Brief Report

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The value of contrast-enhance ultrasound in the diagnosis of hepatic post-transplant lymphoproliferative disease: Four case reports

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SUMMARY Post-transplant lymphoproliferative disease (PTLD) is a rare but life-threatening disease that occurs after organ transplantation. Histopathology is the gold standard for the diagnosis of PTLD. Because of its rarity and atypical symptoms, many patients are misdiagnosed with liver abscess, liver cancer, or missed diagnosis long before pathological diagnosis is obtained, thus delaying treatment. Early and accurate diagnosis, in addition to histopathological examination, is difficult. Contrast-enhanced ultrasound (CEUS) imaging techniques have overwhelming advantages of being safe (noninvasive, radiation-free) and sensitive for evaluating the microcirculation of lesions, thus making them widely used in the diagnosis of hepatic lesions. Unfortunately, there are few reports of CEUS data on hepatic PTLD (HPTLD). This study reported and analyzed four cases of HPTLD in detail, all of which underwent pre-biopsy CEUS examinations and had a complete diagnosis and treatment process. By offering readers comprehensive knowledge of CEUS in the diagnosis of HPTLD, our study aims to help reduce misdiagnoses and missed diagnoses, thereby improving patient treatment and prognosis.

Keywords post-transplant lymphoproliferative disease, transplant liver, contrast-enhanced ultrasound, diagnosis, image

1. Introduction

Post-transplant lymphoproliferative disease (PTLD) develops in only 1–3 % of liver transplant recipients (*1*). However, as PTLD shows characteristics of high malignancy and mortality rates, early diagnosis is expected to be life-saving (*2*). Contrast-enhanced ultrasound (CEUS) has the advantages of non-radiation, non-invasiveness, cost-effectiveness, portability, good repeatability at frequent intervals, and few side effects (*3*). As CEUS has high accuracy in the detection and diagnosis of hepatic lesions, it is recommended as a first-line examination for high-risk populations of HCC according to the Chinese guidelines (*4*) and World Federation for Ultrasound in Medicine & Biology (WFUMB) guidelines (*5*). In particular, microbubbles of CEUS do not metabolize through the liver; therefore, CEUS (even if used continuously and cumulatively) has no hepatotoxicity. From the perspective of liver preservation, CEUS is safer than contrast-enhanced computed tomography (CT) and magnetic resonance (MR) imaging for follow-up of patients undergoing liver transplantation. CEUS should be used as the first-line imaging modality to screen patients for post-orthotopic liver transplantation complications (*6*).

Currently, there are no English articles describing the imaging features of CEUS for hepatic PTLD (HPTLD) after transplant liver. Research, even with a small-sized case report, is important as it provides a reference for the CEUS diagnosis of HPTLD. To date, 1,300 cases of adult liver transplantations have been performed at the First Affiliated Hospital of Xi'an Jiaotong University, ranking among the leading positions in China (*7*). With abundant sources of liver transplantation cases at hand, we searched for HPTLD cases that had undergone CEUS examinations over the past seven years (when CEUS technology was introduced as a routine clinical examination for patients). We hope that this research can provide valuable information and inspiration for doctors/scholars studying PTLD from a pre-operative imaging perspective.

2. Patients and Methods

2.1. Clinical data

Patient data were retrospectively collected from the First Affiliated Hospital of Xi'an Jiaotong University, China. The research period was from January 2017 (Since CEUS began to be performed in our hospital) to June 2024, based on the pathologic diagnoses of HPTLD by searching the hospital's electronic pathology reporting system. From this period, only four patients had a definite histopathological diagnosis of PTLD and underwent pre-biopsy CEUS examinations. We retrospectively collected their general clinical data (sex, age, etiology, medical history, and treatment process, especially the regimen of using immunosuppressants after transplantation), as well as data on laboratory examinations (tumor markers, liver function, *etc*.) from the electronic medical record system.

Written consent was obtained from the selected patients or their immediate families for the publication of this case report and any accompanying images. All data collection and diagnostic and therapeutic procedures were performed in accordance with the principles of the Declaration of Helsinki. This study was approved by the Research Ethics Committee of the First Affiliated Hospital of Xi'an Jiaotong University (XJTU1AF2023LSK-363) on June 6, 2023.

2.2. Grayscale ultrasound (US) and CEUS examination

A LOGIQ E9 US system (GE Healthcare, Milwaukee, WI, USA), equipped with native tissue harmonic grayscale imaging and CEUS functions, was used. Convex and microconvex transducers with frequencies of 1–6 and 2–5 MHz, respectively, were used. A 2.0–2.5 mL dose of sulfur hexafluoride microbubbles (SonoVue, Bracco, Milan, Italy) was injected into the antecubital vein at 0.2 mL/s *via* a 20-gauge cannula, followed by 5 mL of 0.9% sterile sodium chloride solution. CEUS images were acquired during three contrast phases: arterial phase (AP) (10–20 s to 30–50 s after contrast injection), portal venous phase (PP) (30–50 s to 120 s), and delayed phase (DP) (>120 s, until bubble disappearance).

2.3. Histological diagnosis

Shortly after the CEUS examination, all four patients underwent US-guided percutaneous transhepatic needle biopsy. Hematoxylin-eosin (HE) staining and immunohistochemical examination with cluster of differentiation (CD)19, CD20, and Epstein–Barr virus-encoding region in situ hybridization (EBER-ISH) were performed. Pathologists with more than 10 years of experience in liver pathology reviewed all specimen slices.

3. Results and Discussion

3.1. Clinical data

Baseline patient characteristics, treatment, and prognosis are summarized in Figure 1. All four patients with HPTLD were middle-aged and had no gender differences. All the patients underwent classic orthotopic liver transplantation for cirrhosis. They all have preexisting conditions, such as viral hepatitis or alcohol use, which precipitate end-stage liver disease and are reported to be non-negligible incentives for PTLD (*8*). After liver transplantation, all four patients were treated with continuous immunosuppressive therapy.

The medication regimens are displayed in Supplemental Table S1 (*https://www.irdrjournal.com/action/getSupplementalData.php?ID=213*). The lymph system is widely known as a part of the immune system, which explains why immunosuppressive therapy is widely recognized as a critical cause of PTLD. Whole blood tacrolimus (FK506) concentrations were measured regularly to maintain the level between 50 and 80 ng/mL. All patients had persistent fever of unknown origin (body temperature > 39°C) after liver transplantation. The quantitative DNA tests of Epstein-Barr virus (EBV) and cytomegalovirus (CMV) in these patients before liver transplantation were within normal limits. However, EBV infection was confirmed by increased levels of IgG antibodies of NA and VCA against EBV, which are serologic tests most often used for EBV serostatus assignment (*9*). However, except that patients No. 1 and 4 had a high level of EBV DNA quantification (1.01×10^4 copies and 3.17×10^4 copies, respectively), DNA quantification of EBV and Cytomegalovirus (CMV) for two other patients was within the normal range. CMV-IgG was positive in all four patients. A thorough laboratory workup on admission showed that the routine blood, urine, feces, serum glucose levels, electrolytes, liver function, and kidney function were all within normal ranges. Tumor markers, including alpha-fetoprotein, carcinoembryonic antigen, protein induced by vitamin K absence or antagonist-II, and carbohydrate antigens 19-9 were also unremarkable.

Patient 1 had better prognosis. This finding suggests that if HPTLD occurs late, therapy begins when the lesion is small, and chemotherapy is repeated regularly, the patient would enjoy favorable survival. In this setting, an accurate diagnosis at the early onset of HPTLD is of crucial importance.

3.2. Grayscale US and CEUS examination

Three patients had solitary lesions in the hilar part of the liver, whereas one patient had multiple lesions in both lobes of the liver. As US is the first-line routine follow-up examination for patients after liver transplantation, all HPTLD lesions were first detected using US. Early

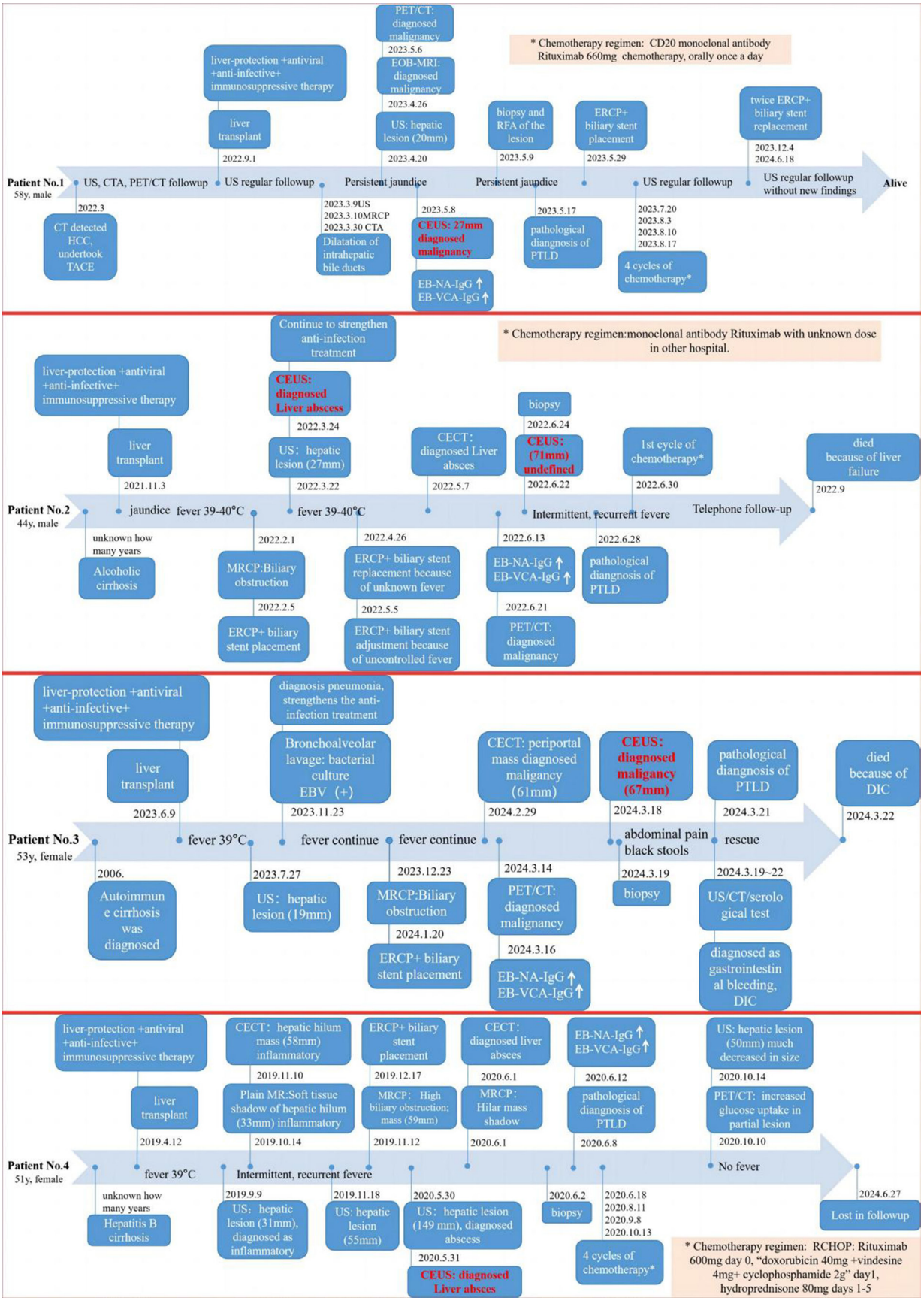

Figure 1. Time-line of diagnosis and treatment of the four patients. Abbreviations PTLD, hepatic post-transplant lymphoproliferative disease; EB(V), Epstein-Barr virus; CMV, Cytomegalovirus; US, ultrasound; CEUS, contrast-enhanced US; CECT, contrast-enhanced computed tomography; MR, magnetic resonance; MRCP, magnetic resonance cholangiopancreatography; ERCP, endoscopic retrograde cholangiopancreatography; PET, positron emission tomography; DIC, disseminated intravascular coagulation; NA, nucleoantigen; VCA, capsid antigen.

lesions usually occur within one year of transplantation, and the first year post-transplant is a risk factor for PTLD (*10*). Consistently, all HPTLD lesions in our study were first detected within one year. From the difference between the size when first discovered and the size when CEUS was performed, it can be seen that the lesions grew rapidly in a short period of time.

The diagnosis of PTLD based on radiological studies, especially at the early stage of the disease course, is thought to be challenging. Positron emission tomography (PET)/CT is recommended for PTLD staging according to guidelines and expert consensus (*9,11*). However, perhaps because PTLD is rare and poorly understood, the best imaging modality for diagnosing it has not yet been determined. Four patients in our study underwent imaging examinations including endoscopic retrograde cholangiopancreatography (ERCP), magnetic resonance cholangiopancreatography (MRCP), CECT, PET/CT, and CEUS. However, PET/CT is radioactive, while CECT and MRI have the allergic and nephrotoxic potential of the employed contrast materials (*12*). Even worse, none of the patients in this study made a diagnosis, or even a suspected diagnosis, of PTLD.

Encouragingly, there were some common US and CEUS features in these four cases. As seen in Table 1 and Supplemental Figure S1–S4 (*https://www.irdrjournal.com/action/getSupplementalData.php?ID=213*), all these lesions displayed similar appearances as inhomogeneous hypoechic/extremely hypoechoic with clear boundaries and irregular shapes in the US images. The appearance of hypoechic/extremely hypoechoic lesions can be explained by the histopathological features of PTLD. The lesion is characterized by high cellularity and single-cell composition (mature medium-large B cells). Therefore, the difference in US acoustic impedance was small (*13*). All lesions grew in hilar areas. Owing to the rapid growth and volume of the lesions, they gradually develop to closely surround and even compress the hilar biliary tract and blood vessels. This is the reason why patients 1 and 2 presented with jaundice, and patient 4 had severe life-threatening post-puncture bleeding complications.

As shown in Table 2 and Supplemental Figure S1–S4 (*https://www.irdrjournal.com/action/getSupplementalData.php?ID=213*), on CEUS, the mass was enhanced earlier than that in the surrounding tissues. In contrast to hepatocellular carcinoma or hepatic hemangioma, there are intratumoral perfusion defects in AP that are independent of lesion size; in other words, even small lesions (No.1 patient and the first CEUS of No. 2 patient) have perfusion defects. The clearance of contrast microbubbles was rapid and occurred during the late AP phase. These characteristics may play a role in the diagnosis of common hepatocellular carcinoma and other benign lesions. There was no significant change in the lesion range after CEUS compared with grayscale US, which was different from that of inflammatory/abscess lesions. In DP, the contrast with the surrounding

Table 1. US features of four enrolled HPTLD lesions

Patient No.	Size (mm)*	Time interval (US first detection~transplantation)	Location	Intrahepatic biliary tract dilatation	Numbers of lesions	Echogenicity	Tumor border	Homogeneity
No. 1	20	7.6 months	S4	Yes	Solitary	Hypoechoic with peritumoral "halo" sign	Ill-defined	Heterogeneous
No. 2	27	4.6 months	S4	Yes	Solitary	Hypoechioc with internal scattered hyperechoic	same**	same**
No. 3	19	1.6 months	S4	Yes	Solitary	Extremely-hypoechoic	same**	same**
No. 4	31	4.9 months	/	Yes	Multiple	Hypoechioc with internal scattered hyperechoic	same**	same**

*The size measured when first detected by US. **This index is exactly the same as the above row of the same column. HPTLD, hepatic post-transplant lymphoproliferative disease; US, ultrasound; S4, segmental 4.

Table 2. CEUS features of four enrolled HPTLD lesions[1]

Patient No.	Size (mm)*	Time interval (CEUS~transplantation)	Perfusion level in AP	Wash-in perfusion pattern in AP	Perfusion level in PP	Perfusion level in DP	Homogeneity	Tumor border	Vascular floating sign
No. 1	37	8.2 months	Local hyperenhancement	Peritumoral ring-like	Hypoenhanced	Hypoenhanced	Heterogeneous	Ill-defined	Absence
No. 2	71	4.6 and 7.6 months**	same***	Peritumoral ring-like +internal sparse scattered dot-like	same***	same***	same***	same***	Presence in the 2nd CEUS
No. 3	67	9.3 months	same***	same***	same***	same***	same***	same***	Presence
No. 4	149	13.7 months	same***	same***	same***	same***	same***	same***	Presence

*The size measured when undertaken CEUS examination. **This patient has taken CEUS examination twice at different time. ***This index is exactly the same as the above row of the same column. HPTLD, hepatic post-transplant lymphoproliferative disease; CEUS, contrast-enhanced ultrasound; AP, arterial phase; PP, portal venous phase; DP, delayed phase.

tissue is very strong and seems to show the sign of "black hole" as metastatic carcinoma.

Deep explored the CEUS images of four patients and reviewed the related literature, and we concluded two typical characteristics of HPTLD. The first is the wash-in perfusion pattern of the microbubbles in the AP. All lesions in the four patients showed peritumoral ring-like perfusion, as described above. Three of these exhibited internal sparse-scattered dot-like enhancement. Peritumoral ring-like enhancement is not well demarcated and has been detected in some cases of liver lymphoma. This marginal enhancement in AP was attributed to vasculitis due to involvement of the liver parenchyma adjacent to the lesion (*14*). This may explain why the lesions were misdiagnosed as inflammatory/abscess. The few areas of intratumoral enhancement in the AP are consistent with the fact that, based on cellular morphology and tissue of origin, lymphoid neoplasms are classified as "round cell neoplasia" rather than "mesenchymal neoplasia" and "epithelial neoplasia" (*15*); thus, they rarely invade the blood vessels. In agreement with our study, under the microscope, HPTLD lesions showed dense and tightly arranged large B cells and lacked vascular endothelial cells. It is worth noting that the second feature is "vascular floating sign" or "vessel penetration sign", which was reported as a specific characteristic for the diagnosis of hepatic lymphoma by CECT (*16*) or color doppler US imaging (*17*). The "vascular floating sign" is a blood vessel passing through the tumor without signs of stenosis or invasion in the blood vessel itself (*18*). The cause of the "vascular floating sign" in the images may be that extranodal lymphoma originates from the interstitium of the organs, which infiltrates and grows along the stroma (rather than the blood vessels). Therefore, the original vascular anatomical structure of the organ remains intact (*19*). In patients No. 2 (2nd CEUS), No.3, and No.4, the vessels walked through the lesion completely, naturally, and without invasion. The reason why the lesions of No.1 patient and the 1st CEUS for No.2 patient did not manifest this phenomenon might be that the lesions were at an early stage and had a relatively small size (patient 1:37 mm, patient 2:27 mm). At that time, the lesion had not grown to the extent surrounding the blood vessels.

We regrettably found that there is still some appearance of CEUS for our HPTLD that cannot be well explained. The first is the anechoic "halo" sign surrounding the lesion in No. 1 case, which has not been reported in any published studies. Secondly, compared with the surrounding liver tissue, all these four cases exhibited the rapid "wash in" and "wash-out" phenomenon, even though perfusion was sparse. This "rapid wash-in and wash-out" phenomenon is usually explained by the low resistance of blood flow and tumor neovascularization (*20*). However, the specific reason for this phenomenon from the perspective of HPTLD formation and progression cannot be clearly explained.

3.3. Histological diagnosis

As shown in Supplemental Figure S1–S4 (*https://www.irdrjournal.com/action/getSupplementalData.php?ID=213*), hematoxylin-eosin (HE) staining showed diffuse infiltration of dysplastic lymphocytes (most medium-large B cells), with a large nucleus, scant cytoplasm, and abundant mitoses. The routine immunohistochemical markers for diagnosing a lymphocyte tumor are cytoplasmic CD19 and CD20, which appear to be focal or diffusely positive for staining of the cytoplasm of larger lymphoid cells. EBER-ISH further classifies lymphocyte tumors as a source of EBV-positive large B-cell lymphoma. Both HE staining and CD series immunohistochemistry staining only detected large and medium-sized B cells with dysplasia and relatively simple shapes, and no plasma cells, monocytes, or T cells were found. Therefore, these findings consistently support the diagnosis of PTLD with clear categories of monomorphic B cells. According to the literature, the most common biopsy proved that the histopathological type and subtype are monomorphic and diffuse large B-cell lymphomas, respectively (*21*). After solid organ transplantation, EBV-specific cytotoxic T cells may be completely lost within 6 months of transplantation, which induces the proliferation of latently infected B cells and results in PTLD.

In conclusion, we found that the four cases in this study had some common characteristics in the diagnosis of HPTLD. This is the first English study to summarize the CEUS features of HPTLD after liver transplant. The typical features of CEUS that suggest the possibility of HPTLD are "vascular floating signs" and wash-in perfusion patterns in the AP. In particular, most CEUS findings of HPTLD found in our study can be reasonably explained from the perspective of the histopathology of lymphoma development. Nevertheless, further case studies are needed to confirm whether these imaging findings are unique to the diagnosis of HPTLD. In summary, we recommend CEUS as the preferred modality for the preoperative diagnosis of HPTLD.

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Conflict of Interest: The authors have no conflicts of interest to disclose.

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