Serological diagnosis and its applicability to the prophylaxis and therapy of hydatid cyst in human patients

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Abstract. The parasites of genus Echinococcus are small cestodes (1-6 mm), which, according to the species, can be found in dogs (E. granulosus), small foxes, coyotes or wolves (E. multilocularis). In larval stage, they are etiological agents of human echinococcosis (hydatid disease or echinococcal disease). Diagnosing the hydatid infection is complex and requires multiple paraclinical, lab investigations and imaging techniques (X-ray, ultrasound, CT and MRI), which usually confirm the clinical suspicion. The results of serological examinations always have to be linked related to clinical diagnosis, imaging and other lab techniques. The study was made in 2007-2009 and included 279 symptomatic and asymptomatic patients from endemic and non-endemic areas of Cluj and Salaj. In the present study we used the ELISA method and German NovaTec kits in order to detect specific antibodies of class IgG. The results came in (NTU) NovaTec units. The results show the relevance of running the serological tests in order to confirm or refute the clinical suspicion of hydatidosis and to apply a differential diagnosis. The results obtained show that serologic examinations allow active identifying of people with hydatidosis, in the early phases.

Keywords: Hydatid cyst, serological diagnosis.

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The parasites of genus Echinococcus are small cestodes (1-6 mm) which, according to the species, can be found in dogs (E. granulosus), foxes, coyotes or wolves (E. multilocularis). In larval stage, they are etiological agents of human echinococcosis (hydatid disease or echinococcal disease) (Gherman, 1994).

In case of E. granulosus infection, the etiological agent of cystic echinococcosis, the cysts are mainly localized in the liver (50-70% of cases), lungs (25% of cases), and rarely in CNS, spleen, kidneys, bone tissue, bone marrow, heart. Most of the times, the primary infection consists of a single cyst, but in 20-40% of the cases multiple cysts may appear, residing in the same of different organs. The hydatid disease may evolve silently/asymptomatically, for months or years. The evolution depends on the age of the patient, the location, number and dimension of the cysts, the quality of the membranes of the cysts (normal, cracked, thick, calcified), the
aspect of the hydatid liquid (clear, infected) (Junie, 2000).

The *E. multilocularis* infection mainly affects the liver, which is invaded by hydatid vesicles located in the thickness of parenchyma, having the specific aspect of crumb (alveolar echinococcosis). Without treatment, the infection may be lethal (Popescu, 1998).

Diagnosing the hydatid infection is complex and requires multiple paraclinical, lab investigations and imaging techniques (X-ray, ultrasound, CT and MRI), which usually confirm the clinical suspicion. The results of serological examinations always have to be linked related to clinical diagnosis, imaging and other lab techniques (Coroiu and Junie, 1995).

The purpose of the study were: (1) to perform a serological study by ELISA method in symptomatic and asymptomatic patients from Cluj and Sălaj counties; (2) to elucidate the epidemiological, therapeutic and prognosis-related aspects of the cystic echinococcosis and (3) to analyze the serological tests (the ELISA method) as screening and diagnosing methods of cystic echinococcosis. All of these aims intend to state the real incidence of hydatid cyst in the studies areas and to describe the distribution of cystic echinococcosis according to their habitats and gender.

Using the ELISA method, we also inteded to diagnose precociously cystic echinococcosis and actively tracking down patients with cystic echinococcosis in precocious, detectable and non-detectable stages by means of imaging techniques, patients who suffers from atypical clinical signs (abdominal pain, rash, migraine, nausea and vomiting).

**Materials and method**

The study was made in 2007-2009 and included 279 symptomatic and asymptomatic patients from endemic and non-endemic areas of Cluj and Sălaj counties.

The patients taken into consideration for this study were clinically suspect of suffering from hydatid cyst, complicated or non-complicated, located in different organs: liver, lungs and brain. According to the location of hydatid cyst, patients suffered from uncharacteristic clinical reactions (malaise, progressive asthenia, loss of appetite, loss of weight), hepato-biliary reactions (hepatomegaly), pulmonary reactions (cough, shortness of breath, pain in the chest, hemoptysis), neurological reactions (focus issues, headache, dizziness) and allergic reactions (pruritus, edema, eosinophilia). The complications of hydatid cyst reside in the infection of the cyst (hepatic, pulmonary abscess), in hepato-biliary compressive phenomena resulting from the pressure lain upon by the cyst (jaundice and pain in the right hypochondrium; cholangitis as a consequence of the obstruction of biliary ducts), the tearing (biliary fistulas) or rupture of the hydatid cyst (spitting hydatid debris) with excreting the hydatid debris in the feces (hidatoenterita), vomiting (hidatoemeza); severe anaphylactic reaction (in major ruptures of the cyst) (Eckert and Deplazes, 2004).

In humans, the infection with *Echinococcus granulosus* determines the emission of circulating serum antibodies class IgG, IgM, IgA and IgE.

In the present study we used the ELISA method and German NovaTec kits in order to detect specific antibodies of class IgG. The results came in (NTU) NovaTec units.

**Interpretation**

- Positive results: values ≥ 11 NovaTec units (NTU)
- Negative results: values ≤ 9 NovaTec units (NTU)
- Inconclusive results: values between 9 and 11 NTU

**Interpretation of the results obtained after running serological tests**

**Positive serological tests**

- The immune response in hydatidosis resides in inducing the specific antibodies, but is influenced by certain factors
- IgG antibodies persist more months or years after the radical surgical intervention,
for which reason it is difficult to assess the therapeutic success.
- High volume of antibodies can be found in serum, irrespective of the location of the hydatid cyst. Hepatic cysts are associated with a more intense humoural response than the cysts located within other organs.
- The level of antibodies is reduced in patients with intact cysts and especially in case of old, calcified cysts.
- The tearing or rupture of a cyst is closely linked to a significant simulation of the production of antibodies.
- The antibodies may be a result of some virulent parasite strains or of an exaggerated immune response which led to the destruction of the parasite.
- The presence of other pathogens is not excluded; in this case a heterologous immune response is possible.

Negative serological tests do not exclude the hydatidosis diagnostic and can be seen in:
- the incipient stages of the infection, due to the low level of antibodies (negative or ambiguous results)
- recurrent infections, in which there is usually no sign of a significant growth of the level of antibodies
- the ELISA test must be repeated within 2-4 weeks, if there is clinical suspicion of hydatidosis.

Results and discussion

Hydatid cyst in patients serologically tested with the ELISA method from Cluj and Sălaj

Out of the 279 patients tested in both counties (Cluj and Sălaj), 22 patients were seropositive, which shows a 7.89% incidence of the hydatid cyst tracked down by means of the ELISA serological tests, in the tested patients.

According to the location of the hydatid cysts, it was established that 4.66% of hydatid cysts were located in Cluj and 3.22% in Sălaj, which proves that the incidence of hydatid cyst tracked down by means of serological ELISA tests is low in both counties.

<table>
<thead>
<tr>
<th>Table 1. Distribution of hydatid cyst in Cluj and Sălaj</th>
</tr>
</thead>
<tbody>
<tr>
<td>Tested patients</td>
</tr>
<tr>
<td>Positive patients</td>
</tr>
<tr>
<td>4.66%</td>
</tr>
</tbody>
</table>

The serological tests have confirmed the suspicion of hydatidosis in 4.66% of the patients with the clinical suspicion of hydatidosis in Cluj and in 3.22% of the patients with the clinical suspicion of hydatidosis in Sălaj and refuted the suspicion of hydatidosis in 92.1% of the total tested patients.

Thus, it can be stated that in 2007-2009 the incidence of hydatid cyst in Cluj and Sălaj was 7.89%, higher in Cluj than in Sălaj.

The results show the relevance of running the serological tests in order to confirm or refute the clinical suspicion of hydatidosis and to apply a differential diagnosis. It is important the differential diagnosis with: amoebian cyst, amoebian abscess, congenital cyst, some neoplastic processes, simple biliar cysts, teratomas, congenital lung malformations, pulmonary TBC (tuberculum, filled cavern), malign, primitive or metastatic lung tumors, benign lung tumors (hamartom, leiomiom, hamangiom), pulmonary sequestration, arterial aneurysm, intrapulmonary arterio-venous aneurysm, encysted pleurisies especially the intrascizural ones, some mediastinal tumors (timom, lipom), pleuropericardic cyst, pulmonary or hepatic abscesses, hematomas, arterio-venous malformations.

The distribution of seropositive patients according to their habitat in Cluj and Sălaj

Out of the total number of tested patients (279), from Cluj (138) and Sălaj (141), 139 (49.82%) came from the urban area and 140 (50.18%) came from the countryside; thus
there is a relatively constant distribution according to the habitat (urban/rural). Out of the total number of the tested patients from Cluj and Sălaj, 2.87% of the patients coming from the urban area and 5.01% of the patients coming from the countryside developed positive serological results.

The study shows a higher incidence of hydatid cyst in the rural area (5.01%) than in the urban area (2.86%), but does not show a significant difference as the one mentioned in the medical studies, according to which the hydatid disease has a high incidence in our country, namely 5-6 cases out of 100 000 people, predominantly in the rural areas (Georgescu et al., 2003).

The fact that most of the patients come from rural areas makes the disease part of zoonosis, as these patients are most of the time exposed to animals (Horvat et al., 1997).

The higher incidence of hydatid cyst in rural areas than in urban areas requires greater efforts in tracking down hydatid cyst, by means of serological methods in rural areas, as well as by means of prevention and control strategies (media, mandatory veterinary check ups).

Table 2. Distribution of seropositive patients according to their habitat in Cluj and Sălaj

<table>
<thead>
<tr>
<th>Tested patients</th>
<th>Cluj</th>
<th>Sălaj</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td>U</td>
<td>R</td>
<td>U</td>
<td>R</td>
</tr>
<tr>
<td>Total no. of tested patients</td>
<td>75</td>
<td>63</td>
<td>64</td>
</tr>
<tr>
<td>Total no. of positive patients</td>
<td>5</td>
<td>8</td>
<td>3</td>
</tr>
<tr>
<td>%</td>
<td>1.7</td>
<td>2.8</td>
<td>1.1</td>
</tr>
</tbody>
</table>

U – urban; R – rural.

The results are shown in figure 3 as well.

Figure 2. Distribution of seropositive patients according to their habitat in Cluj and Sălaj

The distribution of seropositive patients in Cluj and Sălaj according to gender

The case study is based on 128 males (45.9%) and 151 females (54.1%). There is a slightly higher incidence of hydatid cyst in males, 4.3%, than in females 3.85%.

Echinococcosis is a disease closely related to certain jobs: butchers, farmers, shepherds, and the geographic distribution of the disease follows the curve of the infection in shepherds and is determined by the level of pastoral hygiene (Burlui and Roșca, 1977).

Table 3. The distribution of seropositive patients according to gender in Cluj and Sălaj

<table>
<thead>
<tr>
<th>Tested patients</th>
<th>Cluj</th>
<th>Sălaj</th>
</tr>
</thead>
<tbody>
<tr>
<td>F</td>
<td>M</td>
<td>F</td>
</tr>
<tr>
<td>Total patients</td>
<td>87</td>
<td>54</td>
</tr>
<tr>
<td>Positive patients</td>
<td>4</td>
<td>5</td>
</tr>
<tr>
<td>%</td>
<td>4.59%</td>
<td>9.25%</td>
</tr>
</tbody>
</table>

F – female; M – male.

The results are shown in figure 3 as well.

Figure 3. The distribution of seropositive patients according to gender in Cluj and Sălaj

Epidemiology of hydatid cyst in Cluj County

From 138 investigated persons through ELISA serological tests, in Cluj County, 9.42% presented positive results at the ELISA serological tests.
Figure 4. Detection of hydatid cyst of the persons investigated through ELISA serological tests in Cluj County

We can observe that in Cluj County, hydatid cysts detected through serological methods were more numerous in rural areas (5.79%) than urban ones (3.62%).

Figure 5. Distribution of seropositive people in terms of area of origin (rural/urban) in Cluj County

Distribution of seropositive persons by gender in Cluj County

From 138 people tested, it can slightly be observed a higher incidence of hydatid cyst at men 5.1% than at women 4.35%.

Epidemiology of hydatid cyst in Sălaj County

Following the origin of those investigated through ELISA serological tests in Sălaj County, there were 6.38% persons detected with hydatid cyst.

Figure 6. Distribution of seropositive persons by gender in Cluj County

The study shows that the incidence of hydatid cyst in Sălaj is higher in rural areas, given that 4.2% of hydatid cysts were diagnosed at people from rural areas compared to only 2.12% of seropositive persons from urban areas.

Figure 7. Detection of hydatid cyst of the persons investigated through ELISA serological tests in Sălaj County

The distribution of seropositive persons by gender in Sălaj County

From 141 tested persons, there were 3.55% men and 2.84% women. It can be noticed a slightly higher incidence of hydatid cyst at males comparing to females.
From the information obtained comparing the results of the two counties, it reveals a higher incidence of hydatid cyst in Cluj County, 3.62%/2.12% which explains the higher incidence of hydatid cyst in Cluj County towards the rural area from Sălaj County 5.79%/4.2% and a higher incidence at men 5.1%/3.55% comparing to women 4.32%/2.83%.

Epidemiological studies highlight the need for intensifying detection efforts and for preventing from hydatid cyst in both counties but mostly in Cluj County.

Imaging methods

From the total of persons with positive ELISA serology (IgG antibodies anti-Echinoccocus positive), we investigated 63.63% individuals through imaging methods. As imaging methods in order to confirm the diagnosis of hydatid cyst detected through serological tests we used: CT cranial, chest RX, CT thorax and abdomen-pelvic ultrasound. At 57.14% of people who presented themselves for investigation, it revealed during the abdominal examination, cystic structures, with suggestive images of liver hydatid cyst, taking into account the classification of Gharbi.

At 7.14% of those investigated, positive serology could specify the diagnosis of hydatid pulmonary cyst. It proved to be a broken and partially eliminated cyst. Without the possibility of a parasitological examination of the vomiting fluid, the diagnosis was based on positive serology and chest ultrasound, and in this case emphasizing the efficiency of the serological diagnosis, combined with diagnostic imaging.

Positive serology permitted also the confirmation of the diagnosis of renal hydatid cyst from 7.14% of the investigated persons who presented through ultrasound, a renal cystic formation, claiming the differential diagnostic of the formation, with the following diseases: malignant tumor, polycystic renal disease, renal abscess, hydronephrosis and inside the cyst hemorrhage, taking into account the diseases mentioned.

Malignant tumor shows a more serious clinical picture and the imagistic computed tomography, ultrasoundography and imaging through magnetic resonance makes the difference. Polycystic kidney disease is always bilateral and the renal function and appearance of arterial hypertension are almost present. Renal abscess comes from skin infection and it ultrasonographically reveals a hypechoegen content of this (Krige and Beckingham, 2001).

Renal hydatid cyst is more difficult to differentiate, specific immunologic tests being useful in the diagnosis of renal hydatid cyst. Infections, hydronephrosis, and inside the cyst hemorrhage are possible complications, but rare for the simple renal cysts (Eckert and Deplazes, 2004).
At 18.18% of the investigated persons, the hydatidosis diagnostic through imaging methods could not be confirmed. Please note that the cystic lesion in order to be highlighted imagistically, should provide a diameter of at least 0.8 cm, so it is likely that people with positive and imagistically unconfirmed serology, present a hydatid cyst with a diameter greater than 0.8 cm, making necessary the investigation through other method (Western Blott, a fine needle aspiration puncture) or an imagistic re-investigation of patients after a certain period of time (Mortelé and Ros, 2001).

**Conclusions**

1. Hydatidosis remains one of the current problems of public health, although the incidence of these infections is noticed to be relatively low.
2. The results obtained show that serologic examinations allow active identifying of people with hydatidosis, in the early phases, undetectable by imagistic examinations, which represents a premise of premature hydatidosis diagnosis and permits therapy and the establishment of drug therapy and avoidance of complicated forms or inter/postoperative complications.
3. ELISA IgG antiechinococcus technique must be associated with other diagnostic methods to confirm results.
4. ELISA is a useful technique for population screening, where corrections of the results can be made through statistical calculation.

5. Antiechinococcus IgG with elevated values are presented in serum regardless the localization of the hydatid cyst and persists many years after cyst removal.

6. Values obtained through ELISA IgG antiechinococcus technique must be interpreted in context and correlation with clinical imagistic symptoms.

7. The implementation of some pre- and postoperative surveillance protocols for persons with hydatid cyst, through serological and imagistic tests could be beneficial for ensuring a measure of quality care and patient service. In this activity, family doctors, serological diagnostic imaging services and surgical internal medicine services must be involved.

8. Stopping hydatidosis in the early stages by drug treatment would avoid repeated hospitalizations, with social, financial, emotional consequences upon those involved, being both in the individual and the society benefit by reducing temporary disability of work and increased costs through prolonged and multiple hospitalizations.

9. Specifying the actual incidence of hydatid cyst, though difficult, is a necessity; this goal being hard to achieve, requiring early detection of infected persons in the territory, by improving the detection methods and a close collaboration between general practitioners, clinicians, statisticians and laboratory, for a correct diagnosis of hydatidosis.

10. It is required the implementation of diagnostic protocols in hydatidosis, to ensure a medical quality treatment and patient benefit.

11. It is required the hydatidosis prevention through intensifying the means of mass popularization of contamination and the significant consequences of this disease on human health.

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