

Original paper

The usefulness of commercially available serological tests in the diagnosis and monitoring of treatment in patients with alveolar echinococcosis

Małgorzata Sulima¹, Beata Szostakowska², Wacław Nahorski^{1,3}, Katarzyna Sikorska^{1,4}, Wojciech Wołyniec⁵, Piotr Wąż⁶

¹Department of Tropical and Parasitic Diseases, University Centre of Maritime and Tropical Medicine (UCMTM) in Gdynia, Medical University of Gdansk, Poland

²Department of Tropical Parasitology, Chair of Tropical Medicine and Parasitology, Institute of Maritime and Tropical Medicine, Faculty of Health Sciences, Medical University of Gdansk, Poland

³Department of Tropical and Parasitic Diseases, Institute of Maritime and Tropical Medicine, Faculty of Health Sciences, Medical University of Gdansk, Poland

⁴Department of Tropical Medicine and Epidemiology, Institute of Maritime and Tropical Medicine, Faculty of Health Sciences, Medical University of Gdansk, Poland

⁵Department of Occupational, Metabolic and Internal Medicine, Institute of Maritime and Tropical Medicine, Faculty of Health Sciences, Medical University of Gdansk, Poland

⁶Department of Nuclear Medicine, Medical University of Gdansk, Poland

Abstract

Aim of the study: To assess the clinical usefulness of serological tests in the diagnosis and monitoring of treatment of patients with alveolar echinococcosis (AE).

Material and methods: The results of serological tests, i.e. Echinococcus multilocularis ELISA (Bordier Affinity Products) and Echinococcus Western Blot IgG (LDBIO Diagnostic), of 66 patients were analysed. Duration of follow-up was two years after diagnosis. In the second phase of the study 11 sera obtained from the patients undergoing surgical treatment, in whom the results of Echinococcus Western Blot IgG assay were still positive, were additionally tested with Anti-Echinococcus EUROLINE-WB (IgG) assay.

Results: Statistically significant negativization of the Echinococcus multilocularis ELISA test was observed in the group of patients who underwent radical surgery or liver transplantation. Negativization of Echinococcus Western Blot IgG assay results was observed in some patients, among both those who received conservative treatment and those who underwent surgery, but no statistically significant differences were found between treatment groups. In 54.5% of cases the Anti-Echinococcus EUROLINE-WB (IgG) test result was negative when the results of the Echinococcus Western Blot IgG assay were still positive.

Conclusions: Echinococcus multilocularis ELISA proved to be useful in assessing the activity of AE in a group of patients who underwent radical surgery or liver transplantation. The results of our study suggest that Anti-Echinococcus EUROLINE-WB (IgG) is a more dynamic test, which at the time of disappearance of AE activity becomes negative earlier.

Key words: serology, echinococcosis, alveolar echinococcosis.

Address for correspondence

Małgorzata Sulima, Department of Tropical and Parasitic Diseases, University Centre of Maritime and Tropical Medicine (UCMTM) in Gdynia, Medical University of Gdansk, 9 B Powstania Styczniowego St., 81-519 Gdynia, Poland, e-mail: m.sulima@poczta.fm

Introduction

Echinococcosis is a parasitic disease caused by tapeworms of the genus *Echinococcus*. The two most common forms of this disease, caused by different tapeworm species, are cystic echinococcosis (CE) and alveolar echinococcosis (AE). CE and AE present different lesion patterns in imaging examinations and are characterized by different clinical course, and thus require different therapeutic approaches. Since the early 1920s, a significant increase in the incidence of AE in Central and Eastern Europe, including Poland [1], has been noted.

Alveolar echinococcosis is caused by the larval form of the tapeworm *Echinococcus multilocularis* [2]. The most frequent site of primary parasitic lesions is the liver [3]. Clinically, the parasitic invasion is characterized by a long-lasting asymptomatic phase. Often, at the onset of symptoms, imaging examination reveals the presence of a large tumour-like mass in the liver suggesting a proliferative process [4, 5]. In some cases, at the time of diagnosis, extrahepatic lesions are also visible due to spread by continuity to adjacent structures or metastasizing via the haematogenic route to distant organs such as the lung or central nervous system (CNS). Patients in such an advanced stage of the disease are deprived of the opportunity of optimal treatment, which is radical surgery combined with temporary pharmacological treatment. Patients who are not eligible for surgery require treatment with benzimidazoles for many years, sometimes for the rest of their lives. In some cases, liver transplantation (LTx) remains the only chance to save the patient's life.

Serological blood tests are, besides imaging studies, as well as histopathological and molecular examinations, important tools in the diagnosis of AE. They are also useful in monitoring the effectiveness of treatment and in follow-up after the termination of pharmacotherapy, due to the risk of recurrence, especially in the group of patients receiving immunosuppression. These tests also proved to be useful in screening examinations carried out in the areas of increased incidence of the disease observed due to the increased number of final hosts [6, 7]. Serological diagnosis is usually based on commercially available immunoenzymatic (ELISA) and immunoblot assays containing recombinant and purified *Echinococcus* antigens. Immunochromatographic tests are mainly useful as a screening tool in endemic areas.

The study analysed the results of serological tests performed at diagnosis and during treatment of AE in patients hospitalized at the University Centre of Maritime and Tropical Medicine (UCMTM) between 2000 and 2016. During this period, the screening test used by the UCMTM was *Echinococcus granulosus* ELISA

(Bordier Affinity Products SA, Crissier, Switzerland) for detecting the presence of IgG antibodies against larval *Echinococcus* antigens. To confirm the diagnosis and differentiate between CE and AE, *Echinococcus* Western Blot IgG (LDBIO Diagnostics, Lyon, France) and *Echinococcus multilocularis* ELISA (Bordier Affinity Products SA, Crissier, Switzerland) detecting Em2plus antigen were used. In 2016, serological diagnostics was extended to include the commercially available tests Anti-*Echinococcus* EUROLINE-WB (IgG) and EUROIMMUN Anti-*Echinococcus* ELISA (IgG), EUROIMMUN US Inc. Medical Diagnostics. Simultaneous use of various serological tests increases the chance of establishing the correct diagnosis of AE, thus avoiding invasive diagnostic methods, such as liver biopsy or exploratory laparotomy. Such an approach is also helpful in assessing the effectiveness of treatment, including determination of the moment when pharmacological treatment can be safely withdrawn. Benzimidazole therapy is associated with potential side effects, including toxic liver damage or leukopenia, and in Poland additionally imposes a large financial burden on the patient.

The aim of the study was to assess the clinical usefulness of commercially available serological tests, *Echinococcus multilocularis* ELISA and the immunoblot *Echinococcus* Western Blot IgG, in the diagnosis and monitoring of treatment of patients with AE, based on the analysis of data from the follow-up of patients treated at the Department of Tropical Diseases and Parasitology, UCMTM. Also, the study aimed to determine whether extended diagnostics including the Anti-*Echinococcus* EUROLINE-WB (IgG) immunoblot assay, performed at the time of *Echinococcus multilocularis* ELISA negativization in the group of patients undergoing surgical treatment in whom the result of *Echinococcus* Western Blot IgG assay is still positive, enables a more accurate assessment of parasitic disease activity.

Material and methods

The analysis included the results of serological tests, i.e. *Echinococcus multilocularis* ELISA (Bordier Affinity Products SA) and *Echinococcus* Western Blot IgG (LDBIO Diagnostics), performed at the time of diagnosis and during the treatment of 66 patients hospitalized in the Department of Tropical Diseases and Parasitology, UCMTM between 2000 and 2016. Study participants were patients with probable and certain diagnosis of AE. According to the WHO-Infomal Working Group on Echinococcosis (WHO-IWGE), the probable diagnosis was based on the positive re-

sults of serological tests and a typical pattern of lesions in imaging examinations. The diagnosis was considered as certain in patients in whom the disease was additionally confirmed by histopathological or molecular (PCR) examination. Serological tests were performed in the Department of Tropical Parasitology, Institute of Maritime and Tropical Medicine according to the manufacturers' instructions. The result of the *Echinococcus multilocularis* ELISA test was considered as negative when the absorbance of the analysed sample was lower than the absorbance of the weak positive (cut-off) serum. The result was positive when the absorbance of the analysed sample was higher than the absorbance of the weak positive serum (IgG antibody concentration against *Echinococcus multilocularis* Em2-Em18 antigens was considered as clinically significant). The *Echinococcus* Western Blot IgG assay is based on antigen extracts derived from the *E. multilocularis* larval form. Bands at 7, 12, 15, 24, and 26 to 28 kDa are common to both *Echinococcus* species. However, their intensity and arrangement in most cases allow for differentiation between CE and AE. There are five band patterns that can be obtained in immunoblot assay – P1 to P5. Patterns P1 (isolated 7 kDa band only) and P2 (7 kDa band + large diffuse 17 kDa band) are indicative of CE diagnosis. Patterns P3 (26-28 band + the narrow 16 and/or 18 kDa bands) confirm the diagnosis of AE. Patterns P4 (isolated 26-28 kDa band only) and P5 (association of the 7 + 26-28 kDa bands) do not allow for differentiation between CE and AE. The patients ($n = 66$) were assigned to one of four

groups depending on the method of treatment used: receiving pharmacological treatment ($n = 25$), radical surgery ($n = 21$), non-radical surgery ($n = 10$) and liver transplantation (LTx) ($n = 10$). In all the patients both tests were performed at diagnosis. During the treatment, serum samples of all patients were assessed using *Echinococcus multilocularis* ELISA. *Echinococcus* Western Blot IgG test was performed in 47 patients (71.2%). Due to the fact that in some patients after surgery or LTx, at the moment of negativization of the *Echinococcus multilocularis* ELISA test, the results of the *Echinococcus* Western Blot IgG assay remained positive, 11 sera obtained from such 11 patients were used in the second phase of the study. Five samples were from patients after radical surgery, two from patients who underwent non-radical surgery and four from LTx patients. The sera were stored under freezing conditions at -60°C . These 11 sera samples underwent additional testing with Anti-*Echinococcus* EUROLINE-WB (IgG) assay, performed in accordance with the manufacturer's instructions. The results were read using EUROLineScan software.

Results

The *Echinococcus multilocularis* ELISA test result was positive in all patients at the time of diagnosis. During the two-year follow-up, statistically significant negativization of the results of this test was observed in the group of patients who underwent radical surgery or LTx (Table 1). The negativization occurred

Table 1. Number of subjects (M) and the percentage values calculated against the sum specified for the given rows (NR) and columns (NC). CTr – patients receiving pharmacological treatment; NRS – patients who underwent non-radical surgery; RS – patients who underwent radical surgery; LTx – patients after liver transplantation. Fisher's exact test for count data was used to examine the independence between the features described in the columns and rows. P -value = 0.000000001392

Patients		Elisa Em2plus test results negative in the course of two-year treatment	Elisa Em2plus test results positive in the course of two-year treatment	All (Elisa Em2plus test positive at the time of diagnosis)
CTr	N	0	25	25
	NR	0%	100%	100%
	NC	0%	64.1%	37.9%
NRS	N	2	8	10
	NR	20%	80%	100%
	NC	7.4%	20.5%	15.2%
RS	N	17	4	21
	NR	81%	19%	100%
	NC	63%	10.3%	31.8%
LTx	N	8	2	10
	NR	80%	20%	100%
	NC	29.6%	5.1%	15.2%
Total	N	27	39	66
	NR	40.9%	59.1%	100%
	NC	100%	100%	100%

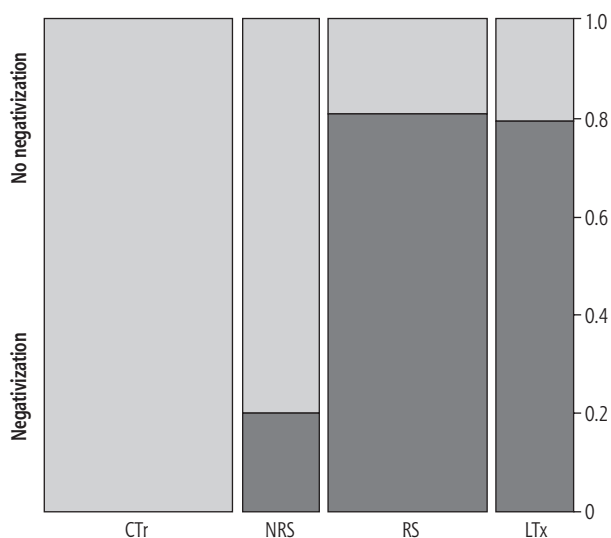


Fig. 1. Percentage of patients with negative result of Echinococcus multilocularis ELISA test during the two-year follow-up according to treatment received. CTr – patients receiving pharmacological treatment; NRS – patients who underwent non-radical surgery; RS – patients who underwent radical surgery; LTx – patients after liver transplantation

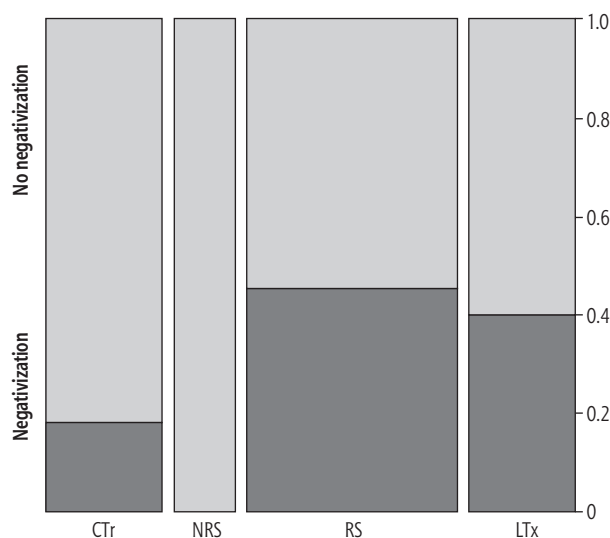


Fig. 2. Percentage of patients with negative result of Echinococcus Western Blot IgG test during the two-year follow-up according to treatment received. CTr – patients receiving pharmacological treatment; NRS – patients who underwent non-radical surgery; RS – patients who underwent radical surgery; LTx – patients after liver transplantation

one month to one year after the surgical procedure. No change in test results was observed in the group of patients receiving pharmacological treatment (Fig. 1).

In 69.7% of the analysed patients, the result of the Echinococcus Western Blot IgG assay indicated AE at the time of diagnosis. Negativization of Echinococcus

Western Blot IgG (LDBIO Diagnostics) assay results was observed in some patients, among both those who received conservative treatment and those who underwent radical surgery or LTx during the two-year follow-up, but no statistically significant differences (Table 2) were found between treatment groups (Fig. 2).

Table 2. Number of subjects (N) and the percentage values calculated against the sum specified for the given rows (NR) and columns (NC). CTr – patients receiving pharmacological treatment; NRS – patients who underwent non-radical surgery; RS – patients who underwent radical surgery; LTx – patients after liver transplantation. Fisher’s exact test for count data was used to examine the independence between the features described in the columns and rows. P-value = 0.1363

Patients		Echinococcus WB IgG test negative in the course of two-year treatment	Echinococcus WB IgG test positive in the course of two-year treatment	All (Echinococcus WB IgG test positive at the time of diagnosis)
CTr	N	2	9	11
	NR	18.2%	81.8%	100%
	NC	13.3%	28.1%	23.4%
NRS	N	0	6	6
	NR	0%	100%	100%
	NC	0%	18.75%	12.8%
RS	N	9	11	20
	NR	45%	55%	100%
	NC	60%	34.4%	42.6%
LTx	N	4	6	10
	NR	40%	60%	100%
	NC	26.7%	18.75%	21.3%
Total	N	15	32	47
	NR	31.9%	68.1%	100%
	NC	100%	100%	100%

Among serum samples from 11 patients after radical surgery or LTx in whom Echinococcus Western Blot IgG assay remained positive, despite negative results of the Echinococcus multilocularis ELISA test, that were additionally examined using Anti-Echinococcus EUROLINE-WB (IgG), negative results were obtained in 6 samples (54.5%). In 2 samples (18.2%) test results were borderline, in 2 samples the results were still positive for AE infection, and in one sample the positive test result did not allow for differentiation between CE and AE.

Discussion

The results of serological tests, Echinococcus Western Blot IgG and Echinococcus multilocularis ELISA, were analysed in patients with probable and certain diagnosis of AE. The Echinococcus Western Blot IgG assay is highly sensitive and can differentiate between CE and AE in 76% of cases [8]. In our group of patients, the test indicated AE at the time of diagnosis in 69.7% of cases. The assay does not allow for determining whether the parasitic infection is active or not [8], although it may be helpful in monitoring the treatment of patients with AE [9]. In the group of patients who underwent radical surgery, a gradual disappearance of 16 and 18 kDa bands (change in the test pattern from P3 to P5) followed by a 7 kDa band (change in the test pattern from P5 to P4) or serological negativization was observed one to four years after surgery [9]. In our study, in the group of patients who underwent radical surgery, negativization of the Echinococcus Western Blot IgG (LDBIO Diagnostics) assay was observed in 45% of patients during two years after surgery and in all patients up to 9 years after surgery. In the remaining cases, i.e. in those patients without test negativization during the two-year follow-up after the surgery, a change in the test pattern from P3 to P5 or P4 interpreted as the disappearance of parasitic lesion activity was observed.

In all patients included in the study, a positive result of the Echinococcus multilocularis ELISA test was obtained at the time of diagnosis. This assay contains the native Em2 antigen isolated from the laminar membrane of the hydatid cyst connected to the recombinant antigen II/3-10 [10] and is characterized by high sensitivity (97%) and specificity (99%) for AE [10]. For this reason, it is useful, like the immunoblot test, in differentiation between *Echinococcus* species [11]. Studies using ELISA with purified native Em2 antigen confirmed that in the group of patients with AE after radical resection of a hepatic lesion, serological negativization was observed in 67% of patients within one

year of surgery [12]. In our study, we observed statistically significant negativization in the group of patients who underwent radical surgery and LTx. It occurred one month to one year after surgery. In patients who underwent non-radical surgery, negativization of the test occurred later. The Echinococcus multilocularis ELISA test was used for assessment of the effectiveness of pharmacological treatment [13, 14]. However, researchers from various centres report long-term maintenance of positive test results in patients with inactive (calcified) lesions. Our study confirms this thesis, because in the group of patients who did not undergo surgical treatment, no case of negativization of the Echinococcus multilocularis ELISA test was observed during long-term follow-up, with the longest follow-up period being 14 years. This fact is most likely related to the laminar membrane antigens that stimulate the immune response. This is supported by serological negativization observed after removal of AE lesions from the liver [12, 13].

The recombinant antigen Em18 (rEm18) is currently considered the most useful in the differentiation between AE and CE [11, 15-17] as well as in the assessment of the activity of parasitic lesions [18, 19]. The use of rEm18 increases the specificity of the test for AE [15], and thus allows for better differentiation between species compared with the Echinococcus multilocularis ELISA test [11]. The Anti-Echinococcus EUROLINE-WB (IgG) assay combines electrophoretically separated native antigens of vesicle fluid of *E. multilocularis* with recombinant, purified antigens specific for *E. multilocularis* (Em18, Em95) and *E. granulosus* (EgAgB). According to the manufacturer's data, the Anti-Echinococcus EUROLINE-WB (IgG) assay has a rate of differentiation between CE and AE of 81%.

The use of the Anti-Echinococcus EUROLINE-WB (IgG) assay in the analysed group of 11 patients who underwent surgical treatment or LTx, in whom persistent positive results of the Echinococcus Western blot IgG test were observed despite the negativization of the Em2plus ELISA test, showed that 54.5% of cases were negative and 18.2% borderline. These results suggest that this assay is more "dynamic" and becomes negative earlier during effective treatment compared with the Echinococcus Western Blot IgG assay. This hypothesis requires further research on a larger group of patients, including patients treated pharmacologically.

In practice, it can be important in monitoring the activity of parasitic disease and making decisions about termination of pharmacological treatment. On the other hand, there are still patients in everyday practice undergoing surgical treatment due to a focal lesion in the liver, who appear at centres dealing with the treatment

of echinococcosis late, even one year after surgery. In some of them, when after radical treatment *Echinococcus multilocularis* ELISA and Anti-*Echinococcus* EUROLINE-WB (IgG) assays are negative, the *Echinococcus* Western Blot IgG test may prove useful in establishing further management of patients with suspected AE, including the implementation of antiparasitic treatment and continuation of follow-up of the patient. When interpreting the results of serological tests, the possibility of cross-reactions should be additionally considered. In the case of the Anti-*Echinococcus* EUROLINE-WB (IgG) assay, false-positive results may occur in other parasitic infections such as *Ascaris lumbricoides* or *Anisakis simplex*. Cross-reactivity may also occur when performing the *Echinococcus* Western Blot IgG assay in the group of patients with neurocysticercosis and schistosomiasis [8, 10]. In contrast, cross-reactivity with the Em2 plus antigen has been observed in fluke infections (fasciolosis, schistosomiasis) and cystic echinococcosis. There is a risk of cross-reactivity of the *Echinococcus multilocularis* ELISA test in patients with malignancies including liver cancer and abdominal tumours [20].

Patients after LTx, due to the immunosuppressive treatment used, are in a special risk group because of the high risk of recurrence of a parasitic disease. While in patients undergoing radical surgery WHO-IWGE recommends treatment with benzimidazoles for 2 years after the surgery, in the LTx group there are no clear recommendations [3]. In the analysed group of LTx patients, one patient had recurrence of a parasitic disease (confirmed in a histopathological examination). It was a patient who had himself stopped taking albendazole in the first year after LTx. The recurrence was preceded by positivization of the *Echinococcus multilocularis* ELISA test and a change in the *Echinococcus* Western Blot IgG test pattern – a change from P4 to P3. In the second case, recurrence of AE was suspected based on imaging examinations performed a year after LTx, and positive results of blood serology using *Echinococcus multilocularis* ELISA and *Echinococcus* Western Blot IgG assays have been consistently observed since LTx, despite the antiparasitic treatment.

The paper emphasizes the necessity of conducting serological diagnostics of AE using simultaneously several assays with different levels of sensitivity and specificity in relation to *Echinococcus* antigens [15]. Interpretation of test results should always take place in conjunction with the results of imaging studies and data on the clinical course of AE, including previous surgical treatment. There is a need to raise awareness among physicians about AE. Invasive diagnostic methods, often performed without previously initiated anti-

parasitic treatment, carry the risk of spreading the infection. Performing serological assays in the first place allows this error to be avoided.

Conclusions

Echinococcus multilocularis ELISA (Bordier Affinity Products) proved to be useful in assessing the activity of AE in a group of patients who underwent radical surgery or LTx. The negativization of the *Echinococcus* Western Blot IgG (LDBIO Diagnostics) assay during the two-year follow-up was not statistically significantly related to the type of treatment used.

The results of our study suggest that Anti-*Echinococcus* EUROLINE-WB (IgG) is a more dynamic test, which at the time of disappearance of AE activity becomes negative earlier – in 54.5% of cases it was negative when the results of the *Echinococcus* Western Blot IgG assay were still positive and presented the P4 or P5 pattern (did not distinguish between AE and CE).

Disclosure

The authors report no conflict of interest.

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