

Original Article

Clinicopathological features of primary hepatic diffuse large B-cell lymphoma: a report of seven cases and a literature review

Jinfeng Zheng¹, Yiliang Hou², Ruting Zhou¹, Dingrong Zhong³

¹Department of Pathology, The General Hospital, Jinan Military Command, Jinan 250031, China; ²Department of Pathology, The Center Hospital of Feicheng Mineral Group Company, Feicheng 271608, China; ³Department of Pathology, Beijing Union Medical College Hospital, Beijing 100005, China

Received August 20, 2015; Accepted September 24, 2015; Epub October 1, 2015; Published October 15, 2015

Abstract: We studied the imaging and histopathological features of primary hepatic diffuse large B-cell lymphoma in order to explore the clinicopathological features, diagnosis, differential diagnoses, and treatment. Immunolabelling was performed in seven cases of primary hepatic diffuse large B-cell lymphoma using histological and immunohistochemical techniques. The clinical manifestations; imaging, histopathological, and immunohistochemical features; treatment; and prognosis of primary hepatic diffuse large B-cell lymphoma were observed and analyzed in light of the relevant literature. The average age of the seven patients was 63.4 years. Moreover, bulge of the upper right abdomen and progressive anorexia and anemia were observed in all seven patients. Computed tomography (CT) revealed the presence of multiple solid hypodense lesions. Further, CT also revealed an enhanced irregular focus. Histopathological analysis revealed the following characteristics: heavy infiltration composed mainly of medium-sized round cells with a lightly stained cytoplasm, prominent nucleoli and vesicular nuclei, nuclear fission and visible sky star phenomena. The tumor cells showed diffuse expression of CD19, CD20, and CD79a, with the percentage of Ki67-positive cells being 75%-80%. All these findings indicated that primary hepatic diffuse large B-cell lymphoma is rare and generally has a poor prognosis. Biopsy and immunohistochemical staining are helpful in its diagnosis. Further, the differential diagnoses include secondary liver diffuse large B-cell lymphoma, low/undifferentiated cancer of the liver, hepatoblastoma, leukemia of the liver, and other tumors. Early surgery and chemotherapy can have a good curative effect.

Keywords: Liver tumor, B-cell lymphoma, clinical pathological features, immunohistochemistry, differential diagnosis

Introduction

Diffuse large B cell lymphoma is a fairly common highly malignant type of non-Hodgkin's lymphoma that develops in the lymph nodes. Primary hepatic diffuse large B cell lymphoma (PHDLBL) is rare, and accounts for 0.016% of all non-Hodgkin's lymphomas [1]. Because the clinical features are not very specific, PHDLBL can easily be misdiagnosed as liver inflammatory disease, benign tumor, or liver cancer. The present study describes the imaging, histopathological, and immunohistochemical features of seven cases of PHDLBL. The imaging characteristics, clinical and pathological features, diagnosis and differential diagnosis, and

treatment and prognosis are discussed in light of other reported cases of PHDLBL.

Materials and methods

Clinical data

We reviewed the cases of seven male patients with PHDLBL admitted to the General Hospital of Jinan Military Area (four cases, 2009-2014) and Peking Union Medical College Hospital (three cases, 2001-2014). The patients were 55-73 years old, and the average age was 63.4 years. In four cases, the tumors were located in the left lobe; in two cases, they were located in the right liver lobe; and in one case, it was locat-

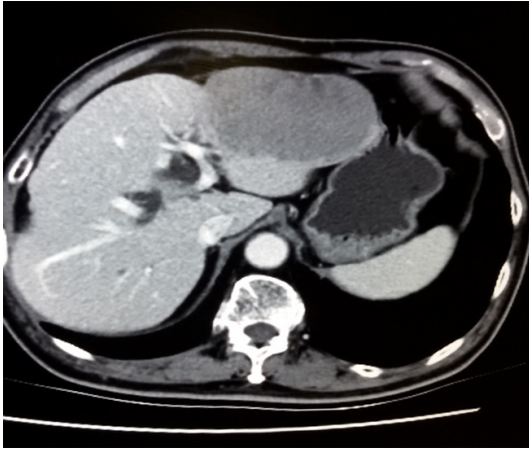


Figure 1. Abdominal computed tomography (CT) scans of a patient with hepatic diffuse large B-cell lymphoma shows an enhanced irregular focus.

ed in the caudate lobe of the liver. In one case, the tumor had invaded the gallbladder. In all the patients, the upper right abdomen displayed a bulge. Further, three of the patients had fever and jaundice, and two patients had common bile duct calculi. Lymph node enlargement was not observed in any of the patients. Computed tomography (CT) revealed diffuse space-occupying nodules in the liver, with a cross-sectional area of 2.5×1.5 cm to 12.5×10.4 cm, and multiple solid hypodense lesions. CT also revealed an enhanced irregular focus (**Figure 1**). There was no evidence of abdominal lymph node enlargement. Laboratory examination showed peripheral blood anemia with a reduced platelet count. Liver function test revealed higher than normal levels of alkaline phosphatase. Two patients were positive for hepatitis B virus, and one patient was positive for hepatitis C virus. The serum α -fetoprotein (AFP) and carcinoembryonic antigen (CEA) levels were normal. The preliminary clinical diagnosis was primary liver cancer in four cases, primary liver cancer with common bile duct calculi in two cases, and hepatic adenoma and cirrhosis in one case. Five of the patients underwent surgery, and two of the patients underwent liver biopsy.

Histological analysis and immunohistochemistry

The specimens used for examination were fixed in 4% formaldehyde. Conventional dehydration, paraffin embedding, sectioning, hematoxylin and eosin (H&E) staining, and light microscopic

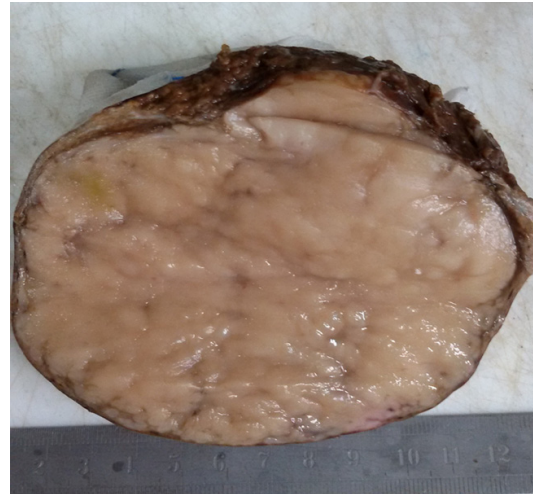


Figure 2. Gross characteristics of the hepatic diffuse large B-cell lymphoma. The grey or grey-black tumor demonstrates clear boundaries, a nodular appearance, and a soft consistency.

observation were subsequently conducted. The EnVison two-step method was used for immunohistochemical staining. Antibodies against CD19, CD20, CD79a, CD3, CD43, CD10, bcl-6, mum1 Protein (mum-1), Hepatocyte-1 (Hep-1), and Ki67 were purchased from Fuzhou Maixin Biotechnology. Diaminobenzidine (DAB) was used for staining. Positive and negative controls were also used. All the procedures were conducted in accordance with the manufacturer's instructions.

Results

Histopathological characteristics

Manual observation and measurement showed that the diameter of the nodular and polypoid tumors in five cases was 12.5 cm, 10.4 cm, 8.5 cm, 6.0 cm, and 4.5 cm. The color of the tumors was grayish black. The surface of the sections was solid and soft, and the boundary with the surrounding mucosa was clear (**Figure 2**). In one case, the tumor had invaded the wall of the gallbladder. In the other two cases of liver biopsy, the diameter of the excised tissue was 0.2 cm, and the length was 0.8-1.0 cm, and the tissue appeared pale and was soft to touch.

Microscopic examination revealed that the morphological characteristics of the tumor tissues and cells were similar in the seven cases. It was also observed that the structure of the hepatic lobule was destroyed. The tumor cells

Primary hepatic diffuse large B-cell lymphoma: clinicopathological features

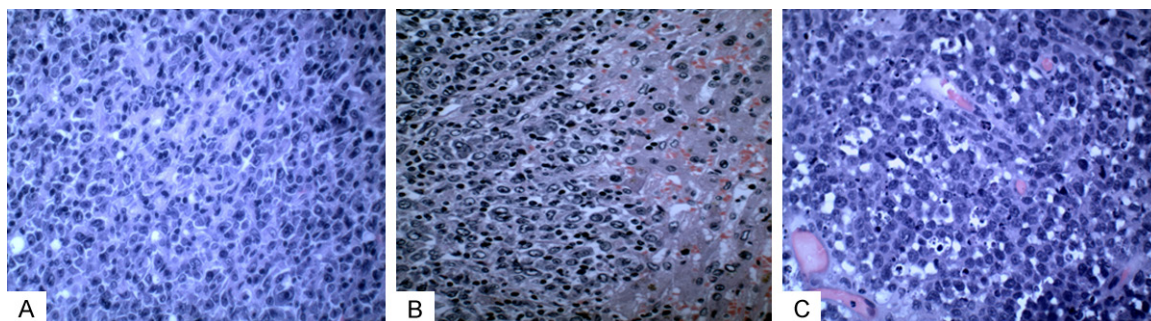


Figure 3. Histology of the hepatic diffuse large B-cell lymphoma. A. Tumor cells are large and round. The chromatin granules are thick and large (H&E, ×400). B. There are distinct boundaries between the tumor (left) and liver (right) tissues (H&E, ×400). C. Mitotic figures and starry sky phenomena are clearly visible (H&E, ×400).

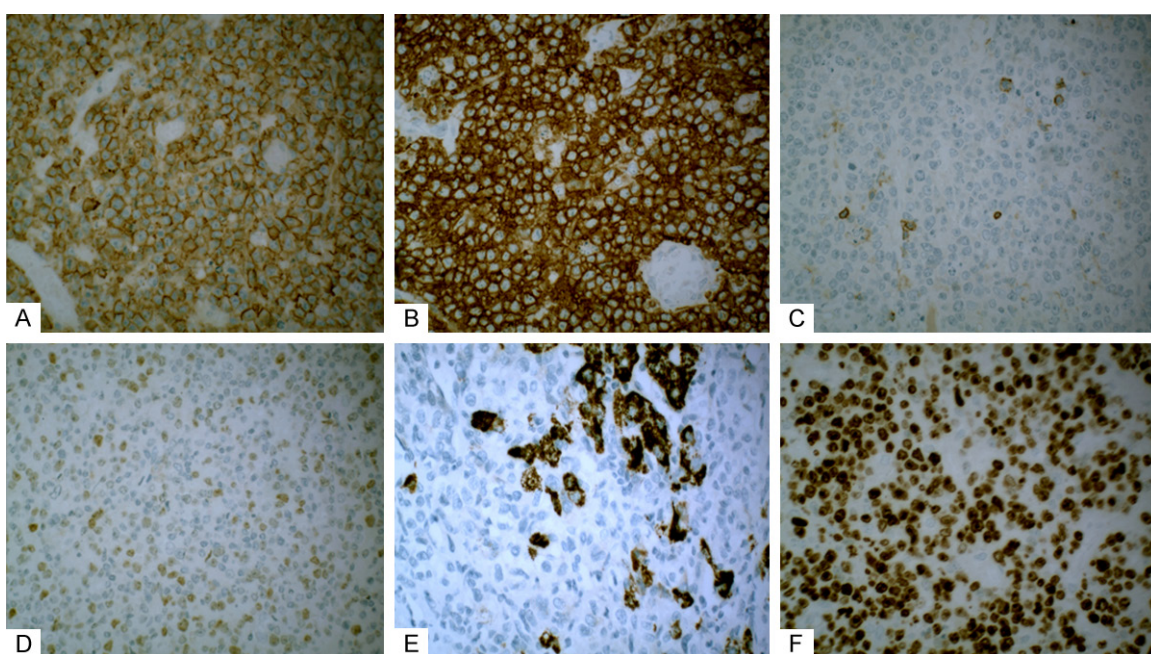


Figure 4. Immunohistochemical staining of hepatic diffuse large B-cell lymphoma according to the two steps EnVision method. A. CD20-positive tumor cells. B. Strong CD79a-positive staining in tumor cells. C. Tumor cell immunohistochemical staining is negative for CD3. D. Tumor cells that are positive for Bcl-6. E. Immunohistochemistry of hepatic cells that are positive for Hep-1. F. The Ki67 index is 75-80%.

were large and round in shape. The chromatin granules were thick and large in size; cytoplasm was found in abundance; Mitotic figures and sky star phenomena were clearly visible (**Figure 3A-C**).

Immunohistochemical staining showed that the tumor cells were positive for CD19, CD20, and CD79a, but negative for CD3, CD43 and Hep-1. The tumor cells in two cases were positive for CD10, and in two cases, they were positive for CD10 and Bcl-6. The percentage of Ki67-positive cells was 75-80% (**Figure 4A-F**).

Diagnosis

The seven patients in this study were diagnosed with PHDLBL, with tumor invasion into the gallbladder wall in one case. In four cases, the tumor was of the germinal center subtype, and in the remaining three cases, it was not of the germinal center subtype.

Discussion

PHDLBL is a type of rare non-Hodgkin's lymphoma in which the lesions are limited to the liver

Primary hepatic diffuse large B-cell lymphoma: clinicopathological features

without any sign of peripheral lymphadenopathy. The vast majority of patients with PHDLBL are elderly men [2], who usually present with abdominal pain, fever, jaundice, splenic enlargement and space-occupying lesions within the liver. PHDLBL has non-specific clinical manifestations, and the rate of clinical misdiagnosis is high. Some cases showed lymphatic metastasis into the surrounding liver tissue and bone marrow involvement. Peripheral lymphadenopathy was not found. CT showed diffuse space-occupying nodules in the liver. The cause of PHDLBL is not entirely clear, but it may be related to viral hepatitis, liver cirrhosis, or the use of immunosuppressant drugs [3, 4]. Subsequent magnetic resonance imaging (MRI) usually shows diffuse hypointense lesions on T1-weighted images and hyperintense lesions on T2-weighted images. Presence of lymphoma in the liver biopsy sample and absence of lymphoproliferative disease outside the liver are considered to indicate PHDLBL. Therefore, liver biopsy or rapid intraoperative pathological examination is of great significance for the diagnosis and differential diagnosis of PHDLBL.

The major histological features of PHDLBL were wide destruction of the hepatic lobule structure with diffuse infiltration of medium to large heterotypic lymphocytes. The tumor cells mainly showed infiltration along the liver sinus and damage to the liver cells. The cytoplasm was stained and abundant in the tumor cells; further, the nuclei were rounded and slightly irregular, with visible vesicular nuclei, nucleoli, and fission. The tumor cells were positive for CD19, CD20, and CD79a, and the germinal center subtype tumors were positive for CD10 and Bcl-6. Tumors that were not of the germinal center subtype were negative for CD10 and Bcl-6, but they were either positive or negative for Mum-1. In addition, 10% of the cases were positive for CD5 positive, and most of the CD5-positive cases were of primary DLBCL.

Due to the lack of specific clinical manifestations and imaging features of PHDLBL, histological analysis of the biopsy sample is important for its diagnosis. If the patients were found to have hepatic space-occupying lesions on imaging, they underwent liver biopsy under B ultrasound or CT guidance. Immunohistochemical staining and flow cytometric analysis were also used to confirm primary non-

Hodgkin's lymphoma. However, because the liver biopsy specimens were very small, the morphological features of the tissue were not clear. Therefore, it is easy to misdiagnose liver lymphoma as liver inflammatory disease or other diseases. It is also important to differentiate PHDLBL from secondary liver diffuse large B cell lymphoma, low/undifferentiated cancer of the liver, hepatoblastoma, leukemia of the liver, and other tumors [5, 6].

In PHDLBL, the tumor boundary in the liver can be distinctly seen. However, secondary liver diffuse large B cell lymphoma is often characterized by hepatic masses with focal or diffuse infiltration, and the main histological feature is the presence of a considerable number of large heterotypic lymphocytes along with small lymphocytes and histiocytes. The tumor cells are mainly found to infiltrate the portal area of the liver.

In the case of low/undifferentiated cancer of the liver, the tumor cells are not large. Although this tumor shares certain characteristics with PHDLBL, immunohistochemical studies show that it is strongly or weakly positive for cytokeratin (CK), positive for Hep-1 in some cases, and negative for CD20 and CD79a. These immunohistochemical features can be used for differentiating it from PHDLBL.

Hepatoblastoma is common in infants and young children, and occasionally occurs in older children and adults. The microscopic features are mainly immature liver cells with or without mesenchymal components. Further, immunohistochemical analysis has shown that hepatoblastomas are positive for EMA, AFP, and HCG; locally positive for CK; and negative for CD20 and CD79a. These findings can be used for differentiating hepatoblastomas from PHDLBL.

Leukemia and other tumors of the liver result in an increase in liver volume with diffused tumor or nodular tumor formation. Further, there is no obvious damage to the hepatic lobules on microscopic examination, and interstitial leukemia cells can be seen occupying the blood sinus. In addition, bone marrow biopsy examination typically shows abnormal lymphocyte hyperplasia.

Surgical treatment, radiotherapy, and chemotherapy have been used alone or in combina-

tion for the treatment for PHDLBL. For solitary liver lesions, complete surgical resection is the first choice of treatment, and it is followed by chemotherapy as the next choice of treatment. Surgical resection only results in an average survival of 22 months. Therefore, simple surgical treatment cannot be used to achieve complete remission in all cases. It has been reported that in addition to surgery, radiofrequency ablation in combination with chemotherapy may have considerably better effects than surgery alone [7, 8]. The traditional chemotherapy regimen used is CHOP (cyclophosphamide, doxorubicin, vincristine, and prednisone), which is an anthracycline-based regimen. Rituxan is a monoclonal antibody that acts against the CD20 antigen, which was the first proven safe and effective treatment against B cell lymphoma. The effect of the R-CHOP protocol is better than that of the CHOP protocol [9, 10]. Emile et al. reported that the prognosis of nodular lymphoma cells in PHDLBL is better than that of diffuse infiltrates [11]. The 1-year and 3-year survival rates of nodular infiltrates of PHDLBL were 70% and 38% respectively, and the rates of diffuse infiltration were 57% and 18% respectively. Among the seven patients, two underwent postoperative R-CHOP chemotherapy and achieved complete remission with follow-up for 10 months and 13 months. Two of the patients were treated with postoperative CHOP for 4 weeks and were stable thereafter; they were followed up for 8 months. Two patients died—one as a result of tumor progression and one as a result of lung infection—after 6 months and 8 months respectively; these two patients did not undergo chemotherapy. In one case, the patient was elderly and refused to undergo chemotherapy; he remained under close clinical follow-up.

In conclusion, PHDLBL is a rare disease that lacks specific imaging and clinical manifestations and biochemical indicators. Its diagnosis is difficult, as lymphoma in other organs or tissues outside of the liver needs to be excluded. When multiple space-occupying lesions are found in the liver but invasion into other organs or tissues is not observed, PHDLBL should be considered as a possible diagnosis and liver biopsy should be performed. If PHDLBL is diagnosed, chemotherapy should be started immediately, and the patient should be closely monitored for adverse reactions and complications.

Acknowledgements

The authors thank the highly qualified editors at American Journal Experts for editing the present paper for proper English language, grammar, punctuation, spelling, and overall style. This study was supported by the President Fund from the General Hospital, Jinan Military Command, China (Grant No.2011M03).

Disclosure of conflict of interest

None.

Address correspondence to: Dr. Dingrong Zhong, Department of Pathology, Beijing Union Medical College Hospital, 41 Big Wooden Warehouse Hutong, Beijing 100005, China. Tel: +86-010 69159370; Fax: +86-010-69159370; E-mail: zhongdingrong@sina.com

References

- [1] Noronha V, Shafi NQ, Obando JA, Kummar S. Primary nonHodgkin's lymphoma of the liver. *Crit Rev Oncol Hematol* 2005; 53: 199-207.
- [2] Ma YJ, Chen EQ, Wang J, Tang H. Progress in research of primary hepatic lymphoma. *World Chinese Journal of Digestology* 2010; 18: 2790-293.
- [3] Bronowicki JP, Bineau C, Feugier P, Hermine O, Brousse N, Oberti F, Rousselet MC, Dharancy S, Gaulard P, Flejou JF, Cazals-Hatem D, Labouyrie E. Primary lymphoma of the liver: clinical-pathological features and relationship with HCV infection in French patients. *Hepatology* 2003; 37: 781-787.
- [4] De Renzo A, Perna F, Persico M, Notaro R, Mainolfi C, de Sio I, Ciancia G, Picardi M, Del Vecchio L, Pane F, Rotoli B. Excellent prognosis and prevalence of HCV infection of primary hepatic and splenic non-Hodgkin's lymphoma. *Eur J Haematol* 2008; 81: 51-57.
- [5] Jaffe ES, Harris NL, Stein H, Vardiman JW. *World Health Organization Classification of tumours pathology and genetics tumours of haematopoietic and lymphoid tissues*. Lyon: IARC Press; 2001. pp. 171-194.
- [6] Harris AC, Ben-Ezra JM, Contos MJ, Kornstein MJ. Malignant lymphoma can present as hepatobiliary disease. *Cancer* 1996; 78: 2011-2019.
- [7] Ng AK. Diffuse large B-cell lymphoma. *Semin Radiat Oncol* 2007; 17: 169-175.
- [8] Leung VK, Lin SY, Loke TK, Chau TN, Leung CY, Fung TP, Lam SH. Primary hepatic peripheral T-cell lymphoma in a patient with chronic hepatitis B infection. *Hong Kong Med J* 2009; 15: 288-290.

Primary hepatic diffuse large B-cell lymphoma: clinicopathological features

- [9] Hamada M, Tanaka Y, Kobayashi Y, Takeshita E, Joko K. A case of MALT lymphoma of the liver treated by RFA and Rituximab. *Nihon Shokakibyō Gakkai Zasshi* 2006; 103: 655-660.
- [10] Ma YJ, Chen EQ, Chen XB, Wang J, Tang H. Primary hepatic diffuse large B cell lymphoma: a case report. *Hepat Mon* 2011; 11: 203-205.
- [11] Emile JF, Azoulay D, Gornet JM, Lopes G, Delvart V, Samuel D, Reynès M, Bismuth H, Goldwasser F. Primary non-Hodgkin's lymphomas of the liver with nodular and diffuse infiltration patterns have different prognoses. *Ann Oncol* 2001; 12: 1005-1010.
- [12] Salmon JS, Thompson MA, Arildsen RC, Greer JP. Non-Hodgkin lymphoma involving the liver: clinical and therapeutic considerations. *Clin Lymphoma Myeloma* 2006; 6: 273-280.
- [13] Noronha V, Shafi NQ, Obando JA, Kummar S. Primary non-Hodgkin's lymphoma of the liver. *Crit Rev Oncol Hematol* 2005; 53: 199-207.