



# Impact of affected lymph nodes on long-term outcome after surgical therapy of alveolar echinococcosis

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Received: 24 January 2018 / Accepted: 5 June 2018 / Published online: 16 June 2018  
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## Abstract

**Purpose** Alveolar echinococcosis (AE) is a life-threatening helminthic disease. In humans, AE mostly affects the liver; the regional hepatic lymph nodes may be involved, indicating dissemination of AE from the liver. To achieve complete removal of the disease, enlarged hepatic lymph nodes may be resected during surgical treatment. We evaluated the frequency of affected lymph nodes by conventional microscopic and immunohistochemical analyses including detection of small particles of *Echinococcus multilocularis* (spem). Furthermore, we analyzed the association of resection of enlarged and affected lymph nodes with long-term outcome after surgical therapy of patients who underwent surgery with curative intent.

**Materials and methods** We identified 43 patients who underwent hepatic surgery with curative intent with lymph node resection for AE. We analyzed the cohort for the manifestation of the parasite in the resected lymph nodes by conventional histology and by immunohistochemistry and compared these data with the further course of AE.

**Results** Microscopically infected lymph nodes (lamina propria visible) were found in 7 out of these 43 patients (16%). In more than three quarters (25/32) of all specimens investigated, lymph nodes showed spems when stained with antibody against Em2G11, a monoclonal antibody specific for the Em2 antigen of the *Echinococcus multilocularis* metacestode. Most frequently, lymph nodes were resected due to enlargement. The median size of microscopically affected lymph nodes was 2 cm (range, 1.2 to 2.5 cm), the median size of immunohistochemically and non-affected lymph nodes was 1.3 cm each (range, “small” to 2.3 or 2.5 cm, respectively). Median follow-up was 8 years for all patients, 5 years for patients with lymph node resection, and 4 years for patients with infested lymph nodes. Overall, recurrent disease was seen in ten patients (10/109; 9%) after a median period of 1.5 years (range, 4 months to 4 years). None of the seven patients with conventionally microscopically affected lymph nodes suffered from recurrent disease. One patient with negative resected nodes and one patient with spems showed recurrent disease after 4 and 35 months, respectively.

**Conclusions** Lymph node involvement in AE is frequent, particularly when evaluated by immunohistochemical examination of lymph nodes with the monoclonal antibody Em2G11. Affected lymph nodes tend to be larger in size. Lymph node involvement is not associated with recurrent disease and therefore warrants further analysis of the biological significance of lymph node involvement.

**Keywords** Alveolar echinococcosis · Lymph nodes · Safe distance

Andreas Hillenbrand and Annika Beck contributed equally to this work.

**Electronic supplementary material** The online version of this article (<https://doi.org/10.1007/s00423-018-1687-9>) contains supplementary material, which is available to authorized users.

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## Introduction

Alveolar echinococcosis (AE) is a severe helminthic zoonosis caused by the larval stage of *Echinococcus multilocularis* (EM). AE is widely distributed in the Northern Hemisphere and is typically maintained in a wild animal cycle including canids as definitive hosts and rodents as intermediate hosts [1]. Humans become infected by ingesting parasite eggs released by the definitive host. In infected humans, AE exhibits a tumor-like growth, predominantly in the liver [2]. Usually, infections become clinically apparent after an incubation period of at least 5 years, but in most cases, development of full-blown disease takes much longer [3]. Without adequate treatment, the growth of AE progressively destroys the liver. In affected patients, a

long-term medical treatment is necessary. Therapy of choice is a complete surgical resection, since the removal of the entire parasite mass offers the most promising likelihood of cure [4]. After complete resection, temporary treatment with benzimidazole derivatives is recommended [4]. Incomplete resection should be avoided since the recurrence rate is high and the surgical benefit is questionable [4]. Infestation with AE may not be limited to the liver, as a lymphatic spread of AE has been shown in both animals [5] and humans [6]. Lymph node involvement is detected microscopically by the larval state of alveolar echinococcosis, i.e., the laminar layer and, in addition, small particles of *Echinococcus multilocularis* (spem) may be detected [7]. These spems can be detected by immunochemistry using the monoclonal antibody Em2G11 specific for the Em2 antigen of the *E. multilocularis* metacestodes.

Since lymphatic spread has been described, routine removal of regional lymph nodes was suggested as a curative approach to avoid incomplete resections and reduce the risk of persistent infection [6]. To date, it is not known whether disease-free survival is influenced by affected regional lymph nodes. Here, we describe the lymph node status and analyze the association between resection of enlarged and affected lymph nodes and long-term outcome after surgical therapy with curative intent.

## Materials and methods

### Ethics statement

The study has been approved by the Ethics Committee of the University of Ulm (No. 440/15) and applies to the Central Ethics Committee at the German Medical Association.

### Patients and tissue samples

We identified between January 1, 2000, and March 1, 2017, 115 consecutive patients who were operated on for AE with curative intent and were medically treated or followed up in the Infectious Disease Department of Ulm University Hospital. The further course and follow-up were only available in 109 out of these 115 patients; therefore, we excluded 6 patients because no follow-up was available (Fig. 1). For the analysis of lymph node resection/involvement and resection margin, we analyzed the surgery and final pathology reports of these 109 patients. In 43 out of 109 patients, lymph nodes were resected. Resected lymph nodes were mostly found to be enlarged intraoperatively ( $n = 27$  patients). The further course and follow-up examinations (computed tomography, magnetic resonance tomography, or ultrasound as well as laboratory results and clinical examination) were taken from the medical charts of our hospital documentation system or asked via telephone calls from patients or further attending physicians. All

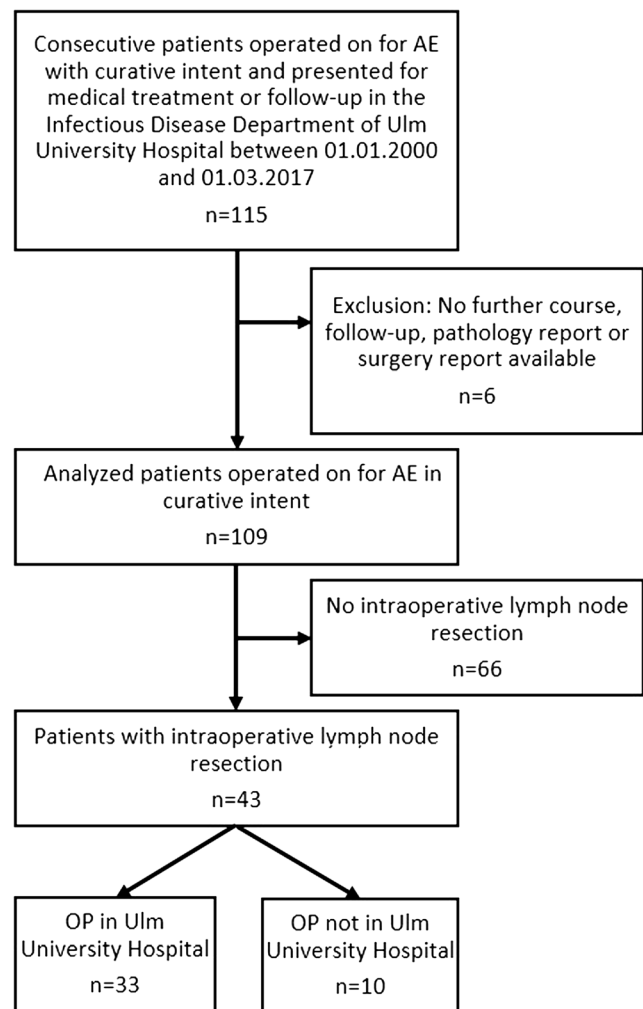


Fig. 1 Flow sheet for study inclusion

109 patients were alive at the time of the data collection. All 109 patients were treated in our Infectious Disease Department, but only 56 patients had surgery in Ulm University Hospital. The other group of 53 patients had surgery for AE in 27 other hospitals. These patients were pre- or postoperatively referred to the Infectious Disease Department of Ulm University Hospital up to 80 months after initial surgery (median 4 months). All patients were staged according to the PNM classification [8]. We have previously shown that recurrent disease is frequently associated with a minimal safe distance achieved intraoperatively (minimal distance of larval tissue to resection margin) [9]. Therefore, we analyzed the minimal safe distance.

### Staining procedure

Hematoxylin-eosin and PAS stainings were performed on paraffin sections as reported [7]. For immunohistochemistry, standard protocols were used. Briefly, for antigen retrieval, the sections were heated in citrate buffer at pH 6 in a

microwave oven for 20 min. The primary antibody was used at a concentration of 0.2057 mg/ml in phosphate buffer saline (PBS); slides were incubated with 50 ml per section in a humid chamber at room temperature for 30 min. As the detection system, we used the EnVision Kit (Dako, Carpinteria, CA, USA) according to the manufacturer's protocols.

The samples were analyzed by the two of us (AB and TFEB) on a multihead microscope using slides stained with H&E and PAS and for immunohistochemical staining as reported.

## Statistical analysis

All values were expressed as median and range. Statistical analysis was performed using Winstat software for Windows (R. Fitch Software Version 2009.1). To test for differences between the two groups, the Mann-Whitney *U* test (MWU) was used, and statistical significance was tested at  $p < 0.05$ . Correlations were estimated by calculating Spearman's correlation coefficients (*r*). Correlation coefficients between 0.3 and 0.7 (−0.3 and −0.7) indicate a moderate linear relationship, and values between 0.7 and 1.0 (−0.7 and −1.0) indicate a strong positive (negative) linear relationship. Statistical significance was tested at  $p < 0.05$ . No adjustments were made for multiple statistical comparisons.

**Data availability statement:** All relevant data are within the paper and its [Supporting Information files](#).

## Results

### Patient characteristics

Patient characteristics are summarized in Table 1. The median age of 43 patients with resected lymph nodes was 41 years (range, 16 to 74 years; 26 women and 17 men). All patients are alive. Median follow-up after surgery was 7.2 years (range, 1 month to 17 years). The liver was affected in all patients; in 19 of the 43 patients, AE had affected adjacent organs and structures ( $n = 12$ ) or lymph nodes ( $n = 4$ ) or both ( $n = 3$ ) (Table 1). According to the PNM classification, parasitic mass was staged in one patient as P1 (peripheral lesions), in 14 patients as P2 (central lesions; two with distant metastasis), in 13 as P3 (central lesions with hilar vascular or biliary involvement of both lobes), and in 15 as P4 (liver lesion with extension along the vessels). Patients with microscopically affected lymph nodes were staged as N1, and patients with immunohistochemically positive lymph nodes (spems) without microscopically detectable lymph node involvement and no further regional involvement were staged as N0.

### Lymph node resection

In 43 out of 109 patients, lymph nodes were resected, most frequently because they were intraoperatively found to be enlarged ( $n = 27$  patients). A systematic lymph node dissection in the hepatoduodenal ligament was performed in 11 out of these 43 patients; 33 of these 43 patients had surgery in Ulm University Hospital; 10 patients with lymph node resection had surgery in 10 different hospitals. In all 33 patients who underwent surgery in Ulm University Hospital, including lymph node dissection, specimens were immunohistochemically stained with antibody Em2G11, a monoclonal antibody specific for the Em2 antigen of the *Echinococcus multilocularis* metacestode. Overall, microscopically infected lymph node fragments of the laminated bodies were visible in PAS staining in 7 out of 43 patients (16%). In the 33 patients with lymph node dissection and surgery in Ulm, 25 patients showed small particles of *Echinococcus multilocularis* (spems) when stained with antibody against Em2G11 (76%; 3 out of these 25 patients had additional microscopically affected lymph nodes; Fig. 2). Overall, the median size of all resected lymph nodes was 1.4 cm (range, “small” to 2.5 cm). The median size of microscopically affected lymph nodes was 2 cm as shown by conventional staining (HE; PAS) (range, 1.2 to 2.5 cm), the median size of immunohistochemically and non-affected lymph nodes was 1.3 cm each (range, “small” to 2.3 or 2.5 cm, respectively). Microscopically affected lymph nodes were not significantly larger than non-affected or immunohistochemically affected lymph nodes ( $p = 0.35$ ).

A systematic lymph node dissection in the hepatoduodenal ligament was performed in 11 patients, in whom between 3 and 12 (median 6) lymph nodes were resected. The size of the largest resected lymph node was given in the pathology report of 9 of these 11 patients. The median size of the largest resected lymph nodes was 1 cm (range, small to 2.5 cm). In two patients, one lymph node in each was microscopically affected (sizes of affected lymph nodes was 1 and 1.2 cm). Seven of these 11 patients had surgery in Ulm University Hospital, and 5 of these 7 patients with systematic lymph node dissection were positive when stained with antibody against Em2G11. About half the lymph nodes in a systematic lymph node dissection specimen were positive on immunohistochemistry. None of the cases analyzed were all lymph nodes positive.

In six patients, Lund's node (sentinel lymph node of the gallbladder) was removed (five times during simultaneous cholecystectomy and once during systematic lymph node dissection). Resected Lund's nodes were small (five were below 1 cm, and the size was not stated in one pathology report). No Lund's node was microscopically affected by a PAS-positive laminar layer; however, in three Lund's nodes, spems were detected by immunohistochemistry.

**Table 1** Patient characteristics

Number of patients	<i>n</i> = 43	F/M, 26/17		
Age	Range, 16 to 74 years	Median, 41 years		
Follow-up	Range: 1 month to 17 years	Median, 7.2 years		
Affected sites	Liver, 43 (all patients)			
Additionally affected sites ( <i>n</i> = 19)	Diaphragm (only)	5		
	Adrenal gland/retroperitoneum + diaphragm	1		
	Pericardium/retroperitoneum + diaphragm	1		
	Lung + diaphragm	1		
	Omentum/mesentery + diaphragm	3		
	Spleen + diaphragm + omentum	1		
	Colon + lymph node	1		
	Hepatoduodenal ligament + omentum/mesentery + lymph node	1		
	Vena cava + diaphragm + lymph node	1		
	Lymph node microscopically	4		
PNM staging	<i>n</i> = 43	Microscopically affected lymph nodes	Only immunohistochemically affected lymph nodes	No affected lymph nodes
Stage 1 (P1 N0 M0)	1	–	–	1
Stage 2 (P2 N0 M0)	12	–	7	5
Stage 3a (P3 N0 M0)	6	–	4	2
Stage 3b (P1-3 N1 M0)	7	1	4	2
(P4 N0 M0)	5	–	3	2
Stage 4 (P4 N1 M0)	9	5	3	1
(P1-4 N0-1 M1)	3	1	1	1

### Follow-up, postoperative course, and medication, minimal safe distance, and recurrence

Median follow-up was 8 years for all 109 patients. Median follow-up was 7 years for patients with lymph node resection and 4 years for patients with infected lymph nodes. Overall, recurrent disease was seen in ten patients (10/109; 9%) after a median period of 17 months (range 4 to 50 months). None of the seven patients with microscopically affected lymph nodes suffered from recurrent disease. One patient with negative resected nodes and one patient with spem showed recurrent disease after 4 and 35 months, respectively (Fig. 3).

Hospital stay of patients with and without lymph node resection was comparable. The lymph node resection was not associated with a higher rate of complications. Apart from a lymphatic fistula treated conservatively with drainage for 15 days (duration of stay 23 days, no revision operation necessary), no specific lymph node resection-related complications are reported.

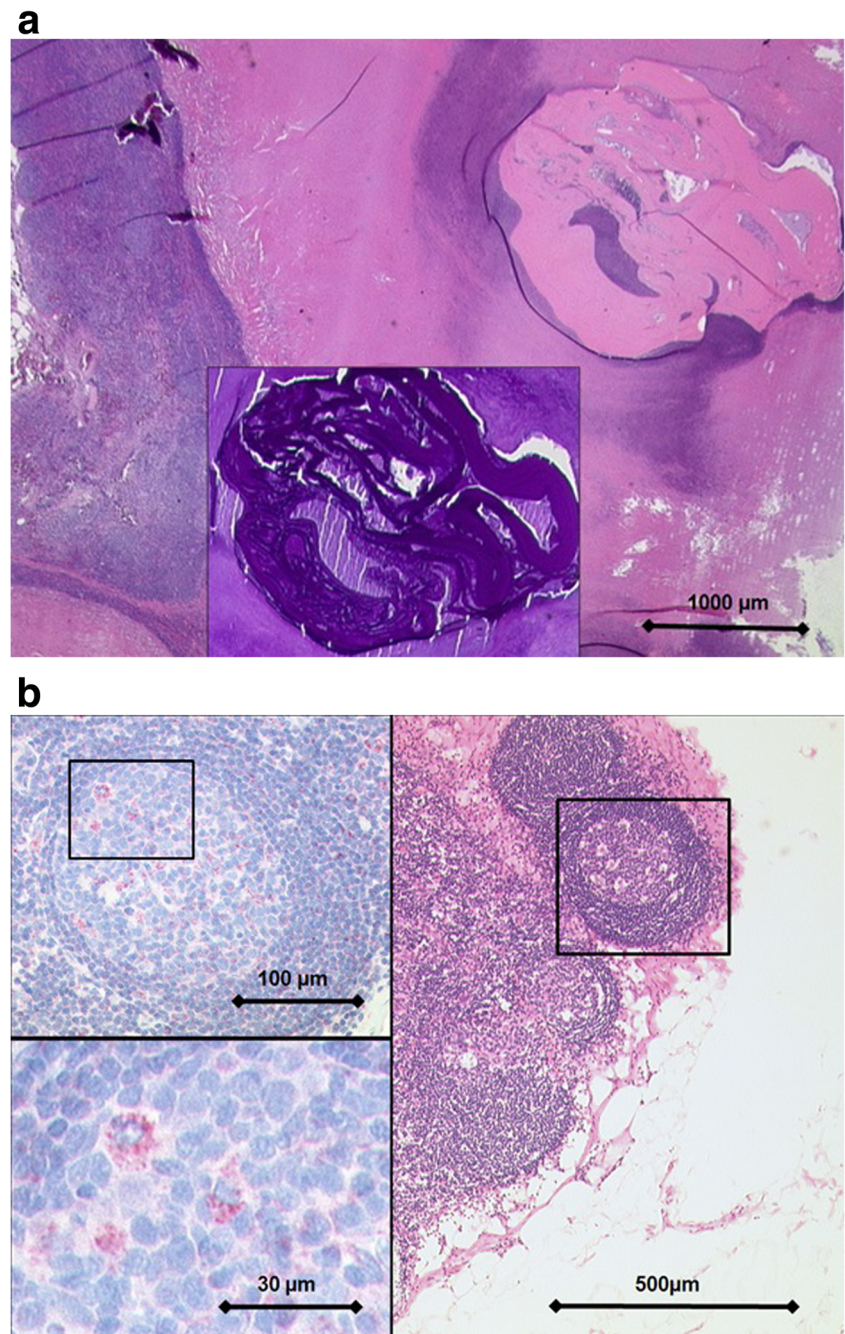
Duration of postoperative benzimidazole derivate therapy was evaluable in 105 out of all 109 patients. No exact data on postoperative medication were available for four patients. Benzimidazole derivates were administered postoperatively in 93 of 105 evaluable patients; 18 out of these 93 patients

received medication for less than 2 years for various reasons (all patients with microscopically unaffected lymph nodes). In the remaining 75 patients, medication was discontinued after 2 years postoperatively or was continued for more than 2 years (*n* = 10). All patients with affected lymph nodes received postoperative antihelminthic medication for at least 24 months.

In 102 patients of the 109 patients included, the safe distance was declared either in the final pathology report or in the surgical report. The median safe distance achieved intraoperatively in all patients was 2 mm (range, resected without any safe distance to 6 cm). In the 43 patients with lymph node resection, median safe distance was 3 mm (range, resected without any safe distance to 6 cm), and in the group with spems and microscopically affected lymph nodes, the median safe distance was 6 and < 1 mm, respectively (range, resected without any safe distance to 6 and 18 mm, respectively).

Ten patients suffered from recurrent disease. All patients had recurrent disease at the liver resection margin. No recurrence was seen outside the liver. In seven of these ten patients, resection was performed without any safe distance. Safe distance in two further patients with recurrent disease was 1 and 3 mm and was not assignable in one patient. In the two patients with recurrent disease and lymph node dissection, resection was performed without any safe distance.

**Fig. 2** Histology of lymph node involvement of the larval state of alveolar echinococcosis. **a** Lymph node with fragments of the lamellar body (upper right; H&E staining). Inset: Intense staining of the lamellar body in a serial section of the affected lymph node (PAS staining). **b** Lymph node with involvement of spems. H&E staining shows an inconspicuous lymph node. Insets: Immunohistochemical staining with the Em2G11 antibody detects numerous spems in the follicle and the perifollicular area (see supplement for negative control).

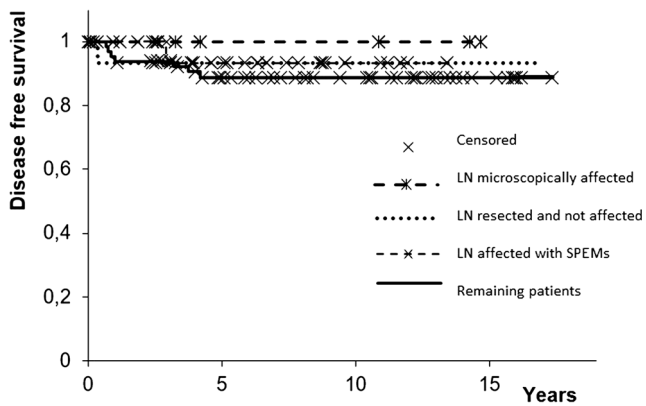


## Discussion

It is evident that lymph nodes can be affected by AE spreading from the liver. Infested lymph nodes tend to be larger in size than non-involved lymph nodes. By means of immunohistochemistry using the monoclonal antibody Em2G11, spems are detected even in lymph nodes that are inconspicuous in standard histochemical staining, such as hematoxylin or PAS. Therefore, the question arises as to how to interpret a lymph node status in surgery for AE with curative intent and thus

what is the prognostic worth of resected lymph nodes. It is not clear whether affected lymph nodes present a risk and/or whether enlarged and affected lymph nodes reflect the immune response of the host.

There is evidence that AE resembles metastatic malignant disease. After infection of *Echinococcus multilocularis* in rodents, a metastatic-like growth of AE is reported [5]. In humans, there are multiple reports in the literature of a metastatic-like spread to other organs with the involvement of the lungs, brain, or bone [10–12]. After liver transplantation



**Fig. 3** Kaplan-Meier curves comparing disease-free survival for the subgroups according to microscopic lymph node (LN) involvement, immunohistochemical lymph node involvement, unaffected and non-resected lymph nodes

in patients with end-stage AE, re-infection of the graft was seen even several years later [13]. This supports the thesis that infected lymph nodes may be a reservoir for re-infection. These arguments are the rationale behind performing a regular lymph node dissection as recommended [6]; this holds particularly true since lymph node involvement cannot be ruled out by preoperative imaging.

In contrast, there are several points arguing that AE does not resemble metastatic malignant disease and a lymph node infection in AE is not comparable with a poorer prognosis, as is the case in malignant disease. In infectious disease, enlarged and potentially affected lymph nodes represent an active status of the immune response. Humans are aberrant hosts rather than intermediate hosts such as rodents. In aberrant hosts, it is assumed that the immune system succeeds in impairing a rapid progression of the disease, e.g., mature protoscolices only develop rarely [14]. In humans, an intense host-parasite interaction is usually seen around the necrotic zone containing the germinal layer of AE. The involvement of cellular immunity and an intense periparasitic granulomatous inflammation represents an active immune response [15]. Affected lymph nodes are at the interface of this interaction of spems with the immune response. In line with this observation, spems increase during the course of treatment [16].

Spems are detected by the monoclonal antibody Em2G11 specific for the Em2 antigen of the metacestodes of *Echinococcus multilocularis*. Em2 antigen is abundantly present in the vesicle fluid and also appears as a major secretory component on in vitro culture of metacestodes [7, 17]. Since AE is an infectious disease, it is therefore conceivable that Em2 antigen is shed to perihepatic lymph nodes during disease progression. Microscopic verification of spems in a lymph node may represent a function of the host immune response that is not necessarily correlated with disease recurrence or progression but may present an epiphenomenon of this disease of yet unknown significance.

Our clinical data support this view: No patient with microscopically affected lymph nodes ( $n = 7$ ) and only 1 of the 22 patients with spem involvement had a recurrence, whereas 10 patients with unaffected lymph nodes suffered from the recurrent disease.

This is even more remarkable when one considers that the seven patients with microscopically affected lymph nodes suffered from more advanced disease stages of AE at the time of surgery compared to the remaining patients. In six of these seven patients with microscopically affected lymph nodes, parasitic mass in the liver was staged as P4 (86%). Seven of the 22 patients (32%) with spems and 15 of the remaining 80 patients (19%) were staged as P4. Therefore, the size of the lesion in the liver seems to impact on the involvement of lymph nodes. We have previously shown that recurrent disease is frequently associated with a minimal safe distance achieved intraoperatively (minimal distance of larval tissue to resection margin) [9]. Patients with microscopically affected lymph nodes had a lower median resection margin compared to patients with non-affected lymph nodes/non-resected lymph nodes. However, none of these patients suffer from the recurrent disease. More importantly, all ten patients with hepatic recurrence had a positive resection margin at the liver. In these patients, no recurrence was seen outside the liver, either at the hepatoduodenal ligament or in the area of resected lymph nodes. Likewise, recurrence of AE after surgery is predominantly reported in the liver at the resection margin and not in the hepatoduodenal ligament or intrahepatically distant from the resection site. If extrahepatic recurrence is reported after surgery, recurrent AE is usually diagnosed within a very short period of time in the lung or spleen after surgery [18]. Detection of recurrence within the first year after surgery is unusual since AE usually develops slowly. Therefore, one hypothesis is a pre-existing (occult) manifestation of AE prior to resection rather than recurrent AE in those patients.

The analysis of our results has several limitations. A retrospective stratification into subgroups according to lymph node involvement may be biased. A further limitation of our study is the low sample size, which implies the necessity of validation of the results by a multicentric study. Lymph nodes were resected in only 43 of 109 patients, and a systematic lymph node dissection was performed in only 11 patients in various hospitals over a long period with various examination and follow-up modalities. In most patients, the indication for lymph node resection was that of enlarged lymph nodes found intraoperatively; however, indication for lymph node resection or even systematic lymph node dissection in the hepatoduodenal ligament was not clearly stated in the operation report in several cases. The histopathologic analysis as described can only be performed after the surgical dissection and has no relevance in terms of preoperative screening.

A further remarkable difference between the groups is that all patients with affected lymph nodes received postoperative antihelminthic medication for at least 24 months, whereas 12 patients of all patients analyzed received no postoperative antihelminthic medication. In patients with affected lymph nodes, postoperative antihelminthic medication is probably administered with higher compliance. Furthermore, follow-up in patients with infested lymph nodes is considerably shorter compared to all patients (4 vs. 8 years), and the median safe distance (distance between the visually affected liver and the cutting edge) in the groups was slightly different, but patients with microscopically affected lymph nodes had the lowest median safe distance of less than 1 mm. Furthermore, in order to be able to differentiate if the affected lymph nodes represent merely an immunologic response of the host or are indeed a reservoir for re-infection, a longer follow-up and a structured, uniform benzimidazole treatment are mandatory before any definitive conclusions can be drawn.

In conclusion, we show for the first time in a larger cohort that lymph node involvement in AE is not a rare event. We found microscopically and immunohistochemically affected lymph nodes in approximately three quarters of patients after surgery with curative intent with lymph node resection. Affected lymph nodes tend to be larger in size when compared to unaffected lymph nodes. We found that lymph node involvement is not associated with recurrent disease; based on our data, the recommendation to perform routine lymph node dissection in patients with AE must be critically questioned. Furthermore, the use of antibody Em2G11 will increase detection rates of spems in resected lymph nodes in terms of a higher sensitivity and specificity; the biological significance of this interaction is uncertain and will therefore fuel further research.

**Authors' contributions** Authorship AH, DHBB, BG, WK, and TFEB contributed to the conceptualization of the study; AH, AB, JS, and BG contributed to the data curation; AH, WK, TG, TFEB, PM, DHB, and BG contributed to the analysis and interpretation of data; AH, AB, and TFEB contributed to the drafting of the manuscript; AH, AB, WK, TG, TFEB, JS, PM, DHB, and BG contributed to the critical revision of the manuscript.

### Compliance with ethical standards:

**Conflict of interests** The authors declare that they have no conflict of interest.

**Ethical approval** All procedures performed in studies involving human participants were in accordance with the ethical standards of the institutional and/or national research committee and with the 1964 Helsinki declaration and its later amendments or comparable ethical standards.

**Informed consent** Informed consent was obtained from all individual participants included in the study. There is no gender defined. We did not perform a breakdown by gender since it is not helpful. Gender-specific data can be consulted on justified request.

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