




CASE REPORT

Programmed cell death ligand 1 expression in a case of poorly differentiated lymphocyte-rich hepatocellular carcinoma

Ginji Omori¹  | Takahiro Osuga¹ | Koji Miyanishi¹  | Kota Hamaguchi¹ | Shingo Tanaka^{1,2} | Hiroyuki Ohnuma¹ | Kazuyuki Murase¹ | Kohichi Takada¹ | Minoru Nagayama³ | Yasutoshi Kimura³ | Ichiro Takemasa³ | Yasuhiro Kikuchi⁴ | Toshihiko Torigoe⁴ | Junji Kato¹ 

¹Department of Medical Oncology, Sapporo Medical University School of Medicine, Sapporo, Japan

²Department of Infection Control and Laboratory Medicine, Sapporo Medical University School of Medicine, Sapporo, Japan

³Department of Surgery, Surgical Oncology and Science, Sapporo Medical University School of Medicine, Sapporo, Japan

⁴Department of Pathology, Sapporo Medical University School of Medicine, Sapporo, Japan

Correspondence

Koji Miyanishi, Department of Medical Oncology, Sapporo Medical University School of Medicine, South-1, West-16, Chuo-ku, Sapporo, Hokkaido 060-8543, Japan.

Email: miyako@sapmed.ac.jp

Abstract

Our pathological study of a case of poorly differentiated lymphocyte-rich hepatocellular carcinoma suggested that immune checkpoint inhibitor may be an effective therapy. The histological type is an important factor in determining treatment choices.

KEYWORDS

hepatocellular carcinoma, immune checkpoint inhibitors, lymphocyte-rich, lymphoepithelial hepatocellular carcinoma, programmed cell death ligand 1

1 | INTRODUCTION

A 70-year-old woman was diagnosed with poorly differentiated lymphocyte-rich hepatocellular carcinoma (HCC). In this case, programmed cell death 1 expression was observed. Immune checkpoint inhibitor treatment may be effective in such cases, although there has been only one report of their use for poorly differentiated lymphocyte-rich HCC.

In the past, poorly/undifferentiated hepatocellular carcinoma (HCC) with high lymphocytic infiltration was classified as lymphoepithelioma-like hepatocellular carcinoma (LEL-HCC).¹ In the 2019 revision of the WHO classification, it was incorporated into the lymphocyte-rich subtype, regardless of differentiation.² We present a case of poorly differentiated lymphocyte-rich HCC, which corresponds to LEL-HCC in the previous classification, and performed a pathological investigation.

This is an open access article under the terms of the Creative Commons Attribution-NonCommercial-NoDerivs License, which permits use and distribution in any medium, provided the original work is properly cited, the use is non-commercial and no modifications or adaptations are made.

© 2021 The Authors. *Clinical Case Reports* published by John Wiley & Sons Ltd.

TABLE 1 Laboratory data on admission

<Hematology>		<Chemistry>		<Serological test>	
WBC	6700/ μ l	TP	8.3 g/dl	CRP	<0.10 mg/dl
RBC	382×10^4 /ul	Alb	4.2 g/dl	HBs-Ag	(+)
Hb	12.9 g/dl	T-Bil	0.7 mg/dl	HBs-Ab	(-)
Ht	38.9%	AST	66 U/L	HBc-Ab	(+)
Plt	12.2×10^4 /ul	ALT	68 U/L	HBe-Ag	(-)
		LDH	196 U/L	HBe-Ab	(+)
		ALP	157 U/L	HCV-Ab	(-)
		γ -GTP	61 U/L		
<Coagulation>		BUN	16 mg/dl	<Genetic test>	
PT	80.5%	Cr	0.6 mg/dl	HBV-DNA	5.6 LogIU/ml
APTT	32.7 s	HbA1c	6.1%		
ATIII	80%	NH ₃	34.7 μ g/dl	<Tumor marker>	
				AFP	3946 ng/ml
				PIVKA-II	15 mAU/ml
				CEA	1.0 ng/ml
				CA19-9	23.5 U/ml

Abbreviations: AFP, alpha-fetoprotein; Alb, albumin; ALP, alkaline phosphatase; ALT, alanine aminotransferase; APTT, activated partial thromboplastin time; AST, aspartate transaminase; ATIII, Antithrombin III; BUN, blood urea nitrogen; CA19-9, carbohydrate antigen 19–9; CEA, carcinoembryonic antigen; Cr, creatinine; CRP, C-reactive protein; Hb, hemoglobin; HbA1c, Hemoglobin A1c; HBc-Ab, hepatitis B-core antibody; HBc-Ag, hepatitis B-core antigen; HBe-Ab, hepatitis B-envelope antibody; HBe-Ag, hepatitis B envelope antigen; HBs-Ab, hepatitis B-surface antibody; HBs-Ag, hepatitis B surface antigen; HBV-DNA, hepatitis B virus-deoxyribonucleic acid; HCV-Ab, hepatitis C virus antibody; Ht, hematocrit; LDH, lactate dehydrogenase; NH₃, ammonia; PIVKA-II, protein induced by vitamin K absence-II; Plt, platelets; PT, prothrombin time; RBC, red blood cells; T-Bil, total bilirubin; TP, total protein; WBC, white blood cells; γ GTP, γ -glutamyl transpeptidase.

2 | CASE REPORTS

In 2019, a 70-year-old woman visited her local doctor with a complaint of 15 kg of weight loss over a month. Abdominal ultrasound revealed a liver tumor, and she was referred to our hospital. There was no history of alcohol intake. Blood examination (Table 1) showed aspartate aminotransferase elevation (AST; 66 U/L, normal <30 U/L), alanine aminotransferase elevation (ALT; 68 U/L, normal <23 U/L), positive HBs antigen, HBV-DNA elevation (5.6 LogIU/ml), and negative hepatitis C virus antibody. Alpha-fetoprotein (AFP) was markedly elevated (3946 ng/ml, normal <7.0 ng/ml). Abdominal ultrasound revealed a 22 \times 20 mm mass with an internal mosaic pattern in segment five of the liver (Figure 1). Angiographic computed tomography (AGCT) scan revealed low absorption in CT during arteriportography (CTAP) and uniformly high absorption in both early and delayed CT during hepatic arteriography (CTHA; Figure 2). EOB-MRI revealed a high signal in early phase, low signal in hepatocellular phase, high signal in diffusion-weighted image, and low signal in ADC (Figure 3). The lesion was solitary and there were no findings suggestive of vascular invasion. Laparoscopic partial hepatectomy was performed subsequent to the diagnosis of classical HCC UICC (Union for International

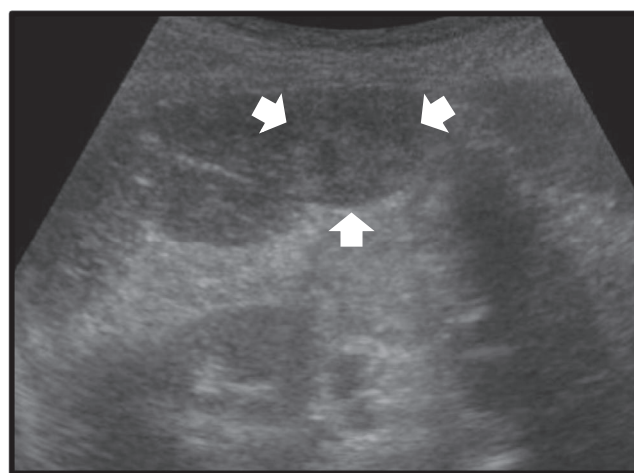


FIGURE 1 Abdominal ultrasonography images. The tumor was present on segment five and showed a mosaic pattern (white arrow)

Cancer Control) Stage Ib. Microscopically, there were many infiltrating lymphocytes in the background, and tumor cells with round to oval nuclei with prominent irregularities and abundant eosinophilic cytoplasm had grown in a plump, sheet-like pattern. Some tubular structures corresponding to the moderately differentiated type

FIGURE 2 CT during angiography. The tumor showed low absorption on CT during arteriopoortography (A) and high absorption on CT during hepatic arteriography both in the early phase (B) and in the late phase (C)

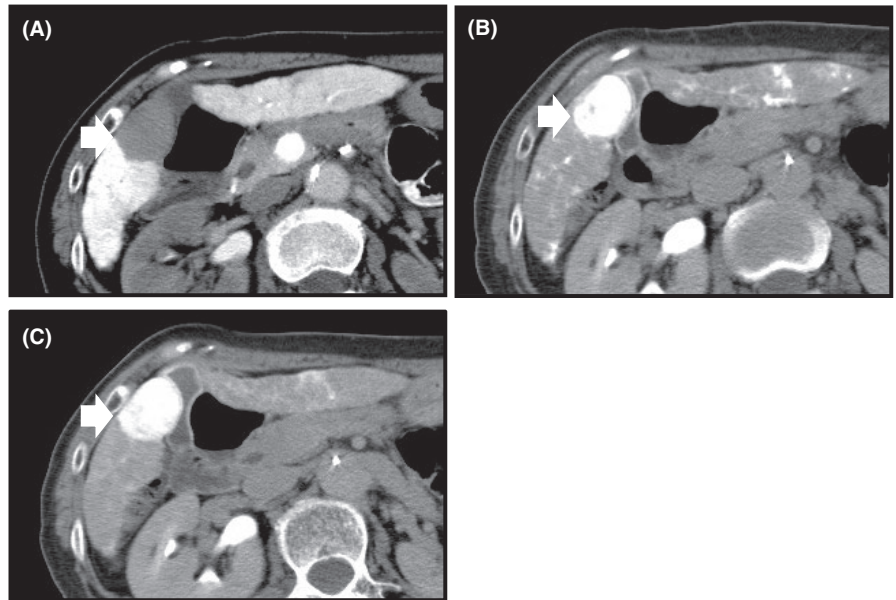
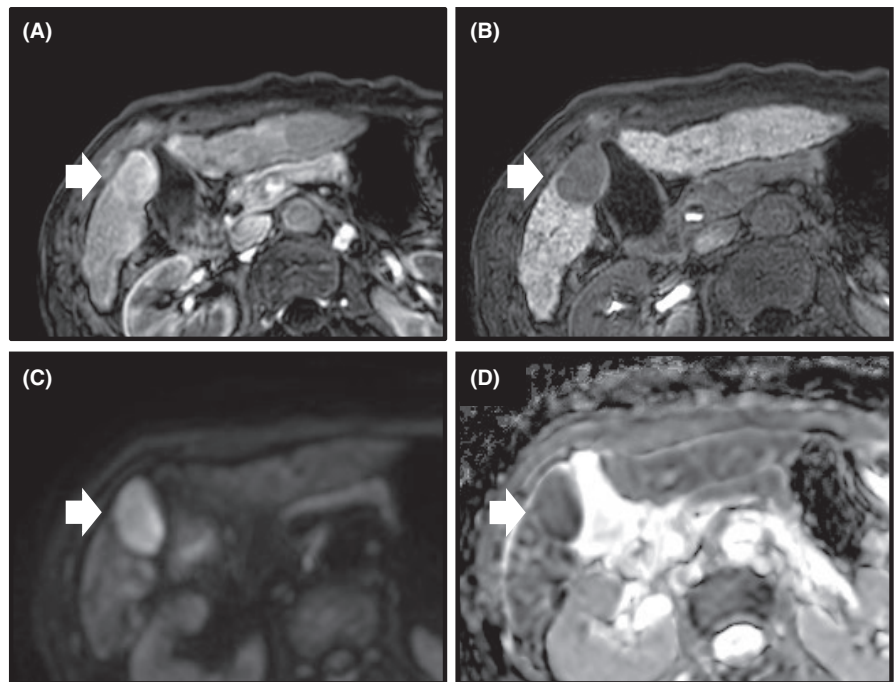


FIGURE 3 EOB-MRI images. The tumor showed a high signal on early phase (A), low signal on T1-weighted hepatocyte phase (B), high signal on diffusion-weighted imaging (C), and low signal on apparent diffusion coefficient (D)



remained at the tumor margins; however, the majority of the cells were the poorly differentiated type (Figure 4). Immunostaining of the tumor cells was positive for glypican 3 and negative for EBER. Programmed cell death ligand 1 (PD-L1) was positive in about 40% of tumor cells, and inflammatory cells around the tumor were also partially positive, showing scattered positive cells in the form of small clusters. The infiltrating lymphocytes showed a mixture of CD3- and CD8- positive cytotoxic T cells and CD4- and FOXP3-positive regulatory T cells (Figures 5-7). Twenty months have passed since hepatectomy and no recurrence has been observed to date.

3 | DISCUSSION

Among undifferentiated cancers with abundant lymphocyte infiltration, those derived from the head and neck region are called lymphoepithelioma.³ Undifferentiated cancers with abundant lymphocyte infiltration in organs other than the head and neck region are called lymphoepithelioma-like carcinomas (LELC), and in the WHO classification 4th edition, LELC in the liver were classified as lymphoepithelioma-like hepatocellular carcinomas (LEL-HCC).¹ However, in the 2019 revision of the WHO classification, HCC with abundant lymphocyte

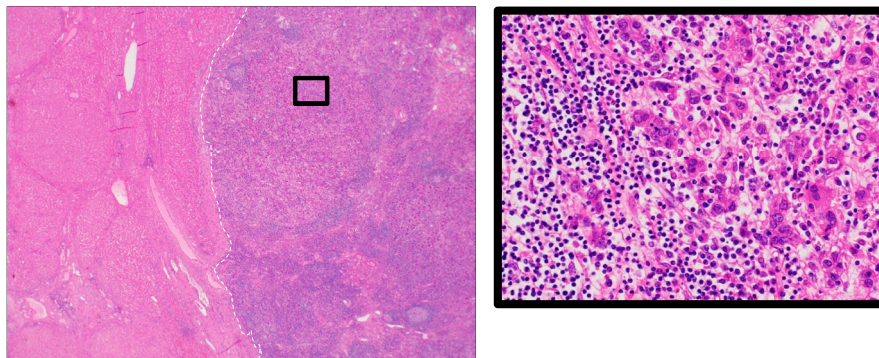


FIGURE 4 Histological findings in resected lymphocyte-rich hepatocellular carcinoma (loupe image, H-E stain). The enlarged image is shown in the black frame. White line shows the border of the tumor and liver background. H-E staining showed poorly differentiated tumor cells with prominent nuclear irregularities and high degree of lymphocytic infiltration in the background

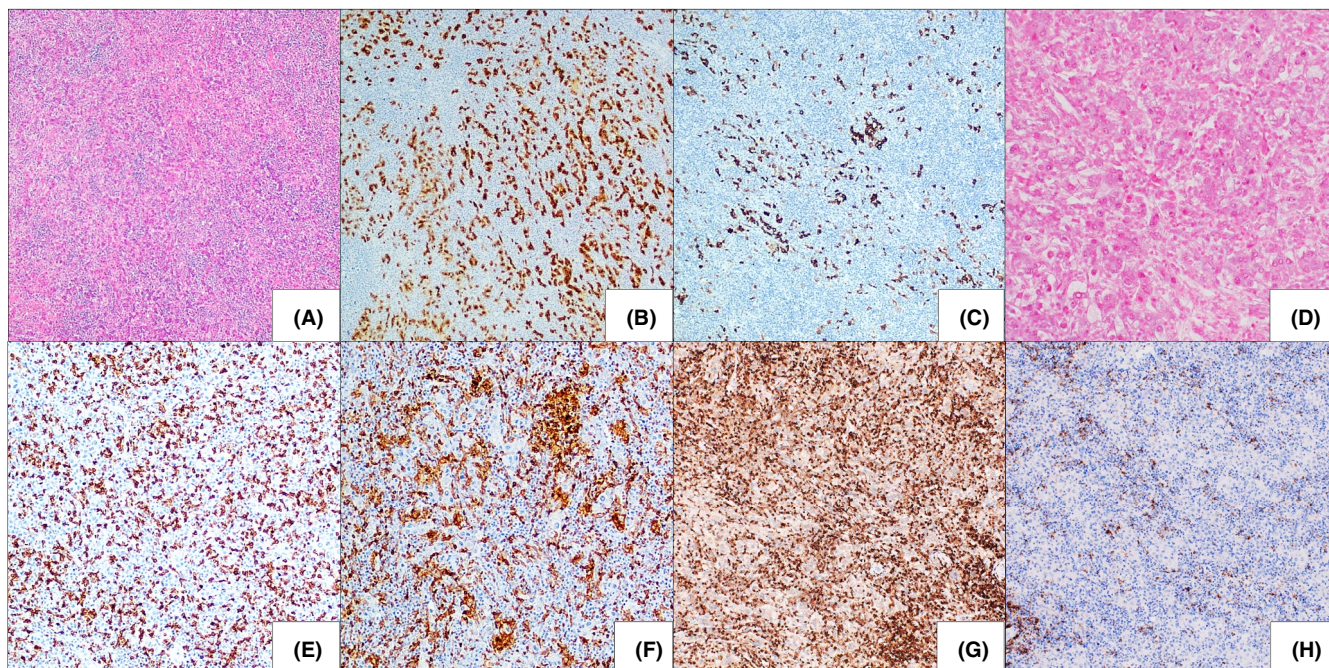


FIGURE 5 H-E staining and immunostaining images of tumor and infiltrating lymphocytes. H-E (A), glypican 3 (B), hepatocyte (C), EBV-encoded small RNA (EBER; D), CD8 (E), CD4 (F), CD3 (G), and CD20 (H). Many infiltrating lymphocytes were found around tumor cells stained with glypican 3 and hepatocyte. EBER was negative. Both CD8 and CD4 were positive in 50%. CD3 was positive in almost 100% and CD20 was negative

infiltration was included in the classification of "hepatocellular carcinoma, lymphocyte-rich" regardless of the degree of differentiation.² The frequency of lymphocyte-rich HCC among all HCC cases is less than 1%, and it is reported that its prognosis is better than that of classical HCC.^{2,4} Most cases of LELC in other organs are associated with Epstein-Barr virus (EBV)⁵ infection, but most cases of HCC, lymphocyte-rich are negative for EBV.⁶ We searched PubMed for articles from 1995 to 2021 using the keywords "lymphoepithelioma-like hepatocellular carcinoma" or "hepatocellular carcinoma lymphocyte rich". As a result, there were 59 cases corresponding to poorly differentiated lymphocyte-rich HCC (Table 2⁷⁻²⁵). We examined a total of

60 cases including the current case. The median age was 61 years; 27 cases were hepatitis B virus (HBV) positive and 19 were hepatitis C virus (HCV) positive. EBV was negative in all but one case. Although there are few reports on the proportion of infiltrating lymphocyte types, there are many cases in which both CD4-positive cells, including regulatory T cells, and CD8-positive cells, considered to be cytotoxic T lymphocytes (CTLs), were present to the same extent. In this case as well, CD4 and CD8 were equally positive. FoxP3 was positive in some of the CD4-positive cells, indicating the presence of regulatory T cells.

In the IMbrave150 trial, the combination of the immune checkpoint inhibitor (ICI) atezolizumab and the

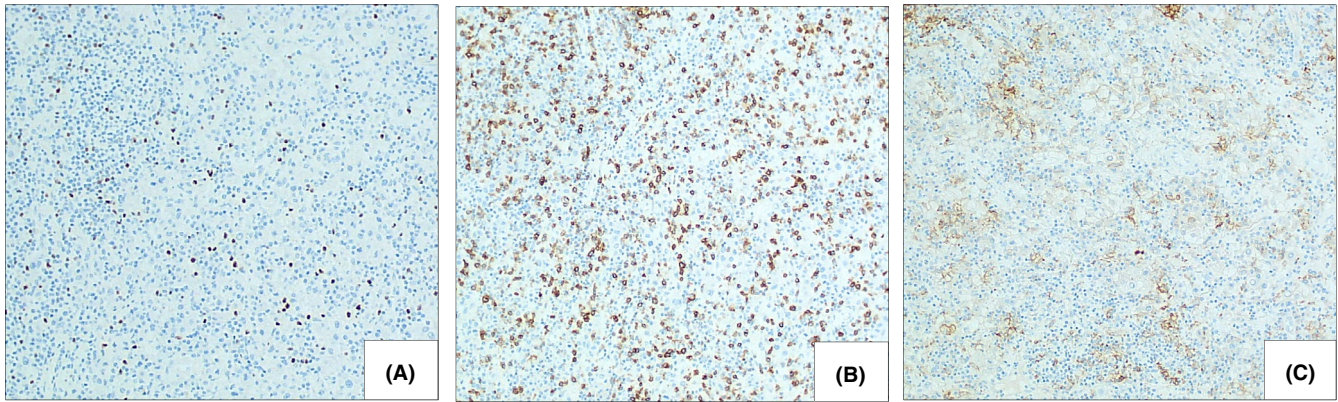
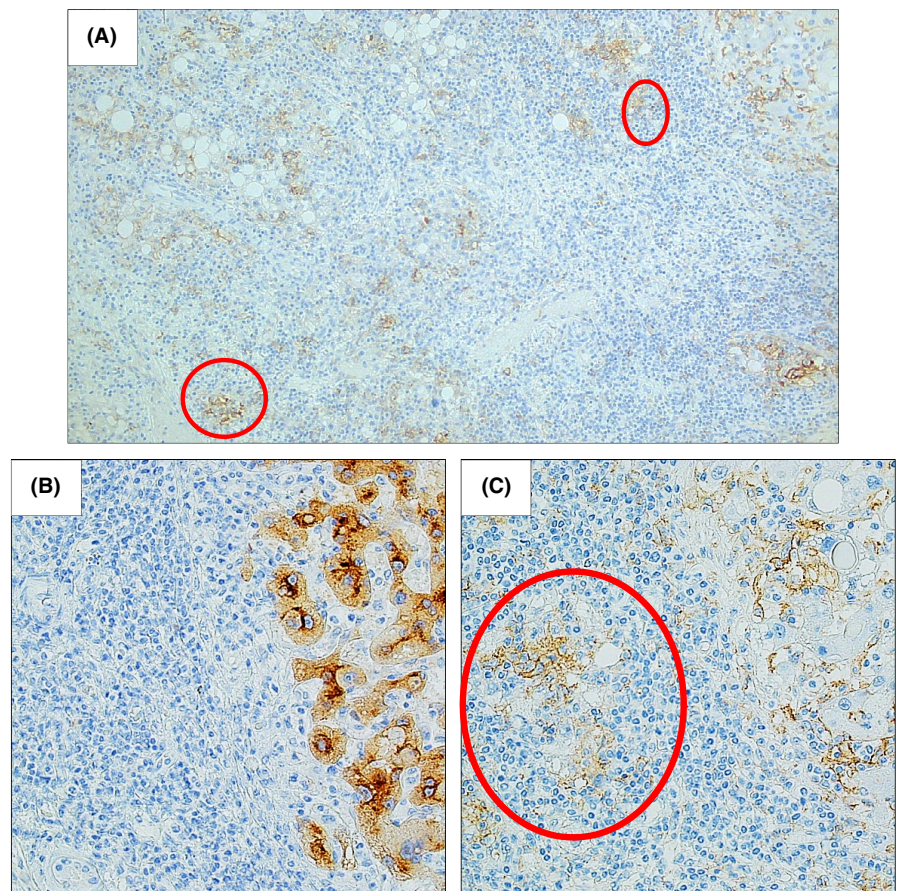


FIGURE 6 Immunostaining images. FOXP3 (A), programmed cell death 1 (PD-1; B), programmed cell death ligand 1 (PD-L1; C). FOXP3 was weakly positive in CD4-positive cells. PD-1 was positive in almost all lymphocytes. PD-L1 was positive in about 40% of tumor cells

FIGURE 7 Low-power field showing immunostaining of PD-L1 (A), high-power field showing glypican-3 immunostaining (B), and PD-L1 (C). Multiple small foci of PD-L1 positive inflammatory cell clusters were founded around glypican 3-positive tumor cells (circles)



angiogenesis inhibitor bevacizumab for unresectable HCC significantly prolonged overall survival compared with sorafenib. As a result, this combination therapy has come to be used clinically for unresectable HCC. The response rate for this treatment in unresectable HCC is 27.3%,²⁶ which is a good result, but it is still necessary to identify biomarkers for predicting the effect of ICIs in terms of pharmacoeconomics and personalized medicine. In addition, Chan et al.²⁷ reported that there are many mutations in the Wnt pathway, including CTNNB1, in normal HCC,

as well many mutations in CCND1 in LEL-HCC. These findings suggest that LEL-HCC may have different tumor properties compared with normal HCC. Therefore, we decided to histopathologically assess the ICI treatment strategy for poorly differentiated lymphocyte-rich HCC. So far, there is only one report for the use of ICIs in LEL-HCC,²⁵ and there have been multiple reports of cases in which ICIs were effective for LELC in other organs.^{28,29} In a study of 217 cases of HCC by Calderaro et al., the PD-L1 positive rate (>1% of tumor cell-positive cases were

Total		n = 60
Age	Median (range)	61 (37–81)
Gender	Male/Female	36/24
Vascular invasion	+/-/NA	11/39/10
HBV	+/-	27/33
HCV	+/-/NA	19/40/1
EBV	+/-/NA	1/56/3
CD4, CD8	mainly CD4/CD4 = CD8/mainly CD8/NA	2/5/2/51

TABLE 2 60 cases of poorly differentiated lymphocyte-rich HCC

Note: We searched PubMed for articles from 1995 to 2019 using the keywords "Lymphoepithelioma like hepatocellular carcinoma" or "Hepatocellular carcinoma lymphocyte rich". As a result, there were 58 cases of poorly differentiated lymphocyte-rich HCC. This table shows a total of 59 cases where we added the case presented.

Abbreviations: CD, cluster of differentiation; EBV, Epstein-Barr virus; HBV, hepatitis B virus; HCV, hepatitis C virus; NA, not applicable.

defined as PD-L1 positive) of LEL-HCC tumor cells was three of 13 cases, which was not different from the positive rate of 17% for all HCC. However, the PD-L1 positive rate of surrounding inflammatory cells was significantly high (PD-L1 expression by inflammatory cells was classified as high if the number of PD-L1 positive clusters around the tumor was equal or superior to six [median of the full series]) in 12 of 13 cases (92%).³⁰ In this case as well, multiple PD-L1-positive immune cell clumps were found around the tumor (Figure 7). It is known that ICIs induce an immune response by inhibiting PD-L1 on tumor-related macrophages,³¹ and ICIs may be effective in poorly differentiated lymphocyte-rich HCC. In the study by Calderaro et al., the analysis of the PD-L1 positive rate of LEL-HCC and tumor cells were limited to only 13 cases. In the present case, 40% of the tumor cells were also PD-L1 positive. There are other reports⁷ that have shown PD-L1 positive in tumor cells and infiltrating lymphocytes of poorly differentiated lymphocyte-rich HCC, which is consistent with our case. To better understand the tendency for PD-L1 expression in tumor cells in poorly differentiated lymphocyte-rich HCC cases, it is necessary to accumulate more cases. Considering the aforementioned details, it is likely that poorly differentiated lymphocyte-rich HCC, which was once designated as LEL-HCC, can be expected to have a good therapeutic response to ICIs, and these findings may contribute to the stratification of HCC treatment in the future.

4 | CONCLUSION

We presented a rare case of poorly differentiated lymphocyte-rich HCC, which may be present in HCCs showing typical imaging findings. For treatment of advanced cases, ICIs may be effective, and it was considered necessary to confirm pathological findings when selecting treatment.

ACKNOWLEDGEMENTS

All authors would like to thank the patient and his family for allowing this case study.

CONFLICT OF INTEREST

The authors have no conflict of interests.

AUTHOR CONTRIBUTIONS

GO, TO, and KM collected and analyzed data and wrote and edited the manuscript. KH, ST, HO, KT, KM, and JK involved in the patient's care. MN, YK, and IT were abdominal surgeons providing hepatectomy. YK and TT were pathologists providing diagnosis.

ETHICAL APPROVAL

This study complied with the standards of the Declaration of Helsinki and the current ethical guidelines.

DATA AVAILABILITY STATEMENT

Data sharing not applicable to this article as no datasets were generated or analysed during the current study.

ORCID

Ginji Omori  <https://orcid.org/0000-0002-4101-9002>

Koji Miyanishi  <https://orcid.org/0000-0002-6466-3458>

Junji Kato  <https://orcid.org/0000-0002-7367-8344>

REFERENCES

1. Bosman FT, Carneiro F, Hruban RH, Theise ND. *WHO Classification of Tumours of the Digestive System*. 4th edn. World Health Organization; 2010.
2. Classification of Tumours Editorial Board. *WHO Classification of Tumours of the Digestive System*. 5th edn. World Health Organization; 2019.
3. Applebaum EL, Mantravadi P, Haas R. Lymphoepithelioma of the nasopharynx. *Laryngoscope*. 1982;92:510-514.

4. Chan AWH, Tong JHM, Pan Y, et al. Lymphoepithelioma-like hepatocellular carcinoma. *Am J Surg Pathol*. 2015;39(3):304-312. <https://doi.org/10.1097/pas.0000000000000376>
5. Iezzoni JC, Gaffey MJ, Weiss LM. The role of epstein-barr virus in lymphoepithelioma-like carcinomas. *Am J Clin Pathol*. 1995;103:308-315.
6. Labgaa I, Stueck A, Ward SC. Lymphoepithelioma-like carcinoma in liver. *Am J Pathol*. 2017;187:1438-1444.
7. Putra J, Anderson TA, Roayaie S, Maeda M, Thung SN. Metastatic lymphoepithelioma-like hepatocellular carcinoma: a potential diagnostic pitfall and demonstration of Pd-11 expression. *Ann Hepatol*. 2017;16:157-159.
8. Shirabe K, Matsumata T, Maeda T, Sadanaga N, Kuwano H, Sugimachi K. A long-term surviving patient with hepatocellular carcinoma including lymphocytes infiltration - a clinicopathological study. *Hepatogastroenterology*. 1995;42:996-1001.
9. Wada Y, Nakashima O, Kutami R, Yamamoto O, Kojiro M. Clinicopathological study on hepatocellular carcinoma with lymphocytic infiltration. *Hepatology*. 1998;27:407-414.
10. Emile JF, Adam R, Sebah M, et al. Hepatocellular carcinoma with lymphoid stroma: a tumour with good prognosis after liver transplantation. *Histopathology*. 2000;37:523-529.
11. Si MW, Thorson JA, Lauwers GY, DalCin P, Furman J. Hepatocellular lymphoepithelioma-like carcinoma associated with epstein barr virus: a hitherto unrecognized entity. *Diagn Mol Pathol*. 2004;13:183-189.
12. Chen CJ, Bin JL, Huang SF. Lymphoepithelioma-like hepatocellular carcinoma. *Chang Gung Med J*. 2007;30:172-177.
13. Nemolato S, Fanni D, Naccarato AG, Ravarino A, Bevilacqua G, Faa G. Lymphoepithelioma-like hepatocellular carcinoma: a case report and a review of the literature. *World J Gastroenterol*. 2008;14:4694-4696.
14. Park HS, Jang KY, Kim YK, Cho BH, Moon WS. Hepatocellular carcinoma with massive lymphoid infiltration: a regressing phenomenon? *Pathol Res Pract*. 2009;205:648-652.
15. Shinoda M, Kadota Y, Tsujikawa H, et al. Lymphoepithelioma-like hepatocellular carcinoma: a case report and a review of the literature. *World J Surg Oncol*. 2013;11:97.
16. Patel KR, Liu TC, Vaccharajani N, Chapman WC, Brunt EM. Characterization of inflammatory (lymphoepithelioma-like) hepatocellular carcinoma: a study of 8 cases. *Arch Pathol Lab Med*. 2014;138:1193-1202.
17. An SL, Liu LG, Zheng YL, et al. Primary lymphoepithelioma-like hepatocellular carcinoma: report of a locally advanced case and review of literature. *Int J Clin Exp Pathol*. 2015;8:3282-3287.
18. Chan AWH, Tong JHM, Pan Y, et al. Lymphoepithelioma-like hepatocellular carcinoma: an uncommon variant of hepatocellular carcinoma with favorable outcome. *Am J Surg Pathol*. 2015;39:304-312.
19. Insilla AC, Faviana P, Pollina LE, et al. Lymphoepithelioma-like hepatocellular carcinoma: case report and review of the literature. *World J Gastroenterol*. 2015;21:10468-10474.
20. Wei J, Liu Q, Wang C, Yu S. Lymphoepithelioma-like hepatocellular carcinoma without epstein-barr virus infection: a case report and a review of the literature. *Indian J Pathol Microbiol*. 2015;58:550-553.
21. Miyasaka C, Ishida M, Ito H, Kaibori M, Uemura Y, Tsuta K. Lymphoepithelioma-like hepatocellular carcinoma: a case report with emphasis on the cytological features. *Int J Clin Exp Pathol*. 2017;10:7893-7897.
22. Wang JK, Jin YW, Hu HJ, et al. Lymphoepithelioma-like hepatocellular carcinoma. A case report and brief review of literature. *Medicine*. 2017;96:e9416.
23. Shih EJ, Chau IY, Yeh YC, Chau GY. Synchronous hepatocellular carcinoma and lymphoepithelioma-like carcinoma arising from 2 different sites of the liver: a case report. *Medicine*. 2018;97:e12548.
24. Filotico M, Moretti V, Floccari F, D'Amuri A. Very rare liver neoplasm: lymphoepithelioma-like (LEL) hepatocellular carcinoma. *Case Rep Pathol*. 2018;2018:2651716.
25. Hermel DJ, Du EZ, Lin R, Frenette CT, Sigal DS. Checkpoint inhibition in the treatment of unresectable, advanced lymphoepithelioma-like hepatocellular carcinoma. *J Clin Transl Hepatol*. 2021;9:265-268.
26. Finn RS, Qin S, Ikeda M, et al. Atezolizumab plus bevacizumab in unresectable hepatocellular carcinoma. *N Engl J Med*. 2020;382:1894-1905.
27. Chan AWH, Zhang Z, Chong CCN, Tin EKY, Chow C, Wong N. Genomic landscape of lymphoepithelioma-like hepatocellular carcinoma. *J Pathol*. 2019;249:166-172.
28. Kim C, Rajan A, DeBrito PA, Giaccone G. Metastatic lymphoepithelioma-like carcinoma of the lung treated with nivolumab: a case report and focused review of literature. *Transl Lung Cancer Res*. 2016;5:720-726.
29. Kumar V, Dave V, Harris J, Huang Y. Response of advanced stage recurrent lymphoepithelioma-like carcinoma to nivolumab. *Immunotherapy*. 2017;9:955-961.
30. Calderaro J, Rousseau B, Amaddeo G, et al. Programmed death ligand 1 expression in hepatocellular carcinoma: relationship with clinical and pathological features. *Hepatology*. 2016;64:2038-2046.
31. Hartley GP, Chow L, Ammons DT, Wheat WH, Dow SW. Programmed cell death ligand 1 (PD-L1) signaling regulates macrophage proliferation and activation. *Cancer Immunol Res*. 2018;6:1260-1273.

How to cite this article: Omori G, Osuga T, Miyanishi K, et al. Programmed cell death ligand 1 expression in a case of poorly differentiated lymphocyte-rich hepatocellular carcinoma. *Clin Case Rep*. 2021;9:e04764. <https://doi.org/10.1002/ccr3.4764>