

Comparative assessment of enzyme-linked immunosorbent assay and Western blot for the diagnosis of toxocariasis in patients with skin disorders

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Summary

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Background The link between various chronic skin disorders and toxocariasis was previously demonstrated by case reports and several case-control studies. However, these previous studies were based only on the *Toxocara canis* excretory-secretory-enzyme-linked immunosorbent assay (TES-ELISA) serological technique, which is not specific due to cross-reactivity with parasites of the genera *Anisakis* or *Ascaris*. Immunoblot analysis is highly specific and can detect very low levels of *Toxocara* antibodies. Therefore, this technique may be useful in the identification of *Toxocara* infection in patients with chronic skin disorders.

Objectives Because urticaria and pruritus/prurigo are skin conditions previously associated with toxocariasis, we carried out a prospective study using both TES-ELISA and *Toxocara* Western blot on 113 patients with either chronic urticaria ($n = 84$) or chronic pruritus ($n = 29$).

Methods Patients were matched with controls according to gender, age and residence location (rural or urban area). Data were analysed using a Mantel-Haenszel χ^2 test. **Results** The proportion of positive TES-ELISA results was not significantly different for patients with chronic skin disorders (urticaria or pruritus/prurigo) from that of control subjects. However, the proportion of positive immunoblot results was significantly higher for patients with chronic urticaria than for control subjects ($P = 0.009$).

Conclusions Our study demonstrates the need to perform Western blotting immunodiagnosis, whatever the TES-ELISA result, to improve diagnosis of human toxocariasis in patients with chronic urticaria caused by *Toxocara* infection.

Human toxocariasis is a widespread, zoonotic, parasitic disease caused by the presence of larvae from *Toxocara canis* or *T. cati* in human tissues. Dogs and cats are the definitive hosts and act as reservoirs. Human infections are usually caused by ingestion of eggs of the parasite from the environment. The larvae hatch and migrate out of the intestine to become lodged in organs or tissues. The persistence of encysted larvae in human host tissues can cause clinical symptoms, depending on the number and location of the larvae in the body and the sensitivity of the host immune system.¹

Two types of tests are available for the immunodiagnosis of toxocariasis: enzyme-linked immunosorbent assay (ELISA) and Western blot (WB), both using *T. canis* excretory-secretory antigens (TES). TES-ELISA was the first method to be employed,²

irrespective of the clinical form of toxocariasis.³⁻⁵ However, the greater sensitivity and specificity of the *Toxocara* WB was demonstrated in 1991.⁶ Moreover, investigations using both methods demonstrate that WB is a better technique than ELISA to investigate patients showing blood eosinophilia^{7,8} or having ocular involvement consistent with toxocariasis.⁹

Cutaneous manifestations of toxocariasis may be the only signs of the disease and are estimated to represent 24% of the symptomatic extraocular forms.¹⁰ Pruritus is one of the clinical symptoms characterizing common toxocariasis.³ Some dermatologists noticed an association between toxocariasis and the presence of chronic pruritus or chronic urticaria. This association was investigated in case-control studies using the TES-ELISA serological test.¹¹⁻¹⁴

A prospective study was carried out to assess the performance of TES–ELISA and *Toxocara* WB in the diagnosis of toxocariasis in patients with chronic skin disorders (chronic urticaria and chronic pruritus).

Patients and methods

Subjects

Inpatients of the Department of Dermatology, University Hospital of Besançon, France, were enrolled prospectively from March to December 1999. Chronic urticaria was defined as an outbreak of oedematous, projecting, well-delimited and rounded papules, persisting for more than 6 weeks.¹⁵ In the chronic pruritus and prurigo group, patients had had pruritus for more than 3 weeks. For each patient, TES–ELISA and *Toxocara* WB were performed to diagnose toxocariasis.

Control population

Control sera from blood donors were collected under an agreement between the French Blood Transfusion Service of Franche-Comté and the Parasitology Department, with the approval of the local ethical committee. Control subjects were informed of the study and signed a consent form. The controls collected were paired with the patients according to sex, age (± 5 years) and provenance (urban vs. rural area). The definition of urban and rural areas was in accordance with the French National Institute of Statistics and Economic Surveys (<http://www.insee.fr>). Urban areas, or urban settlements, correspond to towns offering at least 5000 jobs.

Laboratory tests

The TES–ELISA procedure was carried out using a commercial kit (Bordier Affinity Products, Crissier-Lausanne, Switzerland) according to the manufacturer's instructions. For each serum, the optical density value was converted into *Toxocara* units (TU), a measurement system which was proprietary. The cut-off reference serum had a value of 24 TU.¹⁶ Immunodiagnosis by the WB technique relied also upon a commercial kit

(LDBIO Diagnostics, Lyon, France). A serum was classified as positive when at least two of the highly specific lower molecular weight bands (LMWB) at 24, 28, 30 or 35 kDa were observed by two different operators.

Statistical analysis

STATA statistical software release 9 (StataCorp LP, College Station, TX, U.S.A., 2005) was used for statistical analysis. A Mantel–Haenszel χ^2 test was used to compare the performance of TES–ELISA and *Toxocara* WB in patients with pruritus and with urticaria (Table 1). Statistical significance was set at the conventional 5% level. Results were expressed as P-values, odds ratios and 95% confidence intervals.

Results

Characteristics of the collected pairs

The patient group included 84 patients with chronic urticaria and 29 with chronic pruritus. The chronic urticaria pairs had a mean age of 40 years and 13 of them came from rural areas (13 of 84, 15%). The chronic pruritus pairs had a mean age of 51 years and 10 came from rural areas (10 of 29, 34%).

Statistical analysis

No significant association was observed between the occurrence of chronic skin disorders (urticaria or pruritus) and toxocariasis from the results of TES–ELISA (Table 1). In contrast, a significant difference in *Toxocara* WB results was found between patients and controls for chronic urticaria ($P = 0.009$) (Table 1).

Discussion

This case–control study highlights the link between chronic skin disorders such as urticaria and toxocariasis seropositivity, and demonstrates the efficacy of immunoblot assay in identifying this link. Better evidence would have been the diagnosis of *Toxocara* by identification of the parasite, but because human

Table 1 P-values, odds ratios and 95% confidence intervals (CIs) (Mantel–Haenszel χ^2 test) for *Toxocara canis* excretory-secretory–enzyme-linked immunosorbent assay (TES–ELISA) and *Toxocara* Western blot (WB) in patients with pruritus and with urticaria

	Positive results		Mantel–Haenszel χ^2	P-value	Odds ratio (95% CI)
	Patients	Controls			
Pruritus (n = 29 pairs)					
TES–ELISA	6/29	2/29	2.88	0.131	3.52 (0.62–20.15)
<i>Toxocara</i> WB	16/29	11/29	1.70	0.192	2.01 (0.69–5.89)
Urticaria (n = 84 pairs)					
TES–ELISA	9/84	7/84	0.27	0.600	1.32 (0.47–3.74)
<i>Toxocara</i> WB	36/84	20/84	6.82	0.009	2.40 (1.22–4.73)

Significant results are shown in bold.

toxocariasis is a parasitological dead-end, neither adult roundworms nor eggs can be found in stools to make a direct diagnosis. Moreover, larvae are very rarely found in skin biopsies and the procedure is too invasive for the severity of the disease. Some case-control studies using TES-ELISA did demonstrate a link between a toxocariasis infection and chronic pruriginous lesions (urticaria and pruritus).^{11,13,14,17} However, the antigens used in TES-ELISA are a complex mixture of glycoproteins and cross-reactivity has been demonstrated with other *Toxocara* species and other helminths (nematodes such as *Ascaris* spp., *Enterobius* spp., *Trichuris* spp. and *Anisakis* spp., cestodes such as *Taenia* spp. and *Echinococcus* spp., and trematodes such as *Fasciola* spp. and *Schistosoma* spp.).^{6,18,19} In this study, the use of a highly specific serological assay allowed us to establish a reliable diagnosis of contact with *Toxocara* without direct invasive procedures.

WB is the more sensitive and specific of the two assays available for immunodiagnosis of human toxocariasis. The high specificity of the test is based on the distinction between clusters of lower and higher molecular weight bands (LMWB and HMWB). HMWB are not specific and are suggestive of cross-reactions with other helminthiases, while LMWB show a high level of specificity. Magnaval *et al.*⁶ studied 118 sera from patients with various helminthiases and found only two sera showing a positive LMWB cluster. They corresponded to patients with strongyloidiasis and cystic hydatidosis who could be also infected by *Toxocara*. We considered as positive only WB assays showing the presence of the LMWB cluster, as recommended by Magnaval *et al.*⁶

Our study also shows that some patients would not have been diagnosed by TES-ELISA alone. Initially, the cut-off value for TES-ELISA assays was determined using patients with visceral toxocariasis, characterized by increased IgE, increased eosinophil counts and high levels of toxocaral antibodies.⁴ Later, it was shown that common and covert toxocariasis are associated with lower levels of toxocaral antibodies.²⁰ A modification of the positive cut-off value recommended by the manufacturer should be considered for those patients with chronic pruritus and urticaria. However, because serological tests cannot differentiate between recent and past *Toxocara* infections, physicians should remember that a positive serology is only one piece of evidence and is not proof in itself. Eosinophilic cationic protein concentration has been proposed as a marker to distinguish between active and past toxocaral infection.²¹

Human toxocariasis may be responsible for various clinical symptoms²² and this study highlights its contribution to dermatological symptoms. Toxocariasis cases are probably underestimated, because *Toxocara* WB is routinely performed only to confirm a positive TES-ELISA. Thus, our study underlines the importance of carrying out systematic *Toxocara* WB, whatever the TES-ELISA result, to improve toxocariasis diagnosis in patients with chronic pruriginous skin disorders.

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