Original Article Intravascular cytotoxic T-cell Iymphoma presenting as hydrocele testis: a case report and review of the literature

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Abstract: Intravascular lymphoma (IVL) is a rare entity of extranodal large cell lymphoma. Most cases of IVL are B-cell phenotype, with a few cases of T-cell or NK-cell origin. To our knowledge, only 5 cases of intravascular cytotoxic T-cell lymphoma with sufficient phenotype studies have been reported so far. Here, a case of intravascular cytotoxic T-cell lymphoma initially occurred in epididymis in a 67-year-old male presenting as hydrocele testis was studied to define the clinicopathologic aspects of the rare disease. The lymphoma was studied by standard H.E staining, immunohistochemistry, in situ hybridization, as well as molecular assay for T cell receptor (TCR) rearrangement. Histological exam of epididymis revealed IVL with cytotoxic T-cell phenotype. The neoplastic cells were CD45+, CD3+, Granzyme B+, TIA-1+, CD56-, CD20-, CD79a-, CD30-, and the neoplastic cells were also positive for Epstein-Barr virus-encoded RNA (EBER) by in situ hybridization technique. Moreover, molecular study revealed monoclonal rearrangement for T-cell receptor (TCR). The finding in the present case may address the unique clinicopathologic features of the EBV-associated IVL.

Keywords: Intravascular lymphoma, cytotoxic T-cell lymphoma, NK/T-cell lymphoma, Epstein-Barr virus

Introduction

Intravascular lymphoma (IVL) is a rare subtype of extranodal large cell lymphoma characterized by the presence of neoplastic cells within the lumen of small vessels without obvious invasion of the surrounding parenchyma [1]. About 90% cases of IVL are B-cell phenotype, only less than 10% cases of T-cell or NK-cell lineage [1]. IVL was initially regarded as a malignant endothelial proliferative disorder, but subsequent immunochemical and molecular study revealed its lymphoid nature [2]. Previously reported cases of intravascular NK/T-cell lymphoma indicated that the skin and the central nerve system were easily involved [3-5]. One report showed epididymis had been initially involved by Epstein-Barr virus (EBV)-associated intravascular large T-cell lymphoma, but no data provided about both cytotoxic markers (Granzyme B, TIA-1, Perforin) expression and T cell receptor (TCR) rearrangement status of the tumor cells [6].

Extranodal NK/T-cell lymphoma is characterized by cytotoxic phenotype and association with EBV. Most cases of extranodal NK/T-cell lymphoma appear to be genuine NK-cell neoplasm, while some cases show cytotoxic T-cell phenotype. Because both cytotoxic T-cell lymphoma and NK-cell lymphoma display similar cytotoxic phenotype (CD3+, Granzyme B+, TIA-1+, Perforin+), it may be difficult to differentiate cytotoxic T-cell lymphoma from NK-cell lymphoma without data of TCR rearrangement. Cytotoxic T-cell lymphoma commonly displays TCR monoclonal rearrangement, while NK-cell lymphoma does not show TCR monoclonal rearrangement. In present article, we described a case of intravascular cytotoxic T-cell lymphoma that initially occurred in epididymis.

Case report

A 67-year-old male presented with hydrocele testis two months ago. Type-B ultrasonic scan showed enlargement of the head and body of his right epididymis with echo change. Then, the patient was treated with antibiotics and anti-inflammation drugs, but his condition had no remarkable improvement. His right scrotum continued to enlarge with a slight pain. No nasal



Figure 1. Gross appearance of the epididymis. The epididymis is enlarged and shows pale-white mass with unclear boundary.

symptoms and nasal abnormalities were found. His body temperature was normal. Physical examination revealed that his right scrotum were seriously swollen, which was approximately 15×9 cm in size. Erythematous skin plaques were found on the trunk and the extremities.

Routine blood test revealed a remarkable decreased lymphocyte count $(0.49 \times 10^9/L \text{ vs.} 1.1 \sim 3.2 \times 10^9/L$ for normal) and a decreased lymphocyte ratio (12.1% vs. 20~50% for normal). Blood coagulation function assay showed an elevated level of D-dimer (4350 mg/L vs. 0~550 mg/L for normal). Serum biochemical assay showed an increased LDH level (553 U/L vs. 120~250 U/L for normal). The serum tumor markers levels such as PSA, CA125 and CA199, were in normal limit. Bone marrow biopsy suggested no obvious involvement by lymphoma. Computed tomography scan of brain, chest and abdomen showed unremarkable findings.

After a clinical diagnosis of epididymitis and hydrocele testis had been made, a surgery of right epididymectomy and resection of testicular sheath membrane was performed. After the surgery, the patient went through a low fever between 37.5~38.5°C for more than two weeks. The patient is still under chemotherapy.

Materials and methods

Tissue specimen of epididymis was fixed and paraffin-embedded sections were made by routine procedure. Immunohistochemical staining was performed by the EnVison method. A panel of commercial antibodies (Products of Beijing Zhongshan Golden Bridge Biotechnology, Beijing, China) including AE1/AE3 (pan-Cytokeratin, pan-CK), EMA (epithelium membrane antigen), CD45 (LCA, leukocyte common antigen), CD20, CD79a, MUM-1, CD10, BCL-2, BCL-6, CD2, CD3, CD4, CD5 CD7, CD8, CD30, CD43, CD56, Granzyme B, TIA-1, ALK, TDT, CD99, CD31, CD34 and D2-40, was used for immune phenotype study. Appropriate positive and negative controls were run concurrently for all the markers tested. In situ hybridization assay for Epstein-Barr virus-encoded RNA (EBER) was performed by an EBER Assay Kit (Beijing Zhongshan Golden Bridge Biotechnology, Beijing, China) according the manufacturer's protocol.

DNA was isolated from paraffin-embedded tissue samples and TCR rearrangement was analyzed by PCR method. Four sets of primers (V γ 1-8, V γ 9, V γ 10, V γ 11) to the variable and joining regions of the TCRg γ g chain gene were used in the assay, while positive and negative controls were used in the same time [1]. Analysis of the amplified products was performed by denaturing gradient gel electrophoresis technique.

Results

Grossly, the epididymis was enlarged and showed pale-white mass with unclear boundary on cut surface (**Figure 1**). Microscopically, H.E sections of the epididymis revealed many large atypical lymphoid cells within the lumen of distended vessels. The neoplastic cells were rich in mitosis and contained hyperchromatic nuclei with prominent nucleoli (**Figure 2A**).

Immunohistochemical analysis showed that the neoplastic cells were positive for CD45, CD3, Granzyme B, TIA-1 (Figure 2C, 2D, 2F), and negative for pan-CK, EMA, CD56, CD20, CD79a, CD2, CD4, CD5, CD7, CD8, CD10, CD43, CD30, BCL-2, BCL6, Mum1, TDT, CD99, ALK. The Ki67 positive-index of the neoplastic cells was nearly 90% (Figure 2E), which indicated a very high proliferation activity. The vessels surrounding the neoplastic cells were highlighted by blood endothelium markers, CD34 (Figure 2B) and CD31 immunohistochemical staining, but not by D2-40 (a marker for lymphatic endothelium). The results demonstrated that the neoplastic cells were located in the lumen of the blood vessel.



Figure 2. Histology and immunohistochemistry. (A) Small vessels of epididymis are filled with large atypical cells (H.E. ×200). (B) The atypical cells are confined in blood vessels which can be highlighted by CD34 immune staining. The neoplastic cells are positive for CD3 (C), negative for CD20 (D). (E) Approximately 90% of the neoplastic cells are positive for Ki67. (F) The neoplastic cells are positive for Granzyme B.

The neoplastic cells were universally nuclear positive for EBER by using in situ hybridization assay (**Figure 3**). Molecular study for TCR demonstrated monoclonal rearrangement for V γ 9 and V γ 11 of TCR γ , while negative for V γ 1~8 and V γ 10 (**Figure 4**), which suggested the existence of monoclonal T cells.

Discussion

IVL is a rare subtype of non-Hodgkin lymphoma. Although intravascular large B-cell lymphoma was classified as a distinct entity by WHO classification [7], intravascular NK/T-cell lymphoma (including cytotoxic T-cell lymphoma) is



Figure 3. In situ hybridization. The intravascular neoplastic cells are nuclear positive for EBER (×100).



Figure 4. Results of TCR rearrangement. TCR rearrangement was assayed by PCR using 4 sets of primers. V γ 9 and V γ 11 showed monoclonal bands, while V γ 1~8 and V γ 10 showed polyclonal smear. 74: sample of this case; NC: negative control; 8: positive control for V γ 1~8; 9: positive control for V γ 9; 10: positive control for V γ 10; 11: positive control for V γ 11.

currently not classified as a separate subtype of NK/T-cell lymphoma [7, 8]. Therefore, intravascular NK/T-cell lymphoma has to be temporarily regarded as a variant of extranodal NK/Tcell lymphoma. Some authors suggested that the diagnosis of intravascular NK/T-cell lymphoma should be independent to facilitate further study of this disease [9].

Neoplasm-associated vascular obstruction and local edema may be one of the most obvious clinical features [10, 11]. Most cases of NK/Tcell IVL show aggressive clinical course, therefore, early diagnosis is important for prompt therapy. In the present case, severe hydrocele testis was the main symptom for the patient to seek medical advice. Due to rarity, some cases of reported intravascular T-cell or NK/T-cell lymphoma were finally diagnosed by autopsy [12, 13].

Majority of reported cases of intravascular NK/T-cell lymphoma showed NK-cell phenotype and negative for monoclonal TCR rearrangement suggested they were NK-cell lymphoma [14]. A few cases of the reported intravascular NK/T-cell lymphoma may be cytotoxic T-cell lymphoma, but they were not further confirmed by TCR rearrangement study [15]. To our knowledge, only 5 cases of intravascular cytotoxic T-cell lymphoma with sufficient phenotype studies have been reported so far (Table 1). In the present case, the neoplastic cells within the blood vessels were CD45+, CD3+, TIA-1+, Granzyme B+, EBER+, and CD56-, CD30-, ALK-, as well as positive for TCR monoclonal rearrangement. These data supported the diagnosis of intravascular cytotoxic T-cell lymphoma.

Intravascular cytotoxic T-cell lymphoma or NK/T-cell lymphoma should differentiate from other lymphoma including nasal type extranodal NK/T-cell lymphoma, aggressive NK-cell leukemia, intravascular anaplastic large cell lymphoma. Nasal type extranodal NK/T-cell lymphomas exhibit angiocentric and angiodestructive growth pattern rather than intravascular growth. In addition, coagulative necrosis is very common in nasal type extranodal NK/T-cell lymphoma [7]. Although aggressive NK-cell leukemia often displays identical immunophenotype as extranodal NK/T-cell lymphoma or intravascular NK/T-cell lymphoma, the former is commonly occur in young to middle-aged adults with a high frequency of bone marrow involvement [7]. Moreover, TCRgyg genes are in germ-line configuration for NK-cell leukemia. The morphology and immune phenotype between intravascular anaplastic large cell lymphoma and intravascular cytotoxic T-cell lymphoma may be very similar, but the former are positive for CD30 [7, 16]. Another mimicker of IVL should be take into consideration for differential diagnosis is benign atypical intravascular CD30+ T-cell proliferation, which is characterized by accumulation of large CD30+ polyclonal T cells within lymphatic in close vicinity to ulceration or inflammatory skin disease. There is no association with Epstein-Barr virus infection for benign atypical intravascular CD30+ T-cell proliferation [17].

Some recent studies suggested that intravascular NK/T-cell lymphoma, aggressive NK-cell leukemia and nasal type extranodal NK/T-cell lymphoma were closely related diseases due to

 Table 1. Reported cases of intravascular cytotoxic T-cell lymphoma or NK/T-cell lymphoma showing positive for monoclonal TCR rearrangement in the literature

| Pt. No | S/A | Clinical Features | Organ involve- ment at Presen- tation | Phenotype | TCR | BMI | Treatment | Follow-up | Author Year |
|-----------|------|---|---|--|-----|-----|--|---|-----------------------|
| 1 | M/37 | HIV+ patient with acute renal failure | Kidney (Diagnosed by autopsy) | CD3+, CD8+, TIA-1+; CD4-, CD5-, CD20-, ALK-1-, TDT-, CD30-, CD56-, EBV+ | Mc | Ν | Antibiotics; without chemotherapy | Respiratory, renal failure, dead after 2 W | Merchant [13] 2003 |
| 2 | F/67 | Red-brown lesion on the leg | Skin, brain | CD2+, CD3+, CD8+, TIA-1+; CD20-, CD4-, CD30+/-, CD56-, EBV+ | Mc | Ν | NA | Dead, 1 Mo | Cerroni [22] 2008 |
| 3 | M/62 | Erythematous patches and plaques on the lower extremities | Skin | CD3+, perforin+, GranB+, TIA-1+, CD20-, CD4-, CD8-, CD5-, CD56+, EBV- | Mc | Ν | 6 cycles of CHOP chemo- therapy | No evidence of progression in 24 Mo | Gleason [23] 2008 |
| 4 | F/23 | Erythematous patches on the trunk and extremities | Skin | CD3+, CD8+, GranB+, TIA-1+, CD4-, CD5-, CD20-, CD56-, EBV+ | Ρ | Ν | Chemotherapy+blood stem cell transplantation | Recurrence after 4 Mo, dead 11 Mo after recurrence | Jang [24] 2014 |
| 5 | F/47 | Erythematous patches/plaques on the lower extremities | Skin | CD45+, CD3+, GranB+; CD4-, CD5-, CD8-, CD20-, CD30-, CD56-, EBV+ | ND | ND | Combination chemo- therapy | Alive with no evidence of disease at 18 Mo | Alhumidi [15] 2015 |
| 6 | M/67 | Hydrocele testis, erythematous plaques on the trunk and extremities | Epididymis, Skin | LCA+, CD3+, GranB+, TIA-1+, CD4-, CD5-, CD8-, CD20-, CD30-, CD56-, EBV+ | Mc | Ν | Combination chemo- therapy | Alive during 2 Mo follow-up period | Current |

Pt. No: patient number; S/A: sex/age; TCR: T-cell receptor; M: male; F: female; Mc: monoclonal; P: polyclonal; ND: not done; NA: not available; N: no involvement; W: week (s); Mo: month (s); BMI: Bone marrow involvement; CHOP: cyclophosphamide, doxorubicin, vincristine and prednisone; EBV: Epstein-Barr virus; GranB: Granzyme B. their similar immunophenotypes, TCR rearrangement results and EBV infection conditions [10]. Some features of the three diseases may overlap during the course of the diseases, which may represent different disease states [9]. The relationship among them may need further clarification.

The mechanism for IVL showing a predilection of intravascular growth has not be fully understood, some reports suggested that alteration of adhesion molecules might play a role [11]. For example, the neoplastic cells of B-cell IVL expressed CD11a, CD49d (VLA-4), while endothelial cells expressed CD54 (CD11a ligand), CD106 (CD49d ligand). The interaction of these molecules help the neoplastic cells grow within the vascular lumen [18]. Some other studies showed that lack of CD29 (\beta1 integrin) and CD54 (ICAM-1) adhesion molecules in tumor cells of B-cell IVL might be linked to intravascular growth of the neoplasm [19]. Whether the similar mechanisms exist in intravascular NK/T-cell lymphoma need further investigation.

The prognosis of IVL may be quiet different, depending on the extent of involvement by the neoplasm [14, 20, 21]. Patients with local or single organ involvement might respond well to chemotherapy, while patients with multiple organ involvement or suffered from disseminated disease may have a poor prognosis. Accumulation of more data for this rare disorder may be important for effective therapy.

Disclosure of conflict of interest

None.

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