Turk J Gastroenterol *2014; 25: 398-404*

Diagnostics in cystic echinococcosis: Serology versus ultrasonography

LIVER

Johanna Wuestenberg[1], Beate Gruener[2], Suemeyra Oeztuerk[1], Richard A. Mason[3], Mark M. Haenle[1], Tilmann Graeter[4], Atilla S. Akinli[1], Peter Kern[5], Wolfgang Kratzer[1]

[1]Department of Internal Medicine I, University Hospital Ulm, Ulm, Germany
[2]Section Infection and Clinical Immunology, Department of Internal Medicine III, University Hospital Ulm, Ulm, Germany
[3]Louis Stokes Cleveland VA Medical Center, Cleveland, Ohio, USA
[4]Department of Interventional and Diagnostic Radiology, University Hospital Ulm, Ulm, Germany
[5]WHO Informal Working Group on Echinococcosis, Comprehensive Infectious Diseases Center, University Hospital Ulm, Ulm, Germany

Original Article

ABSTRACT

Background/Aims: Cystic echinococcosis (CE) is the most widespread zoonosis worldwide. The objective of the present study was to compare diagnostic methods in the work-up of suspected cystic echinococcosis of the liver.

Materials and Methods: Data from a total of 68 patients were compiled and analyzed.

Results: A diagnosis of cystic echinococcosis was made in 36.8% of patients. Broken down according to WHO criteria, patients with at least one echinococcus cyst were determined in 12.0% of cases to exhibit cysts consistent with stage 1 disease (CE1), while in 24.0%, cysts consistent with CE2 and CE3 were identified. CE4 and CE5 cysts were identified in 32.0% and 8.0% of patients, respectively. Solitary cysts were found in 60.0% of patients with cystic echinococcosis, while in patients with at least one cystic lesion, there were most often multiple cysts. The indirect hemagglutination test (IHA) and echinococcus-specific immunoglobulin E (IgE) concentration showed a higher sensitivity (60.9%, 68.4%) than did the enzyme-linked immunosorbent assay (ELISA) for *Echinococcus multilocularis* ($Em2^+$) and total IgE (11.1%, 38.9%). The respective specificities of all four serological methods lay between 83.9% and 88.9%.

Conclusion: Our data show that ultrasound remains the diagnostic method of choice in the work-up of cystic lesions of the liver suspected to be due to Echinococcus granulosus. Serological methods can serve an adjunctive role.

Keywords: Liver, *Echinococcus granulosus*, ultrasonography

INTRODUCTION

Cystic echinococcosis (CE) is the most widespread zoonosis worldwide (1). It is endemic in many regions of the world, such as nations bordering on the Mediterranean Sea, as well as in northern and eastern Africa, western and central Asia, China, South America, and Australia (2). The focus of the disease lies in regions with a low hygienic standard in which humans and animals live in close proximity (3).

The causative organism of CE is the dog tapeworm *(Echinococcus granulosus),* a parasite inhabiting the gastrointestinal tracts of canines and other wild carnivores as its definitive host. The primary host for the parasite is the dog; hooved domestic animals, such as sheep, cattle, goats, and camels, serve as intermediate hosts. By contrast, humans represent an aberrant host that is not part of the natural cycle and are infected accidentally (4). Infection usually occurs as a result of oral ingestion of parasite ova excreted with feces in dogs and transmitted through close contact with infected animals (smear infections, direct contact). Also, under discussion as a potential means of infection is the consumption of contaminated food, particularly in developing countries (5).

In humans, the parasite may, in principle, infest all organs but exhibits a predilection for the liver (70%-80% of cases), followed by the lung (20%-30%) (6). Less commonly involved are the spleen, kidney, heart, bone,

Address for Correspondence: Wolfgang Kratzer, Department of Internal Medicine I, University Hospital Ulm, Ulm, Germany
E-mail: wolfgang.kratzer@uniklinik-ulm.de
Received: 3.2.2014 **Accepted:** 11.3.2014

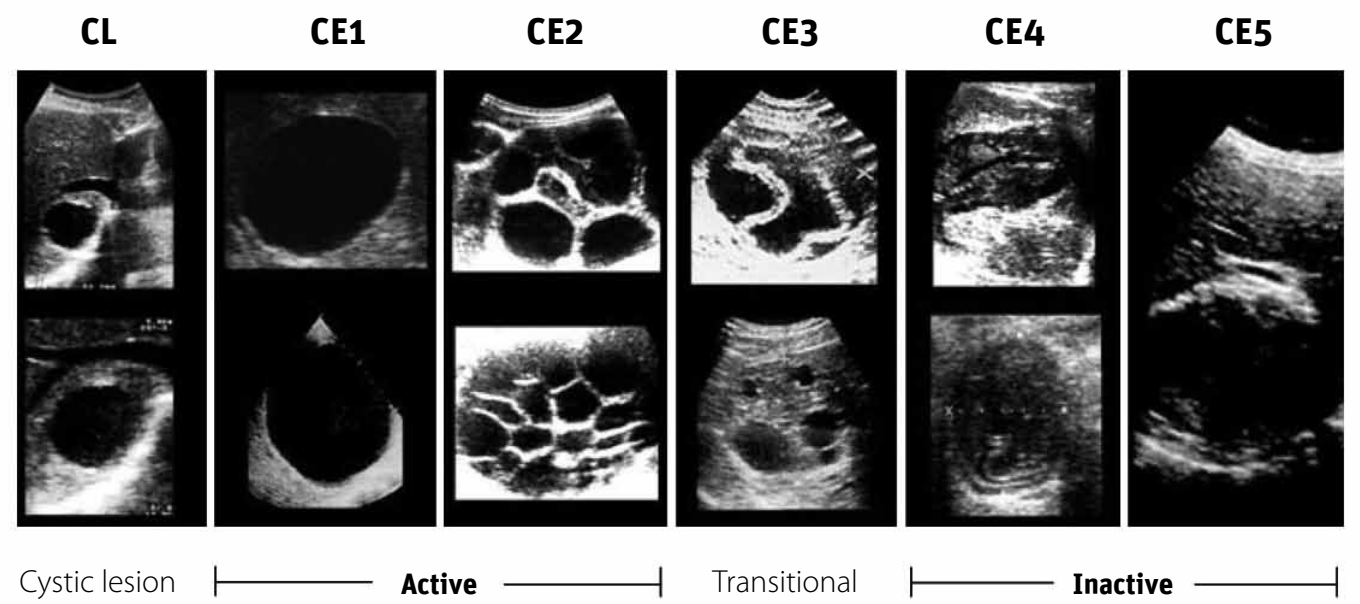

Figure 1. WHO-IWGE classification of cystic echinococcosis.

central nervous system, and other organs (7). CE is a disease that may remain asymptomatic for many years, with symptoms usually appearing only as the cysts increase in size (8).

Treatment of echinococcosis often becomes increasingly challenging as the disease progresses; hence, diagnosis in an early disease phase and differentiation from benign cystic lesions (CL) of the liver play a decisive role for patients' prognosis (9). Cystic lesions of the liver are detected by means of diagnostic imaging methods, such as ultrasonography, magnetic resonance imaging (MRI), or computed tomography (CT) (10). The diagnosis of cystic echinococcosis using serological methods remains controversial (11). Serological methods currently employed in the diagnosis of CE include the enzyme-linked immunosorbent assay (ELISA), the indirect hemagglutination test (IHA), the latex agglutination tests, and immunoblots (12).

The objective of the present retrospective study was to assess the value of different diagnostic methods employed in the work-up of cystic lesions suspicious for CE of the liver in a specialized clinic.

MATERIALS AND METHODS

Patient selection

For the present study, data compiled anonymously from 140 patients with suspected CE examined in a specialized clinic between 1999 and 2009 were analyzed. A total of 68 subjects with cystic lesions in the liver were included in the study. Exclusion criteria included: patients with unclear findings (n=2), misdiagnosed patients (n=1), missing serological findings (n=1), previously confirmed CE (n=50), and patients with cysts of the kidneys, spleen, or bone (n=9). Also excluded from the study was one patient who had been referred to the clinic based on elevated liver transaminase concentrations and who exhibited no other evidence of CE and one further patient who was examined preventively. Patients suffering from alveolar echinococcosis (n=7) were also not included. The study is in line with the Declaration of Helsinki and was approved by the local ethics committee.

Data gathering

The following parameters were reviewed from the hospital's medical record system: ultrasound findings, medical history, clinical symptoms, medication history, current medications, immunosuppression, and laboratory parameters (IHA, enzyme-linked immunosorbent assay (ELISA) for Echinococcus multilocularis ($EM2^+$), total Immunoglobulin E (IgE), echinococcus-specific IgE, aspartate aminotransferase (AST), alanine aminotransferase (ALT), gamma-glutamyltransferase (GGT) and bilirubin). AST, ALT, GGT and bilirubin were measured by using Dimension XL (Dade Behring Inc., Newark, DE 19714, USA). IgE was determined using ElectroChemiLumineszenz ImmunoAssay (ECLIA) MODULAR ANALYTICS E170 (Roche Diagnostics, Rotkreuz, Switzerland). $EM2^+$ was measured by ELISA-Reader Expert 96 (Asys Hitech, Eugendorf, Austria). For IHA Cellognost Echinococcosis test (Dade Behring, Marburg, Germany) was used.

Classification of liver cysts

Patients' ultrasound images were interpreted in accordance with the WHO informal working group on echinococcosis (WHO-IWGE) classification (Figure 1) (13,14). Based on this classification, liver cysts were categorized as cystic lesions (CLs) or cystic echinococcosis in stages 1-5 (CE1-CE5) (15,16). CE stages 1 and 2 are considered active disease stages in the WHO IWGE guidelines.

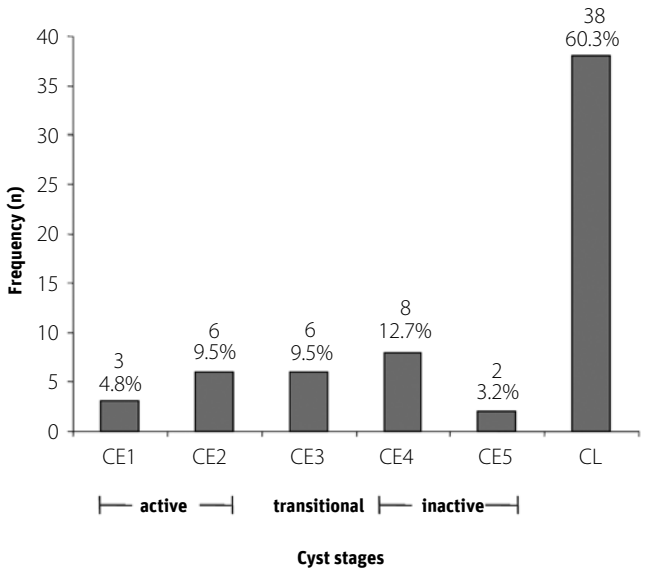

Figure 2. Frequency of different cyst stages.

Patients were classed as "infected" or "not infected" based on the following ultrasound diagnostic criteria. The assignments were then compared with the patients' respective serological findings.

CE1: Unilocular, round to oval, echo-free cysts, often double-walled. "Snowflake effect" apparent on shifting the patient's position due to free-floating protoscolices.

CE2: Daughter cysts visible as "wheel spokes" or "honeycomb" pattern within mother cysts.

CE3a/CE3b: Transitional stage in disease activity still possible. The laminate membrane on the cysts' inner surface dissolves as part of the degenerative process and produces the "water lily" sign (CE3a).

CE4: Heterogeneous internal structure with appearance of a "ball of wool." The cysts are inactive.

CE5: The inactive cyst has a thick, arch-like calcified wall that casts a conical ultrasound shadow. No protoscolices remain visible (17).

The ultrasound examinations were performed by using Philips IU22 and Philips HDI 3000/5000 scanners with C5-1 and C5-2 transducers (Philips, Bothell, USA).

Statistical analysis

Statistical treatment of the data was performed using the SAS 9.2 statistical software package (SAS Institute Inc. Cary, North Carolina, USA). Data were analyzed descriptively with respect to absolute and relative frequencies, means, and standard deviations. The correlation between ultrasound findings and serological results were tested using the chi-square test or, in the case of small numbers of cases, Fisher's exact test. Sensitivities and specificities were calculated for all four serological test methods.

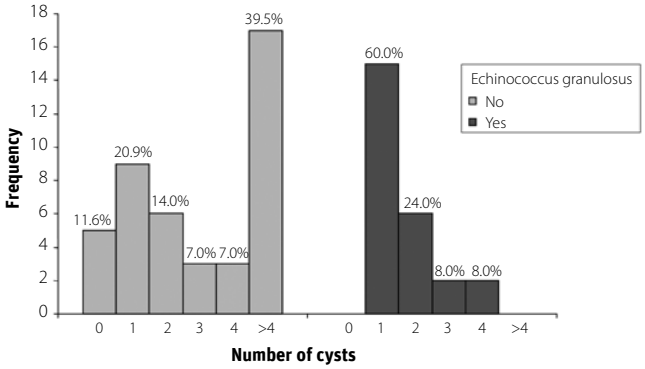

Figure 3. Number of cysts in patients with at least one cystic lesion and in patients with cystic echinococcosis.

RESULTS

Patient demographics

A total of 68 subjects (64.7% females, 35.3% males; mean age 55.6±13.8 years) with cystic liver lesions suspicious for echinococcosis were examined. Cystic lesions (CLs) were identified in 55.9% of patients (n=38). In 7.4% of the referred patients (n=5) in whom suspicion of echinococcosis rested exclusively on serological findings, sonographic examination failed to reveal any liver cysts. Sonographic findings consistent with the WHO IWGE criteria were returned in 36.8% of patients (n=25; 36% females, 64% males). Patients with at least one echinococcus cyst were on average younger than patients with non-echinococcal cystic lesions (50.8±15.4 years vs. 59.3±11.6 years; p=0.0272).

Sonographic findings

Broken down according to WHO criteria, patients with at least one echinococcus cyst were determined in 12.0% of cases (n=3) to exhibit cysts consistent with stage 1 disease (CE1), while in 24.0% (n=6), cysts consistent with CE2 and CE3 were identified. CE4 and CE5 cysts were identified in 32.0% (n=8) and 8.0% (n=2) of patients, respectively (Figure 2). Cysts displayed a visible wall in 90.0% of patients with stage CE1 and CE2 disease. In addition, sonomorphological features consistent with criteria established for the respective disease stages could be documented in all patients with cysts assigned to stage CE2 and stage CE3/CE4 disease. Only in 1 of 2 patients with stage CE5 disease did ultrasound fail to visualize the typical arch-like calcification and conical shadow.

Mean diameter of cysts in patients with CL stood at 46.1±27.7 mm compared with 95.2±116.5 mm in patients with CE; this difference, however, was not statistically significant (p=0.1146).

Solitary cysts were found in 60.0% of patients with CEs. By contrast, in patients with cystic lesions, ultrasound often detected more than four cysts (39.5%; Figure 3). Individual patients ex-

Table 1. Serological findings in patients with a cystic lesion of the liver and in patients with cystic echinococcosis

		Cystic liver lesions	Cystic echinococcosis of the liver	
		n (%)	n (%)	p value
IHA (n=54)	normal	27 (87.1)	9 (39.1)	<.0001*
	elevated	4 (12.9)	14 (60.9)	
Em2⁺ (n=49)	normal	27 (87.1)	16 (88.9)	0.9226**
	elevated	4 (12.9)	2 (11.1)	
Total IgE (n=49)	normal	26 (83.9)	11 (61.1)	0.2023*
	elevated	5 (16.1)	7 (38.9)	
Echinococcus-specific IgE (n=46)	normal	24 (88.9)	6 (31.6)	0.0001*
	elevated	3 (11.1)	13 (68.4)	

* Chi-square test
** Fisher's exact test
IHA: indirect hemagglutination test

hibited multiple cysts in different WHO IWGE stages. For example, one patient exhibited both CE1 and CE3 cysts, while another exhibited both CE2 cysts and non-echinococcal cysts (CL). Finally, one patient was found to suffer from cysts consistent with stage CE3 and CE4 disease.

Serologic findings

Indirect hemagglutination test (IHA)

Antibodies to *E. granulosus* were identified by means of IHA test in 18 of 68 patients. Of these, IHA results were unequivocally positive in 33.3% (n=6), while serum titers were moderately elevated in 22.2% (n=4) and high in 16.7% (n=3). Findings were borderline in another 16.7% (n=3) and weakly positive in 11.1% (n=2). Among the 18 patients with positive ILA test results, a diagnosis of CE was made sonographically in 77.8% (n=14). ILA findings were false-negative in 9 cases. The calculated sensitivity and specificity of IHA were 60.9% and 87.1%, respectively.

Enzyme-linked immunosorbent assay (ELISA) for Echinococcus multilocularis Em2⁺

Em2⁺ test results were elevated in a total of 6 of 68 patients. Sonographic evidence of CE, however, was returned in only 33.3% (n=2). This represents four false-positive results. False-negative findings were returned in 16 patients. The calculated sensitivity and specificity of the Em2⁺ test were 11.1% and 87.1%, respectively.

Total immunoglobulin E (IgE)

Abnormal total IgE concentrations were confirmed in 12 of 68 patients. CE was diagnosed sonographically in 58.7% of these patients (n=7). Results were false-negative in 11 patients. The calculated sensitivity and specificity of echinococcus-specific IgE were 38.9% and 83.9%, respectively.

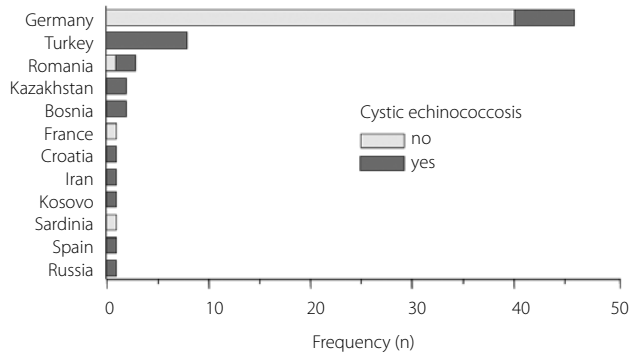

Figure 4. Distribution of patients according to their countries of birth.

Echinococcus-specific IgE (specific IgE)

The echinococcus-specific IgE concentration was elevated in 16 patients, among whom 81.3% (n=13) exhibited sonographic evidence of CE. False-negative were returned in 6 cases. The calculated sensitivity and specificity of specific IgE were 68.4% and 88.9%, respectively.

Among patients with simple dysontogenic liver cysts, findings of IHA were negative for antibodies to *E. granulosus* in 87.1% of cases. Similarly, elevated Em2⁺, total IgE, and specific IgE concentrations were found in 19.9%, 16.1%, and 11.1% of these patients, respectively.

Comparison of the patients' serological results with sonographic findings showed an association between ultrasound and both IHA ($p<0.0001$) and echinococcus-specific IgE ($p=0.0001$). No correlation could be identified for Em2⁺ and for total IgE (Table 1).

Non-specific hepatic tests/transaminases

No correlation could be determined between the concentrations of non-specific liver enzymes, AST, ALT, and GGT and patients' sonographic findings (p=0.7161, p=0.1081, p=0.4853, respectively).

Additional findings and results

Among patients diagnosed with CE, 32% was born in Turkey and 24% was born in Germany. The remaining 44% was born in other countries, mostly in southeastern Europe or the former USSR (Figure 4). In addition, 40% of patients with CE had traveled in foreign countries, including Turkey (60%), the United States, the Russian Federation, Uzbekistan, and the Canary Islands (10%). Whether travel to these foreign destinations resulted in patients becoming infected with *E. granulosus* can not be analyzed on the basis of retrospective data. Contact with animals was reported by 36% of patients with CE. This contact involved dogs in all cases. At the time of diagnosis, 25% of patients (n=17) were asymptomatic. Diffuse abdominal complaints were reported by 77.8% (n=53), while 16.7% suffered from nausea and 5.6% suffered from diarrhea. Weight gain was reported in 5.6% of cases, and weight loss was reported

Original Article

in 11.1%. Reduced physical stamina was reported by 5.6% of patients with CE.

DISCUSSION

With the increasing immigration into Germany and the broad appeal of foreign travel, ever more cases of cystic echinococcosis (CE) are being diagnosed (14). At the same time, the number of routine sonographic examinations has continued to grow, with the result that physicians in a variety of specialties are more and more frequently confronted with cystic lesions of the liver, the differential diagnosis of which includes CE. The retrospective data presented in this study are intended to assist practitioners to better assess corresponding findings in routine clinical settings.

Ultrasonography is the diagnostic method of choice recommended for the work-up of cystic liver lesions and CE (18). Reports in the literature describe a sensitivity of 88%-90% to 93%-98% and a specificity of 93%-100% for this method in the diagnosis of CE (19). One study reported no difference in the detection rates for liver cysts between senior radiologists and residents (20). Ultrasound represents a safe and cost-efficient method that is superior to both computed tomography (CT) and magnetic resonance imaging (MRI) in the visualization of the morphology of cystic lesions (21). In addition, ultrasound facilitates the recognition of CE in asymptomatic disease stages (22). The WHO IWGE guidelines for cyst staging provide us with a worldwide uniform sonographic classification of echinococcosis. The criteria allow for simple assessment and interpretation of cystic liver lesions in routine clinical settings (17). Corresponding to reports in the literature, our patients with positive evidence for infection with *E. granulosus* exhibited solitary cysts in 60% of cases (23), while those with simple cysts more frequently had multiple cysts (24). Also, in accordance with the literature, our findings confirm that simple, non-parasitic cysts are mostly less than 5 cm in diameter (25). The growth of CE cysts ranges from 1 to 50 mm per year (17), an observation that permits a rough estimation of the age of the cyst. Also, the characteristic cyst wall, which we visualized in 90% of cases and which forms within 2 to 4 weeks after infection, is a pathognomonic feature of CE, which facilitates the diagnosis and contributes to identifying a possible point of infection (26). Based on the sonographically visualized internal structure of the cysts, it is possible to follow the transition from the anechoic stage CE1 into the more echo-dense stage CE2 (27). The observation of the so-called "snowflake phenomenon" caused by free-floating protoscolices upon shifting the patient's position provides further evidence for the possible diagnosis of CE (28).

It has been shown that routine hematological and biochemical tests are inadequate for diagnosing cystic echinococcosis of the liver (24). Our collective analysis of hepatic transaminases (AST, ALT, GGT) showed sporadically elevated concentrations both in patients with CE and in those without this diagnosis. Thus, these laboratory parameters do not appear to be a relevant component in the primary work-up of CE (29), nor do they facilitate differentiation between CE and non-echinococcal cystic lesions of the liver.

The currently available immunological methods for use in suspected parasitic liver lesions are ELISA (echinococcus IgG ELISA), IHA, latex agglutination tests, and immunoblots (30). These methods, however, continue to be plagued by inadequate sensitivity, specificity, and standardization (31). In our study, both IHA and echinococcus-specific IgE showed higher sensitivities than did $Em2^+$ and total IgE. The specificities of all four methods fell between 83% and 88%.

In recent years, great efforts have been made to improve these methods using new, recombinant proteins, synthetic peptides, and combinations of defined antigens (26). Nevertheless, and despite the fact that immunoglobulin levels are undetectable, only 60%-80% of infected individuals become seropositive (32). In addition, 10%-15% of serological examinations return negative results due to the wall thickness of the individual *E. granulosus* cystic lesions. Calcified cysts with thick cyst walls also significantly compromise the probability of reliable immunological diagnostics (33). In 10%-20% of cases, no echinococcus-specific antibodies are produced, which leads to false-negative serological findings (34). Reports in the literature describe sensitivities of 50%-100% for IHA (35). False-negative IHA findings have been reported in patients with solitary cysts, cysts less than 9 cm in diameter, intact cysts, extrahepatic cysts, and unilocular or degenerative cysts. Finally, an increased rate of false-negative findings has been reported in children and adolescents between the ages of 10 and 20 years (36).

By contrast, false-positive serological findings are reported in 10%-15% of patients, mainly due to cross-reactions with other parasitic infections, most commonly with *Echinococcus multilocularis* (37). False-positive serological results may also be returned in non-active disease stages, during pregnancy, and in patients with autoimmune or malignant diseases (34). Finally, women seem to be more frequently affected by *E. granulosus* infections (38).

Inadequate standardization is a further problem limiting the reliability of immunological diagnostics, as variations between laboratories may lead to incorrect results (34). At the present time, one immunological method employing new, recombinant proteins and synthetic antigens shows great potential for enhancing the sensitivity and specificity of these methods (39), though a final assessment of its value will require further studies. Because of its acceptable cost and its applicability in a variety of clinical setting and acceptance in the population, together with its clinical value for both diagnosis and monitoring, ultrasonography remains superior to serological methods. Ultrasound is particularly suitable for follow-up monitoring, while serological methods are of only limited value (29).

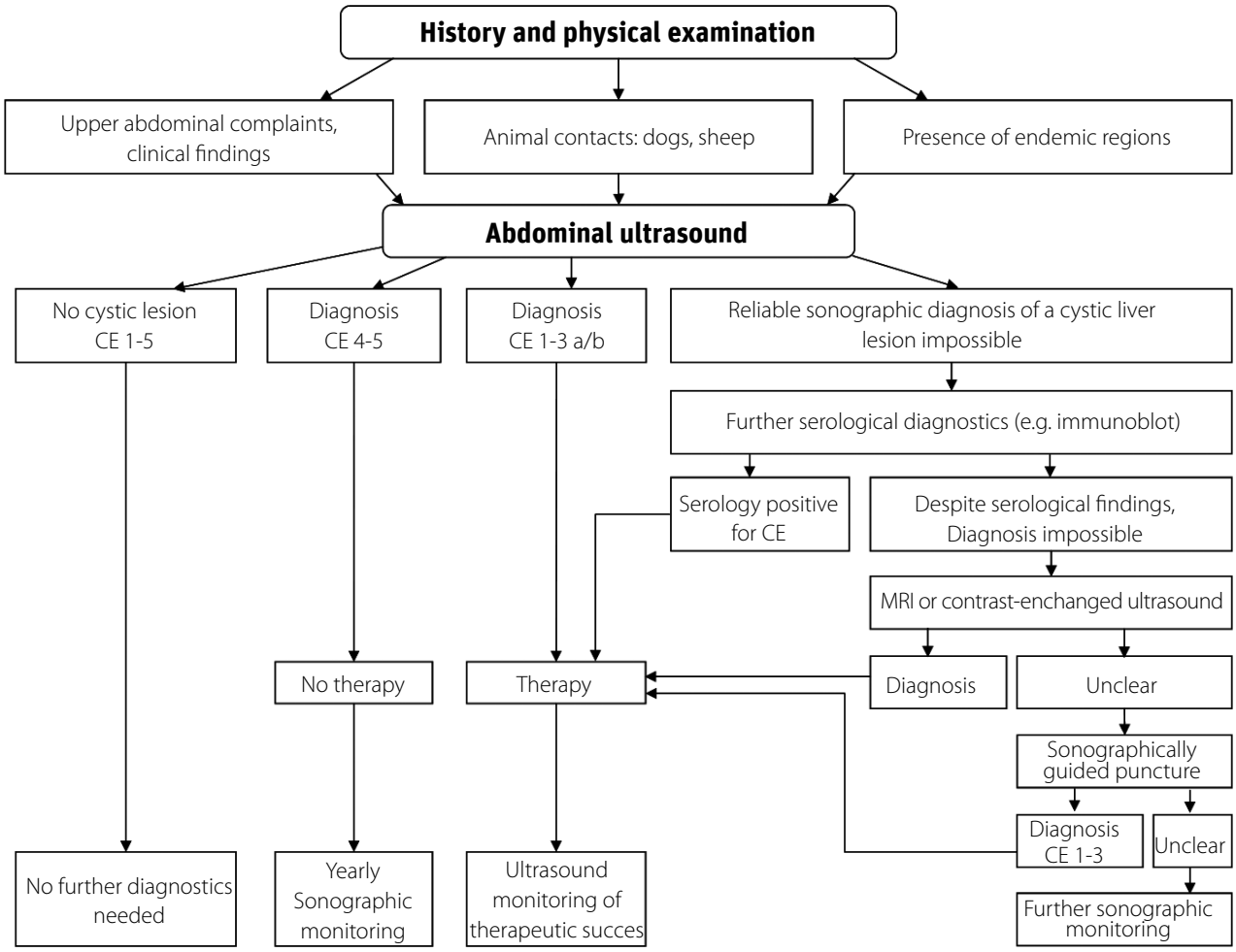

Figure 5. Diagnostic algorithm in suspected cases of cystic echinococcosis of the liver.

In conclusion, we contend that ultrasonography remains the most efficient diagnostic method that not only provides a mobile and non-invasive diagnostic tool in the work-up of cystic liver lesions, even in pregnant women and children, it also enjoys broad acceptance in the population. Ultrasonography provides clinically relevant evidence with respect to the localization of cysts and the stage of the disease. Until more versatile immunological methods become available, we consider ultrasonography to be the method of choice both in the work-up of cystic liver lesions and in the diagnosis and therapeutic monitoring of cystic echinococcosis (Figure 5).

Ethics Committee Approval: Ethics committee approval was received for this study from the Ethical Committee of Ulm University.

Informed Consent: N/A.

Peer-review: Externally peer-reviewed.

Author contributions: Concept - B.G., W.K.; Design - B.G., W.K. ; Supervision - B.G., P.K., W.K.; Resource - B.G., W.K.; Materials - J.W.; Data Collection&/or Processing - B.G., J.W., W.K.; Analysis&/or Interpretation - S.O., R.A.M., M.M.H., T.G.; Literature Search - J.W., A.S.A.; Writing - B.G., J.W., R.A.M., W.K.; Critical Reviews - M.M.H., P.K., T.G., W.K.

Conflict of Interest: No conflict of interest was declared by the authors.

Financial Disclosure: The authors declared that this study has received no financial support.

REFERENCES

1. WHO. Cystic Echinococcosis and Multilocular Echinococcosis. Available from: http://www.who.int/zoonoses/diseases/echinococcosis/en/index.html.
2. Grosso G, Gruttadauria S, Biondi A, Marventano S, Mistretta A. Worldwide epidemiology of liver hydatidosis including the Mediterranean area. World J Gastroenterol 2012; 18: 1425-37. **[CrossRef]**
3. Barnes TS, Deplazes P, Gottstein B, et al. Challenges for diagnosis and control of cystic hydatid disease. Acta Trop 2012; 123: 1-7. **[CrossRef]**
4. Mandal S, Mandal MD. Human cystic echinococcosis: Epidemiologic, zoonotic, clinical, diagnostic and therapeutic aspects. Asian Pac J Trop Med 2012; 5: 253-60. **[CrossRef]**
5. Ernest E, Nonga HE, Kynsieri N, Cleaveland S. A retrospective survey of human hydatidosis based on hospital records during the period 1990-2003 in Ngorongoro, Tanzania. Zoonoses Public Health 2010; 5: 124-9. **[CrossRef]**
6. Derfoufi O, Ngoh Akwa E, Elmaataoui A, et al. Epidemiological profile of cystic echinococcosis in Morocco from 1980 to 2008. Ann Biol Clin (Paris) 2012; 170: 457-61.

7. Vahedi MA, Vahedi ML. Demographics of patients with surgical and nonsurgical cystic echinococcosis in East Azerbaijan from 2001 to 2012. Pak J Biol Sci 2012; 15: 186-91. **[CrossRef]**
8. Eckert J, Deplazes P. Biological, epidemiological, and clinical aspects of echinococcosis, a zoonosis of increasing concern. Clin Microbiol Rev 2004; 17: 107-35. **[CrossRef]**
9. Mastrandrea S, Stegel G, Piseddu T, Ledda S, Masala G. A retrospective study on burden of human echinococcosis based on hospital discharge records from 2001 to 2009 in Sardinia, Italy. Acta Trop 2012; 123: 184-9. **[CrossRef]**
10. Stojkovic M, Rosenberger K, Kauczor HU, Junghanss T, Hosch W. Diagnosing and staging of cystic echinococcosis: How do CT and MRI perform in comparison to ultrasound? PLoS Negl Trop Dis 2012; 6: e1880.
11. Schweiger A, Grimm F, Tanner I, et al. Serological diagnosis of echinococcosis: the diagnostic potential of native antigens. Infection 2012; 40: 139-52. **[CrossRef]**
12. Nunnari G, Pinzone MR, Gruttadauria S, et al. Hepatic echinococcosis: Clinical and therapeutic aspects. World J Gastroenterol 2012; 18: 1448-58. **[CrossRef]**
13. WHO Informal Working Group. International classification of ultrasound images in cystic echinococcosis for application in clinical and field epidemiological settings. Acta Trop 2003; 85: 253-61. **[CrossRef]**
14. Brunetti E, Gulizia R, Garlaschelli AL, et al. Cystic echinococcosis of the liver associated with repeated international travels to endemic areas. J Travel Med 2005; 12: 225-8 **[CrossRef]**
15. Taylor BR, Langer B. Current surgical management of hepatic cyst disease. Adv Surg 1997; 31: 127-48.
16. Cowles RA, Mulholland MW. Solitary hepatic cysts. J Am Coll Surg 2000; 191: 311-21. **[CrossRef]**
17. Brunetti E, Kern P, Vuitton DA; Writing Panel for the WHO-IWGE. Expert consensus for the diagnosis and treatment of cystic and alveolar echinococcosis in humans. Acta Trop 2010; 114: 1-16. **[CrossRef]**
18. Tabain I, Sviben M, Ljubin-Sternak S, Vilibić-Čavlek T, Mlinarić-Galinović G. Seroprevalence of Echinococcus granulosus infection in Croatian patients with cystic liver disease. J Helminthol 2010; 25: 1-4.
19. Macpherson CN, Milner R. Performance characteristics and quality control of community based ultrasound surveys for cystic and alveolar echinococcosis. Acta Trop 2003; 85: 203-9. **[CrossRef]**
20. Larssen TB, Rørvik J, Hoff SR, Horn A, Rosendahl K. The occurrence of asymptomatic and symptomatic simple hepatic cysts. A prospective, hospital-based study. Clin Radiol 2005; 60: 1026-9. **[CrossRef]**
21. Liang P, Cao B, Wang Y, Yu X, Yu D, Dong B. Differential diagnosis of hepatic cystic lesions with gray-scale and color doppler sonography. J Clin Ultrasound 2005; 33: 100-5. **[CrossRef]**
22. Kilimcioğlu AA, Ozkol M, Bayindir P, Girginkardeşler N, Ostan I, Ok UZ. The value of ultrasonography alone in screening surveys of cystic echinococcosis in children in Turkey. Parasitol Int 2006; 55: 273-5. **[CrossRef]**
23. Sarkari B, Sadjjadi SM, Beheshtian MM, Aghaee M, Sedaghat F. Human cystic echinococcosis in Yasuj District in southwest of Iran: an epidemiological study of seroprevalence and surgical cases over a ten-year period. Zoonoses Public Health 2010; 57: 146-50. **[CrossRef]**
24. Akyildiz HY, Akcan A, Karahan I, et al. Recurrent liver hydatid disease: When does it become symptomatic and how does one diagnose it? Clin Imaging 2009; 33: 55-8. **[CrossRef]**
25. Caremani M, Vincenti A, Benci A, Sassoli S, Tacconi D. Ecographic eidemiology of non-parasitic hepatic cysts. J Clin Ultrasound 1993: 21; 115-8. **[CrossRef]**
26. Zhang W, Li J, McManus DP. Concepts in immunology and diagnosis of hydatid disease. Clin Microbiol Rev 2003; 16: 18-36. **[CrossRef]**
27. Rogan MT, Hai WY, Richardson R, Zeyhle E, Craig PS. Hydatid Cysts: Does every picture tell a story? Trends Parasithol 2006; 22: 431-8. **[CrossRef]**
28. Czermak BV, Akhan O, Hiemetzberger R, et al. Echinococcosis of the liver. Abdom Imaging 2008; 33: 133-43. **[CrossRef]**
29. Eckert J, Gemmel MA, Meslin FX, et al. WHO/OIE Manual on echinococcosis in humans and animals: A public health problem of global concern. World Organization for Animal Health (OIE), Paris, France 2001.
30. McManus DP, Zhang W, Li J, et al. Echinococcosis. Lancet 2003; 362: 1295-304. **[CrossRef]**
31. Carmena D, Benito A, Eraso E. Antigens for the Immundiagnosis of Echinococcus granulosus infection: An update. Acta Trop 2006; 98: 74-86. **[CrossRef]**
32. Gavidia CM, Gonzales AE, Zhang W, et al. Diagnosis of cystic echinococcosis, central Peruvian Highlands. Emerg Infect Dis 2008; 14: 260-6. **[CrossRef]**
33. Nepalia S, Joshi A, Shende A, Sharma SS. Management of echinococcosis. J Assoc Physicians India 2006; 54: 458-62.
34. Scherer K, Gupta N, Caine WP, Panda M. Differential diagnosis and management of a recurrent hepatic cyst: A case report and review of literature. J Gen Intern Med 2009; 24: 1161-5. **[CrossRef]**
35. Gonlugur U, Ozcelik S, Gonlugur TE, et al. The retrospective annual surgical incidence of cystic echinococcosis in Sivas, Turkey. Zoonoses Public Health 2009; 56: 209-14. **[CrossRef]**
36. Mzali R, Ben Amar M, Kallel W, Kolsi K, Beyrouti MI, Ayadi A. Liver cystic echinococcosis: Which cysts are correlated with false negative indirect passive hemagglutination (IHA)? Tunis Med 2007; 85: 367-70.
37. Legatt GR, Yang W, McManus DP. Serological evaluation of the 12 kDa subunit of antigen B in Echinococcus granulosus cyst fluid by immunoblot analysis. Trans R Soc Trop Med Hyg 1992; 86: 189-92. **[CrossRef]**
38. Harandi MF, Moazezi SS, Saba M, et al. Sonographical and serological survey of human cystic echinococcosis and analysis of risk factors associated with seroconversion in rural communities of Kerman, Iran. Zoonoses Public Health 2011; 58: 582-8. **[CrossRef]**
39. Manzano-Román R, Siles-Lucas M. MicroRNAs in parasitic diseases: Potential for diagnosis and targeting. Mol Biochem Parasitol 2012; 186: 81-6. **[CrossRef]**