patient with past, self-cured toxocariasis. Comparison of her ECP titer to results concerning both group 2 and group 4, together with the finding of a significant linear correlation between eosinophilia and ECP among toxocariasis patients, strongly suggested the persistence of active larval parasitism. The excellent result of the therapeutic test added further circumstantial evidence to support this hypothesis, since diethylcarbamazine is active only on microfilariae and *Toxocara* larvae.

Nonetheless, more investigations among patients presenting various clinical and laboratory features, along with positive toxocariasis immunodiagnosis, are required to prove that the ECP level correlates with the stage of evolution in toxocaral infection.

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