Original Article
Metastatic and prognostic factors in patients with alveolar echinococcosis

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Abstract: Background: Prognostic predictions in alveolar echinococcosis (AE) are usually based on relapse and metastasis. Thus, assessment of factors associated with metastasis and clinical outcomes in patients with alveolar echinococcosis are of significant important. The purpose of this study was to investigate the correlated factors for metastasis and prognosis for patients with AE. Methods: We analyzed 159 AE patients who underwent curative surgery at the First Teaching Hospital of Xinjiang Medical University from February 2003 to December 2013 by hematoxylin and eosin (HE) staining. Immunohistochemistry (IHC) staining for CD44 was performed in 159 AE and adjacent normal liver specimens. The clinicopathological parameters were analyzed for metastasis and prognosis, including gender, age, size, calcification, necrosis, foreign body granuloma and CD44 protein level. Results: The rate of metastasis was 22.1%. Chi-square test showed that low levels of CD44 was associated with metastasis (P = 0.004). However, multivariate analysis suggested that CD44 expression is not independent prognostic indicators for overall survival (P = 0.356). In addition, no correlations between prognosis and gender, age, size, calcification, necrosis, foreign body granulomatous, metastasis were identified. Conclusions: The finding that a low level of CD44 is associated with metastasis in AE patients. At present, it thus remains question whether CD44 expression is a valid prognostic marker for AE, further investigations are required.

Keywords: CD44, metastasis, prognosis, alveolar echinococcosis

Introduction

Alveolar echinococcosis disease spreading in the majority of the northern hemisphere [1] has a considerable impact on major public health in rural communities. Recent studies in China have shown that E. multilocularis is frequently detected including Xinjiang, Ningxia, Tibet autonomous regions, Qinghai, Gansu, Sichuan and Inner Mongolia [2, 3]. Infected dogs and foxes may act as the principal definitive hosts. The parasite is transmitted to humans occurs when eggs are ingested accidentally [4]. So, herdsmen and hunter are higher risk populations. AE is one of the most lethal parasitic diseases with a high fatality rate and poor prognosis in the absence of appropriate treatment [5]. The identification of prognostic factors is essential for predicting patients' survival and determining optimal therapeutic strategies.

Contrast to cystic echinococcosis (CE), AE is characterized by a tumor-like growth of the E. multilocularis metacestode, spreading aggressively in the liver, producing large irregular masses. Patients with AE had rapid disease progression, and recurrence and metastasis ratio was high even after surgery. For AE, metastasis more likely occurs on lung and brain around the world [6]. However, the metastasis may be seen in any organ or tissue. Animal experiment showed that this metastatic disease can through the detached proliferated bud, even very small or only a few nuclei entering the blood vessels [7]. Metastasis continues to be a major clinical challenge in the treatment of AE. Thus, identifying a sensitive and representative biological marker is extremely important for diagnosis and evaluating prognosis.

Recently, more and more studies gradually reveal that dysregulation of the CD44 plays a pivotal role in the pathogenesis of many human malignancies [8, 9]. CD44 is a transmembrane glycoprotein that was first found in lymphocytes

Table 1. Clinicopathologic characteristics of patients

Characteristics and finding	N = 159
Median age, years (range)	39 (14-69)
Median size, cm (range)	11.79 (2-30)
Gender	
Male	78 (48.8%)
Female	81 (50.6%)
Calcification	
Present	107 (67.3%)
Absent	52 (32.7%)
Necrosis	
Present	115 (72.4%)
Absent	44 (27.6)
Foreign body granulomatous	
Present	74 (46.6%)
Absent	85 (53.4%)
Relapse	
Present	39 (24.4%)
Absent	120 (75.6)
Metastasis	
Present	35 (22.1%)
Absent	124 (77.9%)
Survival	
Alive	144 (90.6%)
Deceased	15 (9.4%)

and macrophages [10]. Increasing evidences have shown that CD44 is involved in the regulations of tumor cell migration, cell adhesion, tumor progression [11, 12]. Low levels of CD44 in colorectal cancer correlate with increased lymph node invasion ability [13]. To date, there have been limited studies of CD44 in AE patients, and the significance of CD44 in the metastatic AE remains unclear. Thus, this study was aimed to the correlated factors for metastasis and prognosis in patients with AE.

Materials and methods

Patients and tissue specimens

For the retrospective study, AE specimens were obtained from 159 patients at the first teaching hospital of Xinjiang medical university from February 2003 to December 2013. In all 159 cases, the liver was the primary location of AE. A total of 35 cases with associated distant (brain, lymph node, pulmonary, multiple organs) metastasis were identified. The patients' patho-

logic features of the primary alveolar echino-coccosis were recorded, including age, size, gender, calcification, necrosis, foreign body granulomatous, recur, metastasis, survival status. Following hematoxylin and eosin (HE) staining, all sections were reviewed and reexamined. Patient survival time was determined from the date of surgery until death or the last follow-up examination. The study was approved by the local ethics committee.

Immunohistochemical staining

Immunohistochemical analysis was performed to study CD44 protein expression in 35 metastatic and 124 non-metastatic AE tissues. The immunohistochemical method was performed as previously described [14]. In brief, paraffin-embedded specimens were cut into 3 μm sections and incubated at 60°C for 60 min. The sections were deparaffinized with xylenes and rehydrated. For antigen retrieval, the section was heated in 10 mM citrate buffer PH 6 in a microwave oven 20 minutes. The sections were incubated with primary antibody at 4°C for overnight. The dilutions used for the primary antibodies were 1:200 for CD44 (Abcam). After washing with phosphate-buffered saline (PBS), the sections were incubated with HRP-conjugated secondary antibodies. Diamino-benzidine (DAB) was used to visualize positive regions, and the sections were counterstained with hematoxylin.

Evaluation of immnunostaining

Quantification of immunostaining was reviewed and scored by two independent pathologists based on the intensity of staining who were unaware of the clinical and pathologic data. In cases of discrepancy, a consensus score was chosen for evaluation. Staining intensity was graded according to the following criteria: 0 (no staining), 1 (weak staining = light yellow), 2 (moderate staining = yellowish brown), and 3 (strong staining = brown). Strong staining was used to define AE with high CD44 protein expression, and no, weak and moderate stains were used to indicate low CD44 protein expression.

Statistical analyses

Statistical analysis was performed with the statistical software program SPSS14.0. The rela-
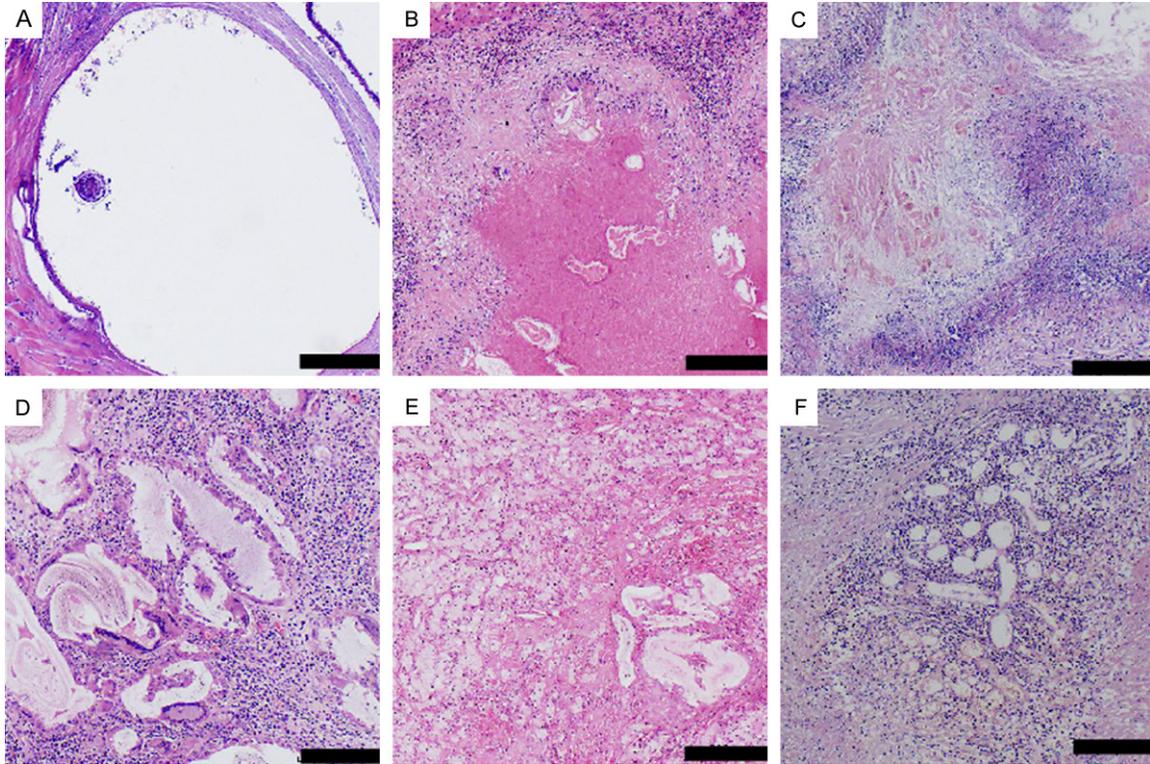
Figure 1. Histologic patterns seen with AE. HE staining clearly shows the protoscolices (A), necrosis (B), calcification (C), foreign body granuloma (D), foam cell (E), new vessels(F), bar = 200 μm.
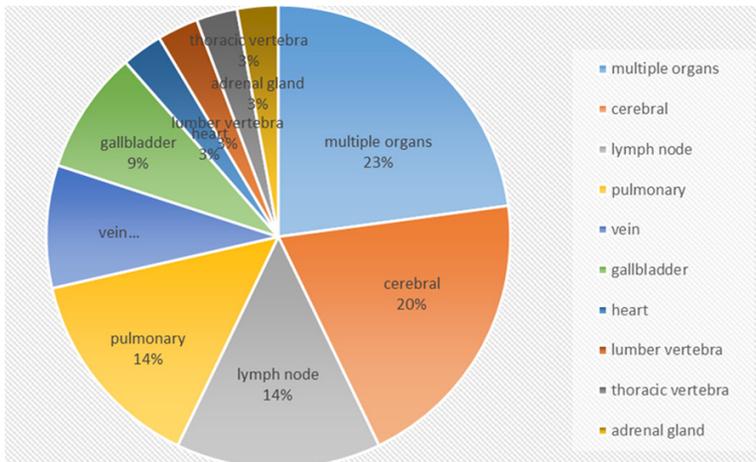

Figure 2. Distribution of metastatic AE.

tionship between metastasis, clinicopathological parameters, and immunohistochemical staining intensity was tested using chi-square test. Relative risks of related death associated with CD44 expression and other predictor variables were estimated by multivariate analyses. Differences were considered significant when *P* value was <0.05.

Results

Clinicopathological characteristics

The characteristics of the study population are summarized in **Table 1**. The median follow-up time was 42 months (range 3 to 252 months). The patients include 78 men and 81 women with a median age of 39 years (range 14 to 69 years). 121 patients (76.1%) were under 50 years of age.

Figure 1 shows representative findings of a HE study for AE. The majority of patients had calcification (107 patients, 67.3%), had necrosis (115 patients, 72.4%), had foreign body granulomatous (74 patients, 46.6%), had foam cell (3 patients, 1.9%), and had new vessels (2 patients, 1.2%). The reason for calcification was slowly clinical progress. The period between exposure and the appearance of
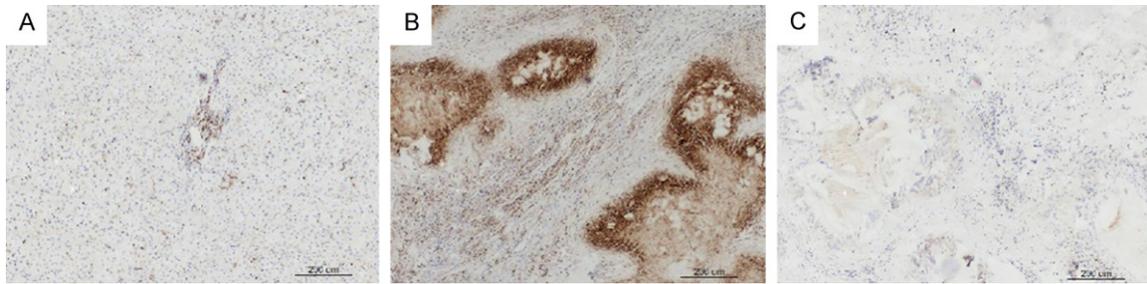
Figure 3. Representative immunohistochemical images of CD44 marker. (A) CD44 expression in adjacent normal liver, (B) strong positive expression of CD44 in AE tissues, (C) negative CD44 expression in AE tissues, bar = 200 μm.

Table 2. Association between metastasis and various clinicopathological factors of AE

Character	Metastatic	Non-metastatic	P
Gender			
Male	15 (42.9%)	63 (50.8%)	0.292
female	20 (57.1%)	61 (49.2%)	
Age, y			
<50	29 (82.8%)	92 (74.2%)	0.181
≥50	6 (17.2%)	32 (25.8%)	
Size			
<15	24 (72.7%)	83 (67%)	0.955
≥15	9 (27.3%)	29 (33)	
Calcification			
Present	25 (71.4%)	82 (66.1%)	0.913
Absent	10 (28.6%)	42 (33.9%)	
Necrosis			
Present	21 (60%)	94 (75.8%)	0.116
Absent	14 (40%)	30 (24.2)	
Foreign body granulomatous			
Present	20 (57.1%)	54 (43.5%)	0.586
Absent	15 (42.9)	70 (56.4%)	
CD44			
Low	30 (85.7%)	46 (37.1%)	0.004
High	5 (14.3%)	78 (62.9%)	

Expression of CD44 in AE patients

In the normal liver specimens, immunohistochemistry showed that CD44 was predominantly localized in liver portal lymphocytes, Kupffer cell and hepatic sinusoidal endothelial cells (**Figure 3A**). The expression of CD44 was immunohistochemically positive in the laminated layer of E. multilocularis, lymphocytes, and histiocyte in AE (**Figure 3B**). Out of 159 patients, there were 83 cases that exhibited a high expression level of CD44 (52.2%), and 76 cases (47.8%) that exhibited a low expression level of CD44.

Correlation between metastasis and clinicopathologic features

We also analyzed the relationships between metastasis and clinicopathological variables of AE patients in **Table 2**. The total low expression rate of CD44 was 85.7% (30/35), while the total high expression rate of CD44 was 14.3% (5/35) in the metastasis AE tissues. The level of CD44 protein expression was found to correlate with metastasis (*P* = 0.004). The gender, age, size, calcification, necrosis, foreign body granulomatous did not show any relationship with metastasis (**Table 2**).

Association between risk factors and survival time

At the time of data analysis, with a median follow-up of 42 months (range 3-252 months), In

symptoms may be as long as several to dozen or more years [15].

During the observation period, 35 of the 159 patients (22.1%) developed metastatic disease (multiple organs in 8, cerebral in 7, lymph node in 5, pulmonary in 5, vein in 3, gallbladder in 3 heart in 1, lumber vertebra in 1, thoracic vertebra in 1 adrenal gland in 1), respectively (**Figure 2**). With regard to organ distribution, approximately 23% (8/35) of the patients developed metastasis in more than one organ system.

Table 3. Multivariate analysis of the association between various paramaters and overall survival in patients with AE

Covariate	HR	95% CI	P
Gender			
Male vs. female	1.407	0.328-6.024	0.646
Age			
<50 vs. ≥50	1.603	0.378-6.79	0.522
Size			
<15 vs. ≥15	2.576	0.617-10.759	0.194
Calcification			
Present vs. absent	0.996	0.178-5.559	0.996
Necrosis			
Present vs. absent	0.640	0.123-3.334	0.596
Foreign bodygranulomatous			
Present vs. absent		0.074-2.224	0.298
Metastasis			
Present vs. absent	1.847	0.460-7.410	0.387
CD44			
Low vs. high	2.042	0.449-9.284	0.356

HR: hazard ration; CI: confidence interval.

144 patients (90.6%) remained alive and 15 patients (9.4%) died. Several studies have shown that great advances in surgery techniques and albendazole have increased the survival rate of patients [16, 17]. Factors with possible prognostic effects in AE were analyzed by multivariate Cox proportional hazard regression analysis. Statistical analysis showed that gender, age, size, calcification, necrosis, foreign body granulomatous, and CD44 had no prognostic value (**Table 3**).

Discussion

In humans, AE has similar features to cancer due to its invasion growth and destruction, and its distant metastases via the blood or lymphatic systems [18, 19]. It is noteworthy that, the most devastating aspect of AE is the emergence of metastases, which is responsible for the majority of deaths from this disease. Thus, to understand the molecular mechanisms of metastasis is one of the most important issues in AE research. In the present study, we investigated candidates of molecular markers in order to predict pathological development.

CD44 was originally identified as the lymphocyte homing receptor in embryonic, hematopoietic, mesenchymal and epithelial cells [20, 21]. According to recently studies, CD44 was asso-

ciated with tumor progression, invasion and metastasis in cancer stem cells [22, 23]. In the current study, we sorted and identified AE from clinical specimens, observing that the relationship between metastasis and the clinicopathological characteristics of AE. We found that CD44 expression was decreased in metastasis AE when compared to the control group by IHC. Additionally, it was identified that CD44 expression was associated with metastasis. However, no correlations between metastasis and gender, age, size, calcification, necrosis, foreign body granulomatous were identified. Thus, CD44 protein may be highly related to metastatic disease.

Furthermore, we found gender, age, size, calcification, necrosis, foreign body granulomatous, were no significantly associated with overall survival. As shown in **Table 2**, the expression of CD44 was associated with metastasis, while the expression of CD44 was not a prognostic predicator. The first reason is multimodality treatment. Mortality from AE has markedly declined, mainly owing to the advances in surgical techniques and albendazole treatment [24, 25]. In the present series of 159 AE patients with long-term follow-up observations averaging >42 months, the fatality rate was 9.4%. The second reason is follow-up times. To facilitate better observe the patient's prognosis, we should extend the follow-up time. Life expectancy in this group is substantially prolonged compared to previous observation. In addition, we hope to go on further study with larger Sample size in the near future.

Although correlation between CD44 expression and patient survival did not reach statistical significance, as determined by multivariate analysis, CD44 immunoreactivity may have the potential to be a good predictor of metastasis in AE patients. With regard to other cancer, CD44 expression in lymphoma, melanoma, vulvar cancer, cancers of the colon, breast, stomach, ovary, cervix, thyroid and lungs was correlated with disease progression, metastases and worse prognosis [26-28]. Further studies are need to clarify this tissue.

In conclusion, our study identified the decreased expression of CD44 was related to metastasis of AE, which may be a potential bio-

marker for the metastasis. In short, molecular immunological diagnosis for human AE needs further studies.

Disclosure of conflict of interest

None.

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