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Short Communication

Programmed death-ligand1 is a determinant of recurrence in alveolar echinococcosis

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ABSTRACT

Objectives: Alveolar echinococcosis (AE) recurrence is one of the major stakes in patients undergoing surgery, the main curative treatment. Preliminary data demonstrated an effect of programmed death-ligand1 (PD-L1) inhibitors on AE proliferation in animals. The current study aimed to analyze the prognostic value of PD-L1 expression in tissue samples of patients with AE undergoing surgery.

Methods: A cross-sectional study of patients operated for AE between 2002 and 2017 was performed. Patients with recurrence were matched 1: 2 with patients without recurrence. The matching criteria were PNM staging (P = hepatic localization of the parasite, N = extra-hepatic involvement of neighboring organs, and M = absence or presence of metastasis), resection status, preoperative albendazole treatment, and lesion size. PD-L1 immunohistochemistry staining was performed in surgical liver specimens. The expression of PD-L1 was assessed in immune cells. Disease-free survival was calculated using the Kaplan-Meier method.

Results: Among 68 consecutive patients, eight patients with recurrence were matched to 16 patients without recurrence. PD-L1 was overexpressed in patients with recurrence (recurrence: PD-L1 <1%: one, PD-L1 ≥1%: seven; no recurrence: PD-L1 <1%: nine, PD-L1 ≥1%: seven, $P = 0.040$). Moreover, patients with lower PD-L1 expression (<1%) showed better median disease-free survival (120 months, 95% confidence interval 104-135 vs 74, 95% confidence interval 44-104, $P = 0.050$).

Conclusion: These findings highlight the proof of concept of PD-L1 in AE, but further data on its prognostic importance and the role of immune checkpoint blockade as a promising therapeutical strategy are needed.

Introduction

Echinococcosis, a parasitic disease caused by *Echinococcus* tapeworms, is classified as cystic (hydatid disease) or alveolar echinococcosis (AE) [1]. Most of the time, patients with cystic echinococcosis (CE) are asymptomatic but parasitic cysts can enlarge and cause various symptoms depending on the lesion location [2]. AE caused by *Echinococcus multilocularis* induces parasitic tumors that can invade most often the liver, lungs, or brain. AE unlike CE behaves similarly as a cancer with the propension to invade the hepatic parenchyma and develop distant metastatic lesions.

The therapeutical arsenal for AE is restrained. Partial hepatectomy remains the main curative approach but is only indicated when complete parasitic lesion removal is achievable [3–6]. Benzimidazoles are the only drugs recommended in the adjuvant setting but display limited efficacy and non-negligible side effects [7]. Recently, programmed cell death protein 1 (PD-1) and its ligand (PD-L1) have been described as immune system regulators [8]. As an immune checkpoint, PD-1/PD-L1 are interesting targets to alter the potential immune response and autoimmunity. In oncology, several specific PD-1/PD-L1 inhibitors stimulated the immune response toward malignant cells, leading to better clinical outcomes [9]. This pathway has been extensively investigated in cancers but

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data on its potential role in infectious diseases remain scant [8,10]. In infectious diseases, interactions between PD-1 and PD-L1 have been shown to control the induction and maintenance of peripheral T cell tolerance, therefore protecting the infected organs from autoimmune aggression [8]. Moreover, some bacteria and parasites use the PD-1/PD-L1 pathway to decrease the antimicrobial immunity that can lead to chronic infection [8]. Preclinical data demonstrated that PD-1/PD-L1 pathway blockade by anti-PD-L1 antibody triggered a host immune response capable of controlling the parasitic proliferation in mice with AE models [11]. A recent study in humans suggested that PD-L1 expression in serum rapidly decreased after surgery but the correlation with outcomes was not evaluated [12]. Altogether, the clinical and biological similarities between AE and cancers, the promising response to PD-1/PD-L1 inhibitors in various cancers, and preclinical data in AE murine models provided the rationale to explore the association between PD-L1 expression in human liver samples of AE and the clinical outcomes of patients with AE.

This study assessed PD-L1 immunohistochemical expression on resected AE specimens and analyzed its impact on recurrence.

Methods

PD-L1 immunochemistry was performed using SP142 clone staining [13]. The evaluation of PD-L1 expression in the immune cells was estimated as previously described [14]. Only the immune cells in the vicinity of the lesion were considered. The expression score was determined by the number of PD-L1-positive immune cells divided by the total number of viable immune cells [14]. The expression score was reported as <1%, 1-4.9%, and ≥5% [14].

The complete methods are summarized in the Supplementary Material.

Results

A total of 68 patients underwent surgery for AE. Demographics, intraoperative details, and postoperative outcomes are summarized in the Supplementary Material. The median follow-up after surgery was 120 months (interquartile range 91-139) and eight patients developed recurrence (8/68, 12%). These eight patients were matched 1: 2 (matching criteria: age, PNM staging (P = hepatic localization of the parasite, N = extra-hepatic involvement of neighboring organs, and M = absence or presence of metastasis), resection status, preoperative albendazole treatment, lesion size) with 16 patients without recurrence.

Recurrence versus no recurrence

Preoperative characteristics, intraoperative details, and postoperative outcomes of patients with and without recurrence are detailed in the Supplementary Material. The groups were comparable after matching.

Immunohistochemical staining and PD-L1 assessment

The data regarding immunohistochemical stainings are summarized in Table 1. No difference in inflammation degree was observed between the groups with and without recurrence.

Most patients (22/24) harbored a moderate-to-high number of cluster of differentiation (CD)3-positive lymphocytes by immunohistochemistry in the lesion vicinity (interface between the lesion and adjacent hepatic parenchyma; seven in the recurrent and 15 in the nonrecurrent group, $P = 0.602$). In the surrounding liver, three patients with recurrence had no or only a small number of CD3-positive lymphocytes, whereas seven patients without recurrence had no or only a small number of CD3-positive lymphocytes ($P = 0.770$). SP142 clone staining for PD-L1 showed a punctate or granular cytoplasmic staining pattern in immune cells (regulatory T cells). A high expression of SP142 staining was more frequent in patients with recurrence (recurrence: <1%: one, ≥1%: seven; no recurrence: <1%: nine, ≥1%: seven, $P = 0.040$).

Table 1
Summary of immunohistochemical stainings in the groups with and without recurrence.

	Recurrence (n = 8)	No recurrence (n = 16)	*P*-value
Degree of inflammation[a]			
Weak	1	1	0.602
Moderate	4	10	0.558
Severe	3	5	0.759
Tertiary lymphoid structure			
Absence	2	3	0.722
Presence	6	13	0.722
Clusters of differentiation 3 staining (vicinity)			
Low	1	1	0.602
Moderate	5	11	0.759
High	2	4	1
SP142 staining			
<1%	1	9	**0.040**
1–4.9%	4	6	0.558
≥5%	3	1	0.053

TLS, tertiary lymphoid structures.
[a] Degrees of inflammation were defined as followed:• Weak: mild overall inflammation and/or presence of few TLS;• Moderate: moderate overall inflammation and/or presence of multiple TLS, irregularly distributed;• Severe: strong overall inflammation and presence of multiple TLS.

Patients with PD-L1 ≥1% showed lower mean disease-free survival (DFS) than those with PD-L1 <1% (74, 95% confidence interval [CI] 44-104 vs 120 months, 95% CI 104-135, $P = 0.050$, Figure 1a). Patients with PD-L1 ≥5% had even lower mean DFS than those with PD-L1 = 1-4.9% (40, 95% CI 3-77 vs 87 months, 95% CI 52-121, $P = 0.042$, Figure 1b). In the Cox regressions, biliary invasion (hazard ratio [HR] 4.3, 95% CI 1.1-18, $P = 0.047$), venous invasion (HR 6.6, 95% CI 1.6-27.4, $P = 0.009$), and PD-L1 ≥5% (HR 4.3, 95% CI 1.1-18.5, $P = 0.048$) were identified as prognostic factors of DFS (Supplementary Material).

Discussion

PD-L1 expression on surgical human liver specimens was higher in patients with AE recurrence than in patients without recurrence. Moreover, PD-L1 expression significantly correlated with recurrence and was identified as a prognostic factor of DFS.

This study reports the first results in human highlighting a correlation between PD-L1 and outcomes after AE treatment. These results provide the proof of concept and streamline the idea of testing PD-L1 inhibitors in patients with AE in phase I/II clinical trials. In the future, AE treatments may be synergized by immune checkpoint blockade [3,11]. The rationale of testing anti-PD-L1 antibodies in AE is based on preclinical data and on the fact that immune checkpoint blockade provides immunomodulation. Because infectious pathogens use these inhibitory pathways to escape from the immune system, immunomodulation using anti-PD-L1 might increase the immune response against pathogens. The study results highlight the importance of assessing the immune microenvironment of AE liver lesions that could help to better predict the prognosis. Future data could be drawn from prospective studies on patients with AE evaluating the immune microenvironment more extensively. Moreover, tests on PD-L1 blockers in AE mice and measures of their effect on recurrence could also be follow-up studies.

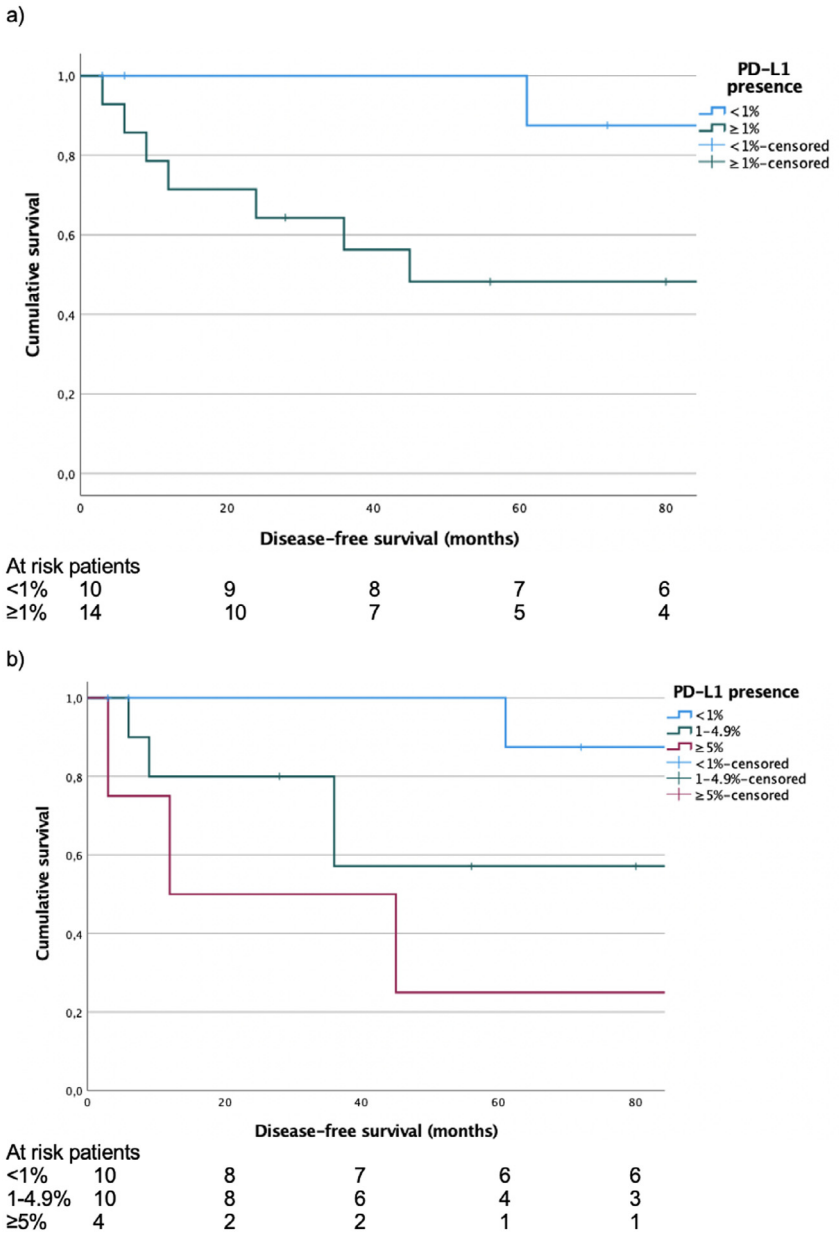

Figure 1. Kaplan-Meier curves of disease-free survivals in function of the presence of PD-L1 in the resected surgical specimen. (a) Mean disease-free survivals: PD-L1≥1%: 74 months, 95% CI 44-104 vs PD-L1<1%: 120 months, 95% CI 104-135, $P = 0.050$. (b) Mean disease-free survivals: PD-L1≥5%: 40 months, 95% CI 3-77 vs PD-L1 = 1-4.9%: 87 months, 95% CI 52-121 vs PD-L1<1%: 120 months, 95% CI 104-135, $P = 0.042$.
CI, confidence interval; PD-L1, programmed death-ligand 1.

This pilot study in humans provides additional data to the studies on PD-L1 in animal AE models [11,15]. Using mice models, these studies suggested that PD-L1 inhibitors decreased the parasitic load of hepatic lesions in acute and chronic AE [11]. More specifically, in chronic murine AE, PD-L1 blockade decreased the number of regulatory T cells, natural killer cells, and natural killer T cells and increased the effector T cell activity and innate immune cell number [15].

This study identified the PD-L1 pathway involvement in the outcomes of patients with an infectious disease, which is an unprecedented finding. PD-L1 may be a potential biomarker to predict AE recurrence and target for AE treatment. These results open new routes and pave the way for future studies aiming to determine whether and how the PD-1/PD-L1 pathway may be targeted in AE.

Declaration of competing interest

The authors have no competing interests to declare.

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Ethical approval

The current study was approved by the appropriate ethics committee Commission cantonale d'éthique de la recherche sur l'être humain (CER-VD). Written informed consent was obtained from all the patients included in the study.

Author contributions

Study design: GRJ, SNMF, IL, CS. Data acquisition: GRJ, IL, SH, CS. Data analysis: GRJ, SNMF, SH, IL, CS. Interpretation of the data: GRJ, SNMF, SH, ND, NH, IL, CS. Manuscript draft: GRJ, IL. Manuscript critical revision: GRJ, SNMF, SH, ND, NH, IL, CS. Final approval of the manuscript version to be published: GRJ, SNMF, SH, ND, NH, IL, CS. Agreement to be accountable for all aspects of the work: GRJ, SNMF, SH, ND, NH, IL, CS.

Supplementary materials

Supplementary material associated with this article can be found, in the online version, at doi:10.1016/j.ijid.2023.01.043.

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