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Case Report Open Access

Unusual Occurrence of Advanced Alveolar Echinococcosis in Young Patients from Poland

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Abstract

Background: Human alveolar echinococcosis is a severe parasitic disease of a progressive and neoplasm mimicking development, which has begun an emerging infection in Poland and some parts of Europe. Due to the very slow development after primary infection in human beings, in most cases alveolar echinococcosis is usually diagnosed in patients older than 40 years old. Advanced cases of *E. multilocularis* infection are sporadically reported in younger patients.

Objective: The aim of this study is to describe an unusual clinical course of severe liver alveolar echinococcosis in young Polish patients which is sporadically observed in other endemic countries.

Methods: Multi-disciplinary clinical and laboratory examinations, including epidemiological interview, imaging, histopathological and immunodiagnostic techniques have been performed during hospitalization in a reference university centre.

Results: We present three exceptionally rare cases of alveolar echinococcosis with final diagnosis established at an age under 30 years old. Thought a medical diagnosis recognised at a young age, only one of those patients could undergo a radical surgical resection of the parasitic mass located in the liver. Two other cases received long-term chemotherapy with albendazole due to an advanced and non-operative parasitic process with distant and contiguous metastases to the lungs and omentum. A possibility of a more intensive invasion with local *E. multilocularis* strains of a higher pathogenicity or acquired in very early infancy has been discussed.

Conclusions: 1. Alveolar echinococcosis should always be taken into consideration in a differential diagnosis of irregular space-occupying lesions located in the liver, especially in patients who live in endemic areas and their epidemiological history indicates potential risk factors for the infection. 2. In young patients alveolar echinococcosis is actually more frequently recognised than previously expected, and a clinical prognosis can be no less severe than in elderly patients. 3. Early diagnosis of *E. multilocularis* infection in humans facilitates the choice of proper and optimal treatment for saving or significantly extending a patient's life.

Keywords: Human alveolar echinococcosis; *Echinococcus multilocularis*; Age; Risk factors; Zoonosis

Introduction

Human alveolar echinococcosis (AE) is a chronic and potentially fatal parasitic disease caused by a metacestode infection of *Echinococcus multilocularis*. In recent years, an increasing number of new cases with alveolar echinococcosis has been reported not only in Poland, but also in other European countries. The infection is caused by the accidental ingestion of invasive tapeworm eggs, which are shed with faeces of definitive hosts and widely spread in the natural environment. In Poland, the main risk factors for this parasitic infection in humans include close contact with red foxes (*Vulpes vulpes*), raccoons (*Nyctereutes procyonoides*), rarely domestic animals - dogs and cats [1], and an occupational or recreational exposure to contaminated soil or forest litter. Professions related to forestry, hunting, gathering brushwood, picking mushrooms or berries have been documented to be potential risk factors for this severe anthropozoonosis, including an increasing predisposition of its occurrence in family agglomerations. AE in humans is classically characterized by a progressive tumour-like growth of the parasite with a tendency for invasive infiltration of neighbouring tissues or organs, and a formation of distant metastases in the lungs and brain, imitating disseminated malignancy. In 96.8% of infected cases, the liver is the primarily affected organ, however rarely primary lesions may be located in the brain and lungs or in other internal organs [2]. Primary alveolar echinococcosis of the adrenal glands has also been reported in literature [3,4]. The disease may develop unnoticeably or even secretly for many years, and may be asymptomatic or subclinical for a long period of time. Usually, lesions located in the liver can be found accidentally during imaging examinations performed due to other conditions or proposed for medical screening (ultrasound or computed tomography of abdominal cavity) [5]. For this reason, a final diagnosis of human alveolar echinococcosis is usually established late, during the advanced stage of infection when irreversible clinical signs associated with obstructive jaundice with liver insufficiency appear. Weight loss including cachexia, prostration, ascites, secondary bacterial infections, peripheral oedema, respiratory distress and focal

neurological deficits are usually related to severe complications or generalised metastases of a very poor clinical prognosis. In Europe, the average age of diagnosing alveolar echinococcosis is 52.5 years, and the infection is occasionally only recognised in persons under 30 years old [2,6].

Until recently, human AE has been sporadically diagnosed in the Polish population, and the age of infected patients did not differ significantly from the European average. Recent epidemiological surveys have shown an increasing incidence of *E. multilocularis* infection among inhabitants from Pomorskie, Warmińsko-Mazurskie, Podkarpackie and Małopolskie districts, located in the forested northern and southern areas of the country, and AE has become an emerging disease in Poland. In Polish patients with confirmed AE and identified under the national control programme for alveolar echinococcosis, the mean age at the time of final diagnosis is 47.6 years, but surprisingly children and young people aged 6-20 years old and infected in early infancy constituted nearly 10% of the patients' population. The diagnosis is the most commonly established in the 41-50 years old group of Polish patients [7]. This study describes three uncommon cases of AE, recently hospitalized in the Department of Tropical and Parasitic Diseases, University of Medical Sciences in Poznań (Poland), who were treated for advanced *E. multilocularis* infection. All the patients were diagnosed at the age under 30 years old.

Case Presentation

Patient 1

A 27-year-old male, who lives in a small village in West Pomeranian Voivodeship, was admitted to the Department for the first time in October 2011 with the clinical suspicion of classic cystic echinococcosis, and a necessity of implementing specific pharmacological treatment for *E. granulosus* liver infection. The epidemiological interview suggested risk factors typical for *E. multilocularis* infection, which included daily work on a farm (red foxes in the yard) and close contact with domestic animals - dogs and cats. For 6 months the patient complained of a nonspecific pain in the right upper quadrant of the abdomen. During an outpatient diagnostic process, the ultrasound examination of the abdominal cavity revealed multiple focal lesions located in liver, with the possibility of their parasitological aetiology. In the patient's medical history there was successful treatment of past pulmonary tuberculosis. During admission to the Department, a physical examination revealed only pain in his right epigastrium. An enzyme-linked imunosorbent assay (ELISA) for *Echinococcus* sp. infection was moderately positive (6.1 Units). The ultrasound image of the abdominal cavity revealed two hypodense lesions located close to one another; the first was 43 mm long and 48 mm wide, the second 53 × 80 mm respectively, with a calcified, hyperdense wall and irregular "empty" spaces in a central area (Figure 1). Those changes were localized in the 7th segment of the liver and suggested degenerating hypo-hyperdense echinococcal cysts of a CE4 type according to the World Health Organization (WHO) classification [8]. On the basis of an imaging examination and positive results of a routine serological test, *E. granulosus* infection was first diagnosed, and classical treatment with albendazole was implemented. The patient was hospitalized in the Department five more times for a clinical follow-up, evaluation of the developmental stage of the disease and continuation of conclusive cycles of pharmacological anti-parasitic treatment. During the fourth hospitalization in July 2013, an abdominal ultrasound revealed new changes in subphrenic parts of the right liver lobe in the form of a hyperechoic, polycyclic area with a size of 94 × 61 × 27 mm, with necrotic lesions observed inside and infiltration of the right liver vein and the diaphragm. A thoracic computed tomography (CT) scan was performed and revealed inflammatory lesions with atelectasis located in the right 8th segment, adjacent to the thickened and infiltrated diaphragm (Figure 2). In laboratory examinations a significant increase in *Echinococcus*-specific immunoglobulin G (IgG) antibody was shown in the classic ELISA test (16 Units), and mild leucocytosis with neutrocytosis was found (11.28 G/l, 72% neutrophils). Moreover, concentrations of gamma-glutamyl transpeptidase (133 U/l) and alkaline phosphatase (141 U/l) were moderately elevated. On the basis of progression observed in the ultrasonography and chest computed tomography scan and poor response to previous medical treatment, a blood sample for a reference Western-blot test for a differential diagnosis of *Echinococcus* species was collected. The Western blot assay showed a typical immunological profile of specific IgG antibodies against 16 and 18 kDa antigens, and clearly indicated *E. multilocularis* infection (Figure 3). A final diagnosis of progressive liver alveolar echinococcosis with metastases to the lungs by contiguous and hematogenous spreading has been documented, and classified as the most advanced P4N1M1 stage according to the reference international AE classification [5]. As surgery has not been suggested, a more intensified and prolonged anti-parasitic chemotherapy with albendazole has been urgently introduced. The clinical follow-up until November 2014 showed no further progression of the disease.

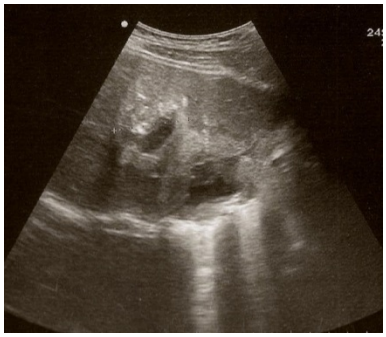

Figure 1: Atypical pathological lesion in the liver caused by *E. multilocularis* mimicking degenerating cystic echinococcosis. Ultrasonography of abdomen (ALOKA ProSound Alpha 7, Japan).

Patient 2

An 18-year-old female, who lives in a small village in Warmian-Masurian Voivodeship, was transferred to the Department from a regional hospital in July 2012 for the implementation of pharmacological treatment for *E. multilocularis* infection. Frequent work in a kitchen garden without gloves, close contact with dogs and seasonal picking of mushrooms and blueberries were potential risk factors of the infection. In January 2012, the patient started to complain of pain in the right epigastrium. An ultrasonography of the abdomen performed in an outpatient clinic revealed irregular focal lesion in the liver of a mixed hypodense-hyperdense structure 81 mm long and 86 mm wide (Figure 4). Before admission to the Department, the patient was hospitalized at oncology and infectious diseases hospitals with different diagnoses, until a positive ELISA test for *E.*

multilocularis-specific Em2plus antigen raised suspicion of alveolar echinococcosis. In April 2012, the patient underwent surgical resection of the 2nd, 3rd, and 4th liver segments.

Patient 3

A 19-year-old male, who lives in a village in Masovian Voivodeship, was admitted to the Department for the first time in 2008 due to multifocal lesions in the liver in a course of alveolar echinococcosis. Foxes present close to the home yard, daily contact with dogs and cats, and history of picking mushrooms and wild fruit were all potential risk factors for *E. multilocularis* infection for this patient. The patient underwent some treatment started in 2005, because he suffered periodically of watery diarrhoea without blood and mucous. Due to these complaints, the patient was frequently hospitalized in local hospitals. A CT scan of the abdominal cavity, taken in 2007, revealed non-homogeneous and hypodense foci located peripherally in the 4th liver segment. A next lesion with the dimensions 35 × 25 × 25 mm with many calcifications and small compartments was located in the 3rd segment, being not well demarked from the tissue of the left liver lobe. In segment 8 another non-homogeneous lesion was described. Those changes were typical for class P3N1 in the WHO PNM classification due to a central localization in the hilus, and affection of both liver lobes and omentum. A traditional ELISA test for *Echinococcus* sp. was positive. The patient underwent diagnostic laparotomy, in which samples from the liver and omentum were taken. A histopathological investigation discovered fibrotic and necrotic tissue with calcifications without neoplastic cells, strongly suggesting alveolar echinococcosis. Retrospective Periodic Acid-Schiff (PAS) staining revealed a multi-alveolar structure of the parasite with characteristic purple walls, and finally confirmed the diagnosis (Figure 5). Outpatient immunoblotting for *E. multilocularis* showed a positive result for the genus of *Echinococcus* of the P5 immunological profile, with specific IgG antibodies against 7 and 26-28 kDa antigens (a lack of specific 16 and 18 kDa bands) (Figure 3). The patient started intensive chemotherapy with albendazole. During admission to the Department in 2008, the patient still complained of a pain in the right upper quadrant, which occurred periodically. Physical examination revealed a scar after laparotomy and mild hepatomegaly (liver was palpable 1.5 cm under the costal margin). Laboratory investigations revealed strongly elevated liver enzymes (alanine aminotranspherase 1293 IU/l, aspartate aminotranspherase 775 IU/l, alkaline phosphatase 154 IU/l, gamma-glutamyl transpeptidase 101 IU/l) with hyperbilirubinemia (total bilirubin 2.6 mg/dl). Infections caused by hepatotropic viruses were excluded. Patient underwent endoscopic retrograde cholangiopancreatography which did not reveal obstruction in the bile ducts. Finally, the elevated liver markers were associated with an advanced stage of alveolar echinococcosis. A reference Western-blot test performed in the Department confirmed infection with *E. multilocularis*. The ultrasound of the abdominal cavity revealed poorly demarcated lesions with many calcifications with a diameter of 3 cm, which were localized in the left lobe of the liver. Similar changes were also described in the right lobe. On the basis of results of imaging and laboratory investigations, diagnosis of alveolar echinococcosis was finally confirmed. The stage of the disease development was evaluated as P3N1M0, according to the international PNM classification, with a documented clinical progression [5]. Due to the advanced stage of the infection, the patient was disqualified from surgical treatment. Specific therapy with albendazole was continued. Then he was hospitalized a few more times in the Clinic for evaluation of the clinical status, staging of the disease and treatment continuation. So far, no further progression of the infection has been observed until November 2014.

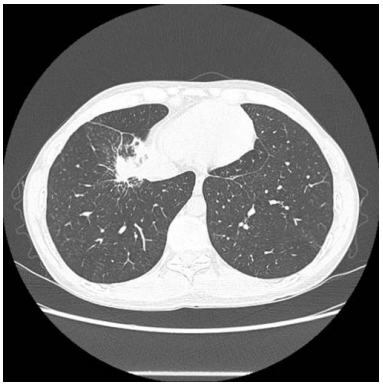

Figure 2: Advanced stage of *E. multilocularis* infection in the liver with infiltration of the diaphragm and right 8th segment of the lung. Computed tomography scan (General Electric Light Speed, United States).

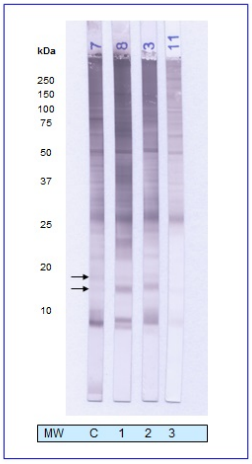

Figure 3: Immunological profiles of specific anti-*E. multilocularis* IgG antibodies using Western blotting in three patients hospitalized in the Department. The arrows show two characteristic bands of *E. multilocularis* - specific antibodies against EM16 and EM18 antigens (LDBIO Diagnostics, Lyon, France). MW: Molecular Weights, kDa: Kilodaltons, C: positive control, 1-3: numbers of consecutive patients.

During first hospitalization in the Clinic in Poznań, the patient complained of abdominal discomfort which occurred periodically. A physical examination revealed a scar after hemihepatectomy, and no other abnormalities, with exception to accelerated erythrocyte sedimentation rate (ESR). A serological ELISA test for *Echinococcus sp.* had a doubtful result. A Western-blot assay showed typical bands of IgG antibodies against 7, 16, 18 and 26-28 kDa antigens specific for *E. multilocularis* infection (Figure 3). A retrospective clinical and imaging analysis allowed to classify the disease to the P3N0M0 stage. Prolonged specific treatment with albendazole was implemented. The

patient was followed-up in the Department four more times (for the last time in November 2014). During all hospitalizations, the ultrasounds of the abdominal cavity did not reveal a reactivation of the infection. A serological ELISA test was still within the borderline limits. The patient over the period of clinical observations was treated with albendazole according to a standard protocol of four-week cycles of therapy with the next two weeks of a break in between. The regular clinical and immunodiagnostic follow-up until November 2014 documented a regression of AE lesions.

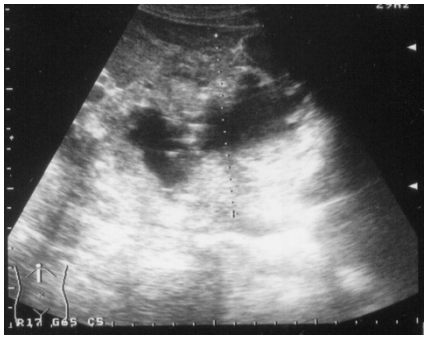

Figure 4: Irregular space-occupying lesion in the liver in a patient with alveolar echinococcosis. Ultrasonography of abdomen (ALOKA ProSound Alpha 7, Japan).

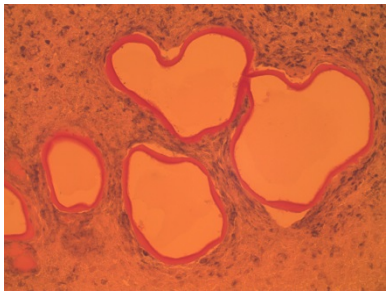

Figure 5: Multi-alveolar structure of *E. multilocularis* metacestode in the histopathological examination of a tumour-like liver lesion. PAS staining (magnification 400X).

Discussion

The presented clinical cases confirmed a nonspecific course of the *E. multilocularis* infection in Poland. Classically, alveolar echinococcosis is diagnosed mainly in elderly people, due to the oligosymptomatic course and long-time evolution of pathological changes in the liver. The primary infection occurs usually many years before the onset of clinical symptoms. Initially, the development of *E. multilocularis* metacestode in the liver may be unnoticed and subclinical, as usually it does not give any clinical signs or symptoms for a long period of time. More frequently, focal pathological lesions in the liver can be found accidentally during imaging investigation ordered due to other conditions [5]. This makes it difficult to establish precisely a time and source of infection. The symptoms may be associated with a large mass of the parasite in the liver, which can invade surrounding tissues, internal organs, veins, as well as biliary ducts. Patients usually complain of abdominal pain localized in the right upper quadrant. Moreover fatigue, malaise, fever, weight loss, diarrhoea, obstruction, and jaundice may occur. It does not exclude the possibility of infection in a young population, especially if it lives in hyperendemic areas with a very high risk of exposure to *E. multilocularis* eggs. Many reasons may lead to the early development of symptoms. A condition of the immunological system may be one of these reasons-polymorphism in the major histocompatibility complex may act as a factor for parasite tolerance [9,10]. The high intensity of infection related to a prolonged time of repeated exposure or high virulence and invasiveness of *E. multilocularis* strains in a given geographic area associated with some genetic factors, as well as different climatic, bio-meteorological, environmental conditions, can all have a direct impact on a much faster metacestode development. An inoculum size of *E. multilocularis* eggs can be higher in children than in adults due to worse hygienic habits and immaturity of the immunological system. A possibility of infection with some local parasite strains of a higher pathogenicity and a more invasive development, and acquired in early infancy cannot be excluded. The very popular Polish tradition of picking mushrooms and blueberries by children as a mass seasonal activity in forested areas probably has a great impact on a more early onset of the infection not reported in other European countries. Even if it is diagnosed in young patients, alveolar echinococcosis can present severe clinical manifestations related to advanced stages of the disease with irreversible complications and a poor clinical prognosis. Our clinical observations showed that alveolar echinococcosis in young patients does not reflect any early phase of a metacestode development nor a more benign clinical expression. In Patient 1, infiltration of the diaphragm and multiple hematogenous metastases to lungs documented a non-operative stage of advanced alveolar echinococcosis. A significant delay in a correct clinical diagnosis was the source of the patient's serious prognosis with a subsequent consequence of life-time chemotherapy with albendazole. In Patient 2, surgical reduction of the liver lesion combined with long-term chemotherapy has been proposed. Whereas, Patient 3 had a central localization of *E. multilocularis* metacestode in the hilus, with infiltration of both liver lobes and omentum, and extension along the main abdominal vessels and the biliary tree. The was no possibility of radical surgical removal of the parasitic mass, so the patient has been managed on prolonged pharmacological treatment with albendazole to obtain the parasitostatic effect and prolong the patient's life. Other actual clinical observations showed that in Poland, alveolar echinococcosis was also reported in children and adolescents [7].

Nonspecific symptoms which occur in the clinical course of AE infection may delay establishing of a proper diagnosis. The lesions observed in imaging investigations (ultrasound, computed tomography scan, magnetic resonance of the abdominal cavity) are usually not well demarcated and invade normal liver tissue, which routinely rehearses a diagnostic process to an oncological path. It is important to remember that primary lesions observed in a course of AE may cause local and distant metastases, which can be compared to advanced stages of generalised malignant neoplasms [11,12]. Of course, such diagnostic procedures are strongly justified due to the much more aggressive progression of primary hepatic carcinoma. This is the most important differential diagnosis of human alveolar echinococcosis. Epidemiological data may be very helpful in the differentiation of aetiology of focal lesions located in the liver. All presented patients lived in rural areas, one of them in a region defined as hyperendemic in Poland-Warmian-Masurian Voivodeship, where

over the last 20 years the largest number of cases has been reported [7]. There is also the highest prevalence of *E. multilocularis* infection in red foxes [13]. The risk factors for AE infection include ingestion of unwashed undergrowth (fruit like strawberries, vegetables) contaminated by fox faeces, as well as direct contact with soil including occupational (forest workers, hunters, farmers) and recreational risk (work in a kitchen garden, vacation camps, picking mushrooms or wild fruit) [14,15].

Diagnosis of AE should be established by taking into consideration multi-variable clinical data: epidemiological interview, symptoms, physical examination, and imaging investigations [7]. Serological assays are very helpful in a non-invasive diagnosis of AE in humans. An ELISA test is the first line laboratory procedure analysing specific antibodies against *Echinococcus* sp. antigens or purified EM2plus antigenic cocktail characteristic for *E. multilocularis*, and very useful for differentiation between *E. granulosus* and *E. multilocularis* infection [16]. The traditional ELISA assay should always be verified using immunoblotting, which determines a specific profile of antibodies against selected parasitic antigens. A positive result of a Western-blot assay based on serum samples is considered as conclusive [17]. Currently, the gold standard for a diagnosis of human AE recommended by the World Health Organization is a direct demonstration of the parasite in biopsy materials using histopathological investigation which reveal Periodic Acid-Schiff (PAS) stain positive structures or a detection of *E. multilocularis* nucleic acids using a PCR technique [18]. A fine needle aspiration biopsy, when primary hepatic carcinoma or human alveolar echinococcosis is suspected, is generally contraindicated, because of the risk of local invasion or generalised spreading, and other possible complications related to this invasive procedure.

A treatment of choice for alveococcal lesions located in the liver is a surgical resection, usually with complete removal of the affected segment or lobe (i.e. partial hepatectomy, lobectomy) combined with specific anti-parasitic chemotherapy [6]. However, this method cannot be used in every case, especially in centrally located lesions with hilar involvement, when a diagnosis is delayed, or established in advanced stages of the disease [18]. The decision of surgical treatment, should strive to maintain 2 cm of margin between parasitical mass and normal liver tissue [19]. It should be highlighted, that despite the young age, lack of co-existing serious chronical disorders, and quite early diagnosis, only one patient from the cases presented above might undergo surgical resection. In contrast to cases of cystic echinococcosis, there are no indications to pre-operative cover with anthelmintics. International PNM (P - parasite mass in the liver, N - neighbouring organs, M - distant metastases) classification, based on findings from imaging investigations, may be useful for qualification for surgery and practical clinical evaluation of progression of the disease [18].

Liver transplantation may be taken into consideration as one of methods of treatment, but its use in alveolar echinococcosis is at least controversial. In Poland, over the last 20 years, 15 liver transplantations were made to patients with very advanced stages of alveolar echinococcosis [7]. It should be highlighted that after transplantation, long-term treatment with immunosuppressive drugs may reactivate parasitic invasion and facilitate a formation of distant metastases to the lungs and brain with following complications and worse clinical prognosis [9].

For a patient who cannot undergo surgery, intensive long-term pharmacological treatment with albendazole is recommended, and a life-time therapy is usually proposed for advanced non-operative cases [18]. Therapy is based on drug intake for a period of 4 weeks with the following two weeks of rest. Adult patients take 800 mg of albendazole in two divided doses daily. In severely infected patients, treatment breaks can be reduced or omitted [6]. However, the treatment is expensive and non-refundable by the National Health Fund in Poland. If the disease is left untreated, it inevitably leads to a poor clinical prognosis including death. In Poland, over the last 21 years of the nation-wide programme for alveolar echinococcosis, 23 deaths due to AE infection were reported. The youngest patient was 24 years old [7].

To conclude, human alveolar echinococcosis is a rare, not well known anthropozoonosis in Poland. However, mainly due to the progressively increasing population of red foxes, a rapid increase of human cases may be expected in risk areas after a period of several years [20]. Similar tendencies are observed in other European countries, like Germany, France, Austria and Switzerland [21]. A significant level of *E. multilocularis* infection in slaughtered pigs in Poland can be a practical indicator of a disease risk for humans [22]. National health services and physicians of various specialties (family doctors, gastroenterologists, hepatologists, surgeons, oncologists, radiologists) should be informed about the significant risk of an accumulative emergence of new cases in endemic forested regions of the country in the near future. Alveolar echinococcosis typically gives clinical symptoms in elderly people after a long asymptomatic period of latent development, but an advanced stage of the disease may also be observed in young patients. Thus, alveolar echinococcosis should be taken into consideration during a differential diagnosis of irregular space-occupying lesions located in the liver, also in young patients who live in highly endemic areas and present a history of typical risk factors for this parasitic infection. A more invasive development and a shorter time for the formation of irreversible sequela with distant metastases in the Polish population at a younger age is very disquieting. An early and proper diagnosis of alveolar echinococcosis has an essential impact on a clinical prognosis and facilitates a selection of optimal methods and duration of effective anti-parasitic treatment, and leads to extend and improve the quality of a patient's life.

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