

Hepatosplenic $\gamma\delta$ T-cell lymphoma

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Received: 2004-04-24 Accepted: 2004-05-09

Abstract

AIM: To investigate the clinicopathologic characteristics, immunophenotype and TCR gene rearrangements of hepatosplenic T-cell lymphoma in eight Chinese patients.

METHODS: Eight Chinese patients with hepatosplenic $\gamma\delta$ T-cell lymphomas were studied. Hematoxylin-eosin-stained slides and clinical histories were reviewed. We also carried out immunohistochemical staining for CD3, CD4, CD8, CD20, CD43, CD56, CD79a, UCHL-1, and TCR $\gamma\delta$. Rearrangements of TCR gamma and delta chain genes were also studied.

RESULTS: The spleens were enlarged and the cut surfaces were homogeneous and red-purple in color without identifiable gross lesions or enlarged hilar lymph nodes. Histologically, lymphoma cells infiltrated the cords of Billroth and often packed the sinuses. Liver biopsy showed lymphoma cell infiltrations in the sinusoids, and three cases showed involvements of the portal tracts. Immunohistochemically lymphoma cells were positive for CD3, CD43, and CD56 in all cases. Four of eight cases were positive for CD8, and all cases were negative for CD4 (6/6). Monoclonal rearrangements of TCR γ gene were demonstrated by PCR analysis in five out of the eight cases. TCR δ gene rearrangements were detected in six out of the eight cases, which demonstrated single bands on PAGE gel, and the amplification products in two cases were confirmed by sequencing.

CONCLUSION: The clinicopathology of hepatosplenic $\gamma\delta$ T-cell lymphoma in Chinese patients is similar to what was previously reported except that the splenomegaly is not so massive, and CD8 is positive.

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Key words: Immunophenotype; Hepatosplenic T-cell

Wei SZ, Liu TH, Wang DT, Cao JL, Luo YF, Liang ZY.

Hepatosplenic $\gamma\delta$ T-cell lymphoma. *World J Gastroenterol* 2005; 11(24): 3729-3734

<http://www.wjgnet.com/1007-9327/11/3729.asp>

INTRODUCTION

Hepatosplenic $\gamma\delta$ T-cell lymphoma is a distinct clinicopathologic entity, and now recognized in the revised European-American lymphoma and World Health Organization classifications. It has uniform clinicopathologic characteristics: predominant occurrence in young male adults, hepatosplenomegaly presentation without lymphadenopathy, and peculiar sinusoidal pattern of infiltration of the spleen, liver, and bone marrow by monomorphic small to medium-sized tumor cells of TCR $\gamma\delta$ immunophenotype^[1].

There were few reports on hepatosplenic $\gamma\delta$ T-cell lymphoma in Chinese patients in the literature. In this paper, we analyzed the clinicopathologic characteristics, immunophenotype and TCR gene rearrangements of hepatosplenic T-cell lymphoma in eight Chinese patients.

MATERIALS AND METHODS

Eight cases of hepatosplenic $\gamma\delta$ T-cell lymphoma diagnosed between 2000 and 2003 were found from the files of the Department of Pathology of Peking Union Medical College Hospital (PUMCH). Spleen and liver biopsies were obtained from seven of the eight cases. Splenectomy was performed on case 3 in another hospital, and the patient was admitted to our hospital for further treatment who finally died. Accessory spleen was found in autopsy. The jurkat cell line (T-cell leukemia) was used as a positive control for the detection of TCR γ gene rearrangements.

Histopathologic studies

Pathological specimens were fixed in formalin. Hematoxylin-eosin-stained slides were prepared from paraffin blocks according to the standard protocol. Immunohistochemical staining was performed using DAKO EnVision and DAB peroxidase detection systems (Dako, Glostrup, Denmark). The antibodies to CD3, CD4, CD8, CD20, CD79a, CD43, CD56, and UCHL-1 were purchased from DAKO (Dako, Glostrup, Denmark) and TCR $\gamma\delta$ antibody from Endogen (Woburn, MA, USA). Normal human spleen tissue (ruptured spleen of a young male adult caused by car accident) was used as a positive control for TCR $\gamma\delta$ immunostaining, and target retrieval solution (pH 9.0) (Dako, Glostrup, Denmark) was used for the best demonstration.

Rearrangements of TCR gamma and delta chain genes

Three to five 10 μ m formalin-fixed and paraffin-embedded

tissue sections were prepared for each sample. After being dewaxed with xylene and ethanol, tissue pellet was digested by proteinase K. Jurkat cells (3×10^6) were collected and cell pellet was also digested by proteinase K. Each DNA sample was verified for its suitability for PCR amplification by amplifying β -actin.

PCR of TCR δ gene rearrangements was performed with the primers J δ 1 and V δ 1b that amplified a fragment of about 120 bp^[2].

Rearranged TCR γ genes was amplified using a method described by Signoretti *et al*^[3]. PCR was carried out using the primers VG1, VG2, VG9, JG12-a, and JGP12-a which described by Muche *et al*^[4]. T-cell leukemia cell line Jurkat was used as a positive control for TCR γ gene rearrangements. Ten microliters of the reaction products was analyzed by electrophoresis on 10% polyacrylamide gels, stained by ethidium bromide and viewed under UV light.

All the amplifications were carried out in a Hermocycle (HYBAID LIMITED, Middlesex, UK). Each sample was amplified at least three separate times to confirm the reproducibility of the results.

Because there was no positive control for TCR δ gene amplification, PCR products of TCR δ gene from cases 6 and 8, which were confirmed to have single target bands, were cut out and purified using a QIAEX gel extraction kit (QIAGEN Inc, Valencia, CA, USA). The purified products were then subjected to direct sequencing by an ABI377 sequencer (Perkin-Elmer Applied Biosystems, Foster City, CA, USA).

Careful measures were taken to avoid contamination during all steps of the analysis.

RESULTS

Clinical findings

The clinical features are summarized in Table 1.

Our study consisted of 6 men and 2 women with a median age of 29 years (ranging from 12 to 52 years). The ages of all the patients were under 36 years except one case whose age was 52 years.

The patients were admitted to PUMC Hospital for fever

and hepatosplenomegaly. Other features included weight loss and abdominal pain, and jaundice. Liver tests of most cases were abnormal, with elevated levels of serum γ -glutamyl-transpeptidase (γ -GT), alanine aminotransferase (ALT) and alkaline phosphatase (ALP) activities. Five cases showed elevated serum lactate dehydrogenase (LDH) levels.

As shown in Table 1, anemia was found in six cases (hemoglobin levels ranging from 79 to 107 G/L). Thrombocytopenia was present in five patients (platelet counts ranging from 49 to 90/L).

All patients had splenomegaly and hepatomegaly at the time of diagnosis. Physical examination revealed no enlarged superficial lymph node. CT scan disclosed no mediastinal or retroperitoneal lymphadenopathy except case 5, in whom mediastinal lymphadenopathy was discovered.

All the patients had no significant past history, including receiving long-term immunosuppressive therapy.

Four cases were followed-up. Three of them died within 1-6 mo after operation, and 1 case continued to receive treatment a year-and-a-half after the diagnosis.

Pathological findings

Pathological features are summarized in Table 2. The spleens were enlarged, weighing 445-1 155 g and the mean splenic weight was 675 g. The cut surfaces were homogeneous and red-purple in color without identifiable gross lesions or hilar lymph node enlargement.

Histologically, there were marked expansion of the red pulp and atrophy of the white pulp in the spleens. Lymphoma cells infiltrated the cords of Billroth and often packed the sinuses (Figures 1 and 2A). Lymphoma cells were usually of small to medium sizes with round or oval nuclei showing slightly dispersed chromatin, and scant to moderate cytoplasm. Seven of eight spleens showed erythrophagocytosis more or less (Figure 3).

Liver biopsy was performed in all the patients except case 3, whose whole liver was available for examination. Lymphoma cells infiltrated and distended the liver sinusoids (Figures 1 and 2B), and three cases demonstrated involvement of portal tract. Liver biopsies of five cases exhibited mild to moderate fatty degeneration.

Table 1 Clinical features of hepatosplenic $\gamma\delta$ T-cell lymphoma in eight Chinese patients

Case Number	Sex	Age (yr)	Clinical symptoms	ALT <40 (U/L)	ALP 27-107 (U/L)	γ -GT 10-50 (U/L)	LDH 97-270 (U/L)	Platelet count 100-350 $\times 10^9$ /L	Hemoglobin >110 (G/L)
1	M	12	Fever, weight loss, hepatosplenomegaly, died 4 mo after diagnosis	199	239	NE	899	104	79
2	M	22	Fever, hepatosplenomegaly	NE	NE	NE	NE	NE	NE
3	M	25	Fever, weight loss, hepatosplenomegaly, jaundice, died 1 mo after diagnosis	775	563	214	775	88	103
4	F	28	Fever, hepatosplenomegaly abdominal pain	55	246	66	Normal	108	100
5	M	30	Fever, weight loss, hepatosplenomegaly	71	506	169	358	49	95
6	F	30	Fever, weight loss, hepatosplenomegaly, abdominal pain	155	500	115	518	74	91
7	M	36	Fever, weight loss, hepatosplenomegaly, jaundice; died 4 mo after diagnosis	409	350	121	389	90	143
8	M	52	Fever, weight loss, hepatosplenomegaly, abdominal pain, jaundice	Normal	159	82	Normal	64	107

NE, not evaluable; normal, within the normal range.

Table 2 Pathological features of hepatosplenic $\gamma\delta$ T-cell lymphoma in eight Chinese patients

CaseNo.	Spleen weight (g)	Gross appearance (cut surface) of spleen	Microscopical findings	Lymphoid involvement	Bone marrow involvement
1	910	Homogeneous red-purple, no nodule, soft	Lymphoma cells infiltrate liver sinus, splenic sinusoids; liver portal tract, liver fatty degeneration	NO	NO
2	800	Homogeneous dull-red, firm	Lymphoma cells infiltrate liver sinus, splenic sinusoids	NE	NE
3	Part of accessory spleen	Homogeneous gray-red, firm	Lymphoma cells infiltrate liver sinus, splenic sinusoids; liver fatty degeneration	NO	NO
4	445 (body weight 45 kg)	Homogeneous gray-red, no nodule	Lymphoma cells infiltrate liver sinus, splenic sinusoids	NO	NO
5	1 155	Homogeneous gray-red, firm	Lymphoma cells infiltrate liver sinus, splenic sinusoids; liver fatty degeneration	Mediastinal lymphadenopathy	NO
6	800	Homogeneous gray-red, soft	Lymphoma cells infiltrate liver sinus, splenic sinusoids; liver portal tract, liver fatty degeneration	NO	NO
7	810	Homogeneous dull-brown, no nodule	Lymphoma cells infiltrate liver sinus, splenic sinusoids; liver portal tract, liver fatty degeneration	NO	Suspected lymphoid cell
8	480 (body weight 45 kg)	Homogeneous dull-red, soft	Lymphoma cells infiltrate liver sinus, splenic sinusoids	NO	Suspected lymphoid cell

NO, not involved; NE, not evaluable.

Table 3 Immunophenotypic features and TCR rearrangements of hepatosplenic lymphoma

	CD20	CD79a	CD3	CD4	CD8	CD43	UCLH-1	CD56	TCR $\gamma\delta$	TCR γ gene rearrangement	TCR δ gene rearrangement
1	-	-	+	-	-	+	+	+	-	-	+
2	-	-	+	-	-	+	+	+	NE	+	+
3	-	-	+	NE	-	+	+	+	+	+	-
4	-	-	+	-	+	+	+	+	+	-	-
5	-	-	+	-	+	+	+	+	+	+	+
6	-	-	+	-	+	+	+	+	+	-	+
7	-	-	+	NE	+	+	+	+	+	+	+
8	-	-	+	-	-	+	+	+	-	+	+

+, positive; -, negative; NE, not evaluable.

Bone marrow aspirate smears were performed in these cases. But only two cases demonstrated abnormal findings suggesting lymphoma infiltrates.

Immunophenotypic findings

As shown in Table 3, neoplastic cells in all cases expressed

T-cell associated markers, i.e., CD3 (Figure 2C), UCLH-1 and CD43, whereas B-cell associated markers including CD20 and CD79A were negative. Tumor cells in all the cases expressed CD56 antigen. Six cases were negative for CD4 (6/6), whereas four cases expressed CD8 (4/8). Positive TCR $\gamma\delta$ immunostaining on routinely formalin-fixed and

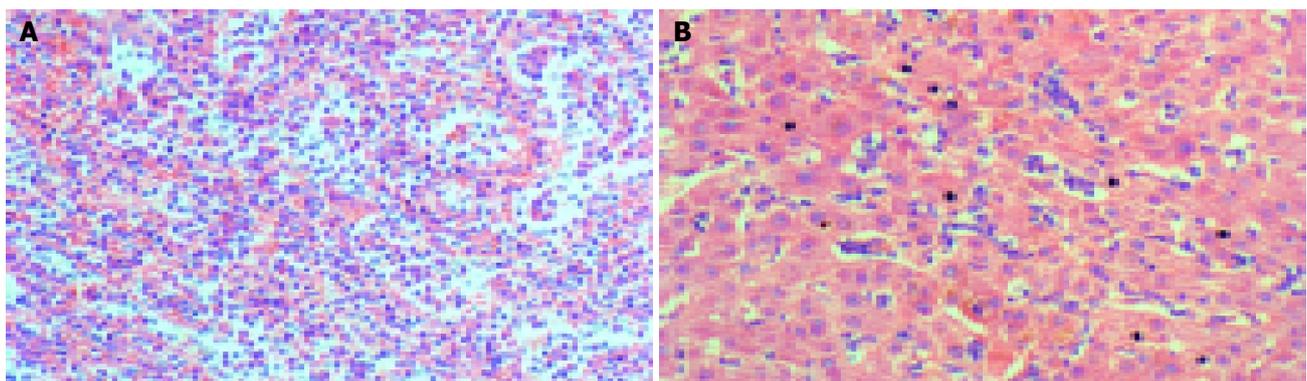


Figure 1 Histology changes in case 8. **A:** medium-sized monomorphic lymphoma cells into the red pulp cords and sinus of the spleen (10 \times); **B:** Distinct

hepatic sinusoidal infiltrates (20 \times).

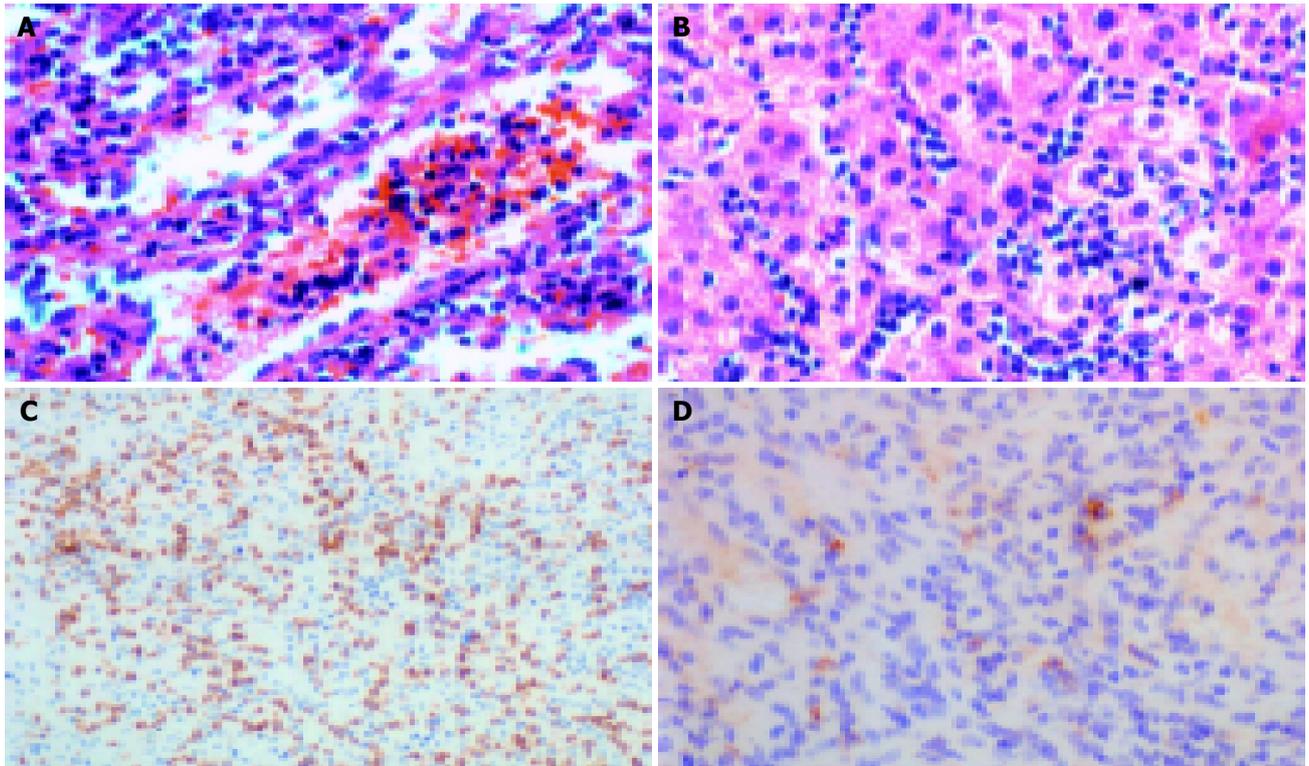


Figure 2 Histological changes and immunophenotypes in case 4. **A:** Infiltration of lymphoma cells into the red pulp cords and sinus of spleen (40x); **B:** Distinct hepatic

sinusoidal infiltrates (40x); **C:** Sinusoidal infiltration of lymphoma cells in spleen demonstrated by CD3 (10x); **D:** TCR $\gamma\delta$ immunostaining of Golgi region (40x).

paraffin-embedded tissue sections was observed in Golgi region of lymphoma cells in five cases (5/7) (Figure 2D).

consistent with the rearranged TCR δ gene through the search of BLAST in PubMed (Figure 6).

T-cell receptor gene rearrangements

Monoclonal rearrangements of TCR γ gene were demonstrated by PCR analysis in five of the eight cases (Table 3), which were single fragments of about 200 bp (Figure 4).

Complete TCR δ gene rearrangements were detected using primers V δ 1b and J δ 1 which amplified fragments of about 120 bp (Figure 5). Six out of eight cases demonstrated single bands on PAGE gel. The amplification products of TCR δ gene in two cases (cases 6 and 8) were also subjected to direct sequencing by an ABI377 sequencer (Perkin-Elmer Applied Biosystems, Foster City, CA, USA). The results were

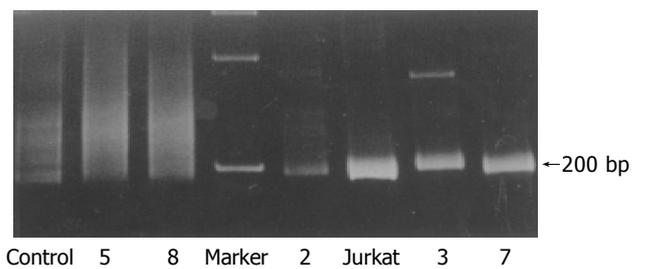


Figure 4 TCR γ gene PCR products in case 2; Jurkat, lanes 3 and 7: single bands of about 200 bp on 8% PAGE gel; Control, lanes 5 and 8 have no distinctive band.

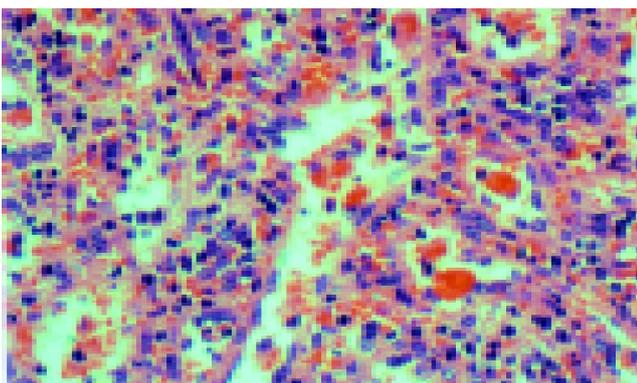


Figure 3 Significant erythrophagocytosis in sinus of the spleen of case 6 (40x).

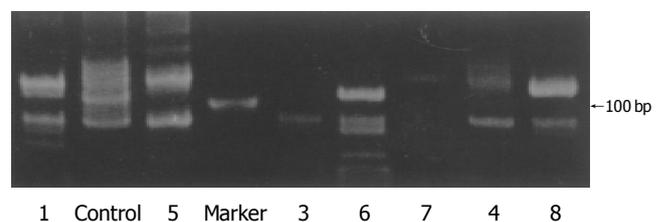


Figure 5 TCR δ gene PCR products in case 1. Lanes 5-8: single bands of about 120 bp on 10% PAGE gel; control and lanes 3 and 4: no distinctive band (the marker band is 100 bp, and the bands under 100 bp are regarded as unspecific amplification).

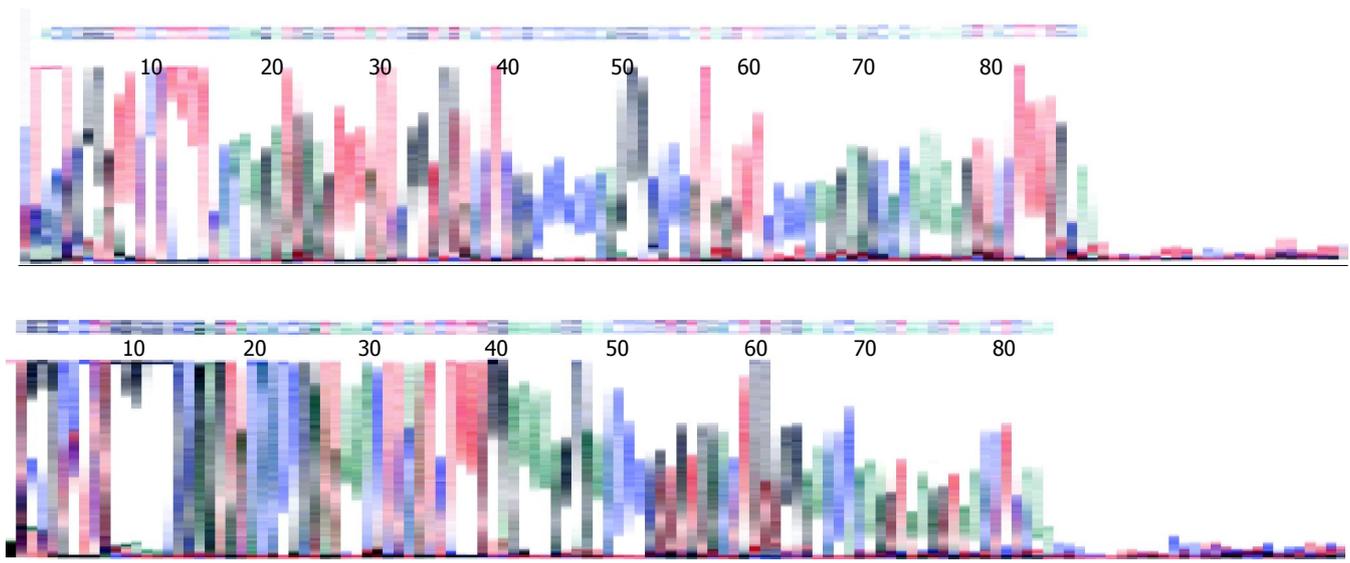


Figure 6 Forward and reverse sequencing results of TCR δ gene rearrangements in case 8, verified as products of TCR δ gene rearrangements

by searching in BLAST (PubMed).

DISCUSSION

In this study we analyzed eight hepatosplenic T-cell lymphomas in Chinese patients. The results confirmed a predilection for young male adults. Splenomegaly and hepatomegaly were present in all cases and coexisted lymphadenopathy was found in one case. The weights of spleens from cases 4 and 8 were 445 and 480 g, respectively. It seemed that the weights were lighter than those reported in the literature, but these two patients were thin, weighing 45 and 47 kg respectively, and their spleens were evidently enlarged, the lower edges were both at the level of umbilicus in physical examination.

The pathological findings were similar to those previously reported in the literature. Neoplastic cells were usually monomorphic small to medium-sized lymphocytes. Tumor cells were located preferentially in sinusoids of the liver, cords and sinuses of the splenic red pulp. However, besides sinusoidal infiltrates of the liver, portal tract involvements were also present in three cases in this series, and liver biopsies of five cases demonstrated mild to moderate fatty degeneration. According to Gaulard *et al*^[1] and Belhadj *et al*^[5], a mild portal and periportal lymphomatous infiltrate might also be observed but was not predominant^[1,5]. However, Weidmann *et al*^[6], indicated that marked or even exclusive portal infiltration could be present. Lymphomatous infiltration resulted in chronic liver injury, and most of our cases revealed abnormal liver function. Perfetto *et al*, described a case of hepatosplenic lymphoma whose clinical course mimicked acute hepatitis. It reminded us that hepatosplenic T-cell lymphoma should be included in the differential diagnosis of acute hepatic dysfunction in young patients who showed no evidence of viral, toxic, autoimmune or metabolic liver diseases.

In previous reports most of TCR $\gamma\delta$ immunophenotypes were demonstrated on frozen sections and it was difficult to detect these antigens on routinely formalin-fixed and paraffin-embedded sections. In this study, we tried to

demonstrated the TCR $\gamma\delta$ immunostaining on paraffin-embedded sections using target retrieval solution (high pH) (Dako, Glostrup, Denmark) following Macon's protocol^[7]. TCR $\gamma\delta$ immunohistochemistry on formalin-fixed and paraffin-embedded sections demonstrated Golgi region staining in five cases. The failure of the rest three cases may be due to the TCR $\gamma\delta$ antigen denaturation caused by formalin fixation. Our results confirmed that TCR $\gamma\delta$ immunostaining could be demonstrated on formalin-fixed and paraffin-embedded sections, although not all the cases revealed positive staining.

For detecting monoclonal TCR γ gene, Southern blot analysis for TCR γ chain gene rearrangements remains a reliable and reproducible technique with minimal false positive results. PCR technique is also a reliable and reproducible technique, but paraffin sample TCR γ PCR testing is less reliable than fresh or frozen tissue reported by most laboratories, and the risk for false negative results in paraffin-embedded tissues should be recognized^[8]. In this study, five of the eight cases demonstrated monoclonal rearrangements of TCR γ gene.

TCR δ gene consists of at least six V δ gene segments. Over 95% of $\gamma\delta$ T-cells express either V δ 1 or V δ 2 gene. Interestingly, normal $\gamma\delta$ T lymphocytes in spleen, thymus, and intestinal epithelium predominantly express the V δ 1 gene, whereas the majority of $\gamma\delta$ T-cells in peripheral blood, tonsils, and skin express the V δ 2 gene. Grzegorz *et al* demonstrated usage of V δ 1 gene and V δ 2 gene were found in hepatosplenic $\gamma\delta$ T-cell lymphoma (10/11 cases) and in $\gamma\delta$ subcutaneous panniculitis-like $\gamma\delta$ T-cell lymphoma (4/4 cases), respectively.

In our study, V δ 1 and J δ 1 gene rearrangements were demonstrated in six of eight cases. The result further support hepatosplenic $\gamma\delta$ T-cell lymphoma is preferentially derived from the V δ 1 subset of $\gamma\delta$ T lymphocytes.

It seemed that the results of rearrangements of TCR γ and TCR δ genes were not accordant. There were two reasons for this discordance. First, in this study, we used

formalin fixed and paraffin embedded samples to perform immunohistochemistry and to extract DNA for PCR amplification because of lack of fresh or frozen tissue. Archival formalin-fixed and paraffin-embedded samples could frequently lead to protein denaturation and DNA degradation. Second, we only employed the most commonly used primers to detect the rearrangements of TCR γ and TCR δ genes. Maybe there were uncommon gene rearrangements in the remaining cases.

In conclusion, the clinicopathology of hepatosplenic $\gamma\delta$ T-cell lymphoma in Chinese patients is similar to what was previously reported in the literature.

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Science Editor Wang XL Language Editor Elsevier HK