

## Serological diagnosis of cystic echinococcosis by the ELISA technique, in the cases hospitalized in the Surgical Clinic no. III and Internal Medicine no. III of Cluj-Napoca, during October 2006 – December 2009

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**Abstract.** The immunodiagnosis, in order to detect serical antibodies, is used to confirm the images that suggest hydatid cyst structures or for the differential diagnosis in case of imprecise tumor images. We have carried out serological tests in 104 individuals in two specialized clinics in Cluj-Napoca, in order to underline IgG type anti-*Echinococcus* specific antibodies. Serological tests were done in people with suspected hydatid disease (82 cases), the secondary and recurrent cystic echinococcosis (7 cases), and the people coming to a check after the intervention for the hydatid cyst (4 cases). Also at 9 persons the antibody titration was carried out before the surgery and 2 months after the surgery.

**Keywords:** Anti-*Echinococcus* antibodies; ELISA test; Individual serodiagnosis; Human cystic echinococcosis.

Received 01/03/2011. Accepted 27/06/2011.

### Introduction

Human infestation with *E. granulosus* determines an increased production of seric immunoglobulins and the formation of IgG, IgM, IgE and IgA specific antibodies (Siracusano et al., 2009). It is known that IgG is

high in the whole current infestations and in any localization, and it persists a different number of years after the ablation of the cyst (Lawn et al., 2004; Rafiei et al., 2008). The main aim of this study is the estimation of the serological tests in order to establish the diagnosis of cystic echinococcosis.

## Materials and methods

### *Hospitalized patients*

We have carried out serological tests in 104 individuals in order to underline IgG type anti-*Echinococcus* specific antibodies:

- 78 individuals hospitalized in the Surgical Clinic no. III of Cluj-Napoca, during October 2006 – December 2009; we have carried out a single titration in 60 individuals who were hospitalized for surgical intervention, in 9 individuals the antibody titration was carried out before the intervention and 2 months after the intervention, 7 individuals were hospitalized again 2 years after the surgical intervention, with suspicion of secondary or recidivating hydatidosis and 2 individuals who come to a check two years after the surgical extirpation of the hydatid cyst;
- 26 individuals hospitalized in Internal Medicine Clinic no. III of Cluj-Napoca, during October 2006 – December 2009; 22 individuals were hospitalized for different digestive problems, and 4 individuals come to a check 1 up to 3 years after the intervention for the hydatid cyst.

### *Immunodiagnosis*

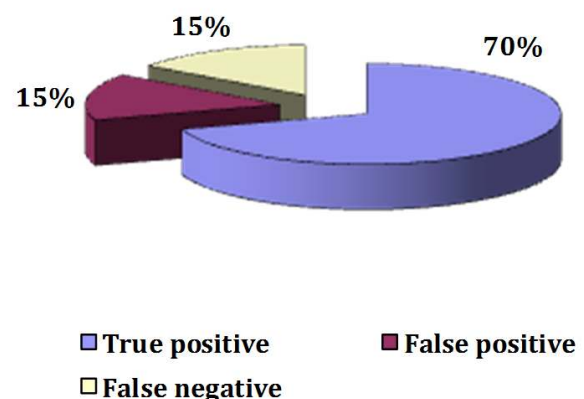
In order to establish the immunodiagnosis, we used the ELISA immunoenzymatic test for IgG antibodies, using German NovaTec kits. The results were expressed in NovaTec kit units (NTU). The values below 9 NTU are considered negative, those between 9 and 11 NTU are not convincing and those above 11 NTU are positive.

## Results and discussion

### *Immunodiagnosis in cases of the Surgical Clinic no. III, Cluj-Napoca*

Among the 60 individuals who underwent surgical intervention and a single titration, 16 had a positive serology, which means a percentage of 26.7%. In other 26 cases (43.3%) the values of the antibody titer were below cut off (the level of the test that is considered positive), but yet important, being above 6

NTU. We think that these values should be considered positive, in the context of obvious clinical and echographic symptoms. Thus, we have a positivity of 70% in the samples discovered with hydatid cyst and serologically tested (42 positive serums of 60 samples). There are 18 cases (30%) of the 60 investigated serums in which the serological results did not coincide with the state discovered during the surgical intervention. In 9 cases (15%) the serological results were false negative, and in 9 cases (15%) the serological results were false positive (figure 1). In human cystic echinococcosis, false negative and false positive results are not surprising. The immune response is influenced by features of the cyst, the serological test used, patient's immunity and the pathogenicity of the parasite (Poretti et al., 1999; Sadjjadi et al., 2009; Sandhu et al., 2009; Siracusano et al., 2009).



**Figure 1.** Comparison of the serological and surgical results

We have succeeded to carry out dynamic serological tests in 9 individuals, in the preoperative stage and one or twice in the postoperative stage, 2 months after the surgical intervention. In the whole 9 cases the diagnosis of hydatid cyst was certainly established in the intraoperative stage. The dynamic serological evolution of the cases is shown in table 1.

In table 1 we may see that there are variations of the level of the antibody titer, which were detected by IgG-ELISA enzymatic test, in the two stages of diagnosis: preoperative and postoperative. In 6 cases the level of anti-

*Echinococcus* antibodies progressively decreased do the elimination of the antigen source that induced the production of antibodies. In one case IgG-ELISA test showed a progressive increase of anti-*Echinococcus* antibodies level; in these individuals it raises the question of a possible secondary cystic echinococcosis by the dissemination of the parasitary elements in the body, possibly during the surgical intervention.

In the situations in which the antibody titer remains increased much time after the intervention, and the echography does not show the presence of a new hydatid in the body, WHO recommends to apply a secondary serological test, in order to confirm the parasitosis (Pawlowski et al., 2001).

In patients suspected with secondary or recurrent cystic echinococcosis (7 patients), after the anamnesis, the abdominal ultrasonography, the serological tests and the surgical intervention, we have established the following cases: one case of secondary cystic echinococcosis, four cases of recidivating cystic echinococcosis, one case of secondary cystic echinococcosis combined with recidivating hydatidosis and one case with a different diagnosis. The situation of the cases that are suspected of secondary or recidivating cystic echinococcosis and that come for a new hospitalization are shown in table 2.

Among the 78 individuals hospitalized in the Surgical Clinic no. III of Cluj-Napoca, which we have tested for anti-*Echinococcosis* antibodies, 2 come for an examination almost 2 years after the surgical intervention for hydatid cyst eradication. Thus, in the first case the titer has decreased from 19 NTU to 18 NTU, and in the second case from 35 NTU to 12 NTU.

*Immunodiagnosis in cases of the Internal Medicine Clinic no. III, Cluj-Napoca*

26 individuals hospitalized in the Internal Medicine Clinic no. III of Cluj-Napoca, were tested for hydatid cyst in order to carry out a differential diagnosis. 22 patients have come to the Internal Diseases Department due to different clinical signs and symptoms, such as: abdominal pains, cutaneous eruptions, migraines, nauseas and vomiting, hepatomegaly, scleral-tegumentary jaundice, etc. Another 4 individuals, who have come to an examination at the Internal Disease Department 1 up to 3 years after the surgical intervention, have negative serological results, and this shows that the antigen source that induced the production of antibodies was eliminated from the body and the secondary cystic echinococcosis did not appeared. The situation of the serological tests carried out in the individuals hospitalized in Internal Medicine Clinic no. III of Cluj-Napoca, suspected of cystic echinococcosis, is shown in table 3.

**Table 1.** Dinamics of anti-*Echinococcus* antibodies in serologically monitored people

Case no.	Preoperative test				Postoperative test I				Postoperative test II			
	Neg.	NTU Val.	Pos.	NTU Val.	Neg.	NTU Val.	Pos.	NTU Val.	Neg.	NTU Val.	Pos.	NTU Val.
1	X	1.68	-	-	X	1.68	-	-	-	-	-	-
2	X	6.29	-	-	X	5.33	-	-	X	4.7	-	-
3	X	8.6	-	-	Ambiguous: 9.1		-	-	-	-	X	11.5
4	-	-	X	36.44	-	-	X	37.55	-	-	-	-
5	-	-	X	21	-	-	X	18	-	-	-	-
6	-	-	X	20.9	X	5.6	-	-	-	-	-	-
7	-	-	X	14.6	-	-	X	12.53	-	-	-	-
8	-	-	X	11	X	7.1	-	-	-	-	-	-
9	-	-	X	19.23	Ambiguous: 10.4		-	-	-	-	-	-

**Table 2.** Situation of the cases suspected of secondary and/or recurrent cystic echinococcosis

Cases	First hospitalization		Second hospitalization	
	Diagnosis	Ac Titer	Diagnosis	Ac Titer
Secondary hydatidosis	HHC segment VII and VIII	-	HC of the abdominal wall	IgG=8.8NTU
Recurrent hydatidosis	CHH segment VIII	IgG=5.6NTU	HHC segment V calcified	IgG=10NTU
Recurrent hydatidosis	HHC segment VI with biliary fistula	IgG=29NTU	HHC segment VII	IgG=23.3NTU
Recurrent hydatidosis	HHC segment VI partially calcified	IgG=2.46NTU	HHC segments IV, V	IgG=20NTU
Recurrent hydatidosis	HHC segment VIII	IgG=1.5NTU	HHC segment III	IgG=2.2NTU
Operated HHC with case history	HHC segment VII corticalized CHH segment IV intraparenchimatous	IgG=4.7NTU	Chronic lithiasic cholecystitis	IgG=12NTU
Secondary hydatidosis with recurrent hydatidosis	HC retroperitoneal latero-iliac	-	Right renal HC HC of the left thigh muscles Recurrent HC of the right thigh muscles	IgG=20.47NTU

HHC-hepatic hydatid cyst; HC-hydatid cyst.

**Table 3.** Situation of the serological tests in people hospitalized in Internal Medicine Clinic no. III, Cluj-Napoca

Diagnosis	No. of cases	Serology	
		positive	negative
Hydatidosis	1	16NTU	-
	3	6-8.5NTU	
	1	-	5NTU
Alveococcosis ?	1	11.68NTU	
Hepatic tumors, carcinomas, hepatic cirrhosis, malignization processes	5	11-16NTU	
	2	6-8.5NTU	
	9		1.3-5.5NTU

It results the fact that, for a good diagnosis of the hydatid disease, in order to implement an appropriate therapy, it is not enough a single investigation, but physicians should associate the serological and imagistic data with the anamnesis of the cases. A good cooperation and an exchange of information between the clinical physician and the laboratory should be imposed.

The implementation of postoperative surveillance protocols of the individuals who underwent hydatid cyst interventions, by serological and imagistic tests, whichever the case may be, should be benefic in order to ensure a qualitative medical act and on behalf of the patient.

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