Pathologic character and diagnosis of female primary genital system diffuse large B cell lymphoma

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Abstract. – OBJECTIVE: We investigated the clinicopathological characteristics and immunophenotype of female genital system diffuse large B-cell lymphoma (DLBCL) in order to improve diagnosis and therapy efficacy.

PATIENTS AND METHODS: The clinicopathologic features of 13 cases with primary DLBCL of the female genital system were studied retrospectively. According to the immunophenotypes, 10 cases were classified as germinal center B-cell-like DLBCL and the other 3 as non-center B-cell-like DLBCL.

RESULTS: The immunohistochemistry data showed that CD20, PAX-5, and CD79a tested positive, while CD3, CD43 and CD45RO tested negative in all 13 cases. In 7 cases EMA was positive, in 9 cases CD10 was positive, in 11 cases bcl-6 was positive, and in 9 cases MUM-1 was tested positive. In one case, the Ki-67 index was less than 59%, in 3 cases Ki-67 index was between 60% to 89% and it was more than 90% in the remaining 9 cases (the median was 90%). All 13 patients underwent hysterectomy, while in 10 of them hysterectomy was followed by chemotherapy. The survival time was 3 to 20 months.

CONCLUSIONS: Primary diffuse large B-cell lymphoma of the primary female genital system is a rare and highly invasive condition that can be easily misdiagnosed. A complete diagnosis is very important because the treatment and prognosis vary in different histological types. Moreover, the immunohistochemistry is an useful diagnostic method for this type of cancer.

Key Words
Reproductive system, DLBCL, Clinical pathology.

Introduction

Lymphoma can be divided into two types: Hodgkin lymphoma (HL) and non-Hodgkin lymphoma (NHL). Diffuse large B-cell lymphoma is classified as a NHL. It has been reported that morbidity of primary cervical lymphoma was 0.12%.

DLBCL is the most common type of non-Hodgkin lymphoma that occurs mainly in the gastrointestinal tract, but it can be also seen in the female genital system. Currently, there is no standard treatment for DLBCL in the primary female genital system, and different modes of treatment are used for this condition: (i) Surgical intervention combined with postoperative chemotherapy or radiotherapy. If lesions are only limited to ovary, womb or vulva, the common operation method is hysterectomy, bilateral salpingo oophorectomy, greater omentum resection and pelvic lymph node dissection. (ii) Chemoradiotherapy, in which female reproductive function can be reserved, and gross tumor volume can be shrunk. The common chemotherapy scheme is CHOP. (iii) Combined treatment: As DLBCL is a malignant lymphoma and a systemic disease, usually single treatment scheme is not enough to cure the disease. Generally, two or three combined treatments are employed. In this study, 10 patients were given combined postoperative CHOP chemotherapy and achieved satisfactory results.

In recent years, literature relating to DLBCL female genital system is mainly case report without a unified understanding of its pathogenesis, clinical pathology, diagnosis and treatment. In this study, we gathered clinical data from 13 patients with DLBCL and investigated the characteristics of this condition in order to improve diagnosis and therapy efficacy.

Patients and Methods

Patients

13 patients were diagnosed with female genital system DLBCL in Xuzhou Central Hospital from January 2007 to August 2012. Diagnosis in all cases was confirmed by 2 doctors titled above associate senior doctor. They used radiographic
results morphological examination, pathological analysis, and immunohistochemistry tests. Combining H & E shape and immunophenotype, 13 cases with DLBCL were classified in histopathology according to WHO classification criterion and were sub-classified according to relevant references. Meanwhile, clinical data of 13 patients were gathered including age, disease course, symptoms and physical signs, primary site, laboratory examination, imaging, and treatment condition. The clinical stages were established based on Ann Arbor staging systems. After pathological diagnosis, 13 patients were followed-up via phone interviews up to October 2012.

Methods

Morphology observation

Patients were classified based on WHO lymph hematopoietic tumor classification (2008 version), and each case was given a histomorphological observation. Tumor infiltration, morphological features of tumor cells, mitotic figure index, infiltration condition of peripheral vessel, tissue necrosis, cell apoptosis and collagen proliferation were observed.

Immunohistochemistry

Tumor tissue samples were collected and cut into slices (4 to 5 µm), and immunohistochemistry tests were done using the method of super Vision (Long Island Biological Technology Co., LTD, Shanghai, China). PBS replaced primary antibody in the negative control, and positive specimens of known antibodies were set as positive control. The main selected antibodies were CD20, CD79α, CD3, PAX-5, CD10, bcl-6, MUM-1, CD5, CD45RO, EMA, CD43, SMA, myeloperoxidase (MPO) and Ki-67 (Dako, Carpinteria, Ca, USA, and Santa Cruz Biotechnology, Santa Cruz, CA, USA). When cell membrane appeared to be brown-yellow, we considered CD20, CD3, CD45RO and CD10 to be positive, and when cell membrane or cytoplasm appeared to be brown-yellow, we considered CD79α to be positive. In those cases where cell membrane was brown-yellow, and a number of positive tumor cells was equal or greater than 30%, then we considered bcl-6 and MUM-1 to be positive.

Statistical Analysis

We used SPSS 13.0 software (SPSS Inc., Chicago, IL, USA) for our statistical analysis. Quantitative data was shown as mean±standard deviation (X ± s), and single factor prognosis analysis was carried on by Log-rank test. p<0.05 was considered statistically significant.

Results

Clinical Features

The age onset of the disease in all cases was 26 to 75 years (average =43.53±9.21). Information on primary site, symptoms physical signs,

<table>
<thead>
<tr>
<th>Case number</th>
<th>Age (years)</th>
<th>Disease course (month)</th>
<th>Stage</th>
<th>Primary site</th>
<th>Clinical symptoms</th>
<th>Treatment condition</th>
</tr>
</thead>
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<tr>
<td>1</td>
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<td>9</td>
<td>I</td>
<td>Ovary</td>
<td>Fever, painless pelvic mass</td>
<td>Operation, chemotherapy</td>
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<td>36</td>
<td>11</td>
<td>I</td>
<td>Endometrium</td>
<td>Menstruation change, contact bleeding</td>
<td>Operation, chemotherapy</td>
</tr>
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<td>3</td>
<td>43</td>
<td>3</td>
<td>III</td>
<td>Ovary</td>
<td>Menstruation change, intermenstrual bleeding</td>
<td>Operation, chemotherapy</td>
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<td>4</td>
<td>45</td>
<td>1</td>
<td>III</td>
<td>Ovary</td>
<td>Intermenstrual bleeding, vaginal secretion increase</td>
<td>Operation, chemotherapy</td>
</tr>
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<td>43</td>
<td>1</td>
<td>I</td>
<td>Cervix</td>
<td>Painless pelvic mass</td>
<td>Operation</td>
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<td>6</td>
<td>37</td>
<td>0</td>
<td>II</td>
<td>Cervix</td>
<td>Vaginal secretion increase</td>
<td>Operation</td>
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<td>7</td>
<td>61</td>
<td>3</td>
<td>II</td>
<td>Ovary</td>
<td>Vaginal secretion increase</td>
<td>Operation, chemotherapy</td>
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<td>8</td>
<td>50</td>
<td>1</td>
<td>II</td>
<td>Ovary</td>
<td>Menstruation change, contact bleeding</td>
<td>Operation, chemotherapy</td>
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<td>Cervix</td>
<td>Pelvic mass</td>
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<td>75</td>
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<td>Ovary</td>
<td>Intermenstrual bleeding, menstruation change</td>
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<td>Cervix</td>
<td>Vaginal secretion increase</td>
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<td>II</td>
<td>cervix</td>
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<td>Operation, chemotherapy</td>
</tr>
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</table>
and clinical stages are presented in Table I. Pelvic mass was detected in 3 cases after auxiliary examination of chest X-ray, CT test, pelvic cavity B ultrasound and ECT test (Figure 1). The pulmonary nodule was detected in 1 case. After ECT test, 2 cases were found to have several confluent lymph nodes in left common iliac and groin, one confluent lymph node in armpit and several confluent lymph node in neck, and there were lymph nodes enlargement and active metabolism at their right groin. The detection of tumor markers, CA125, CA19-9, CEA, AFP, and HCG test did not show any significant abnormality, and peripheral hematogram and bone marrow examination were normal. For treatment, 3 cases in stage I were treated with both radical hysterectomy and pelvic lymphadenectomy, and two cases were treated with postoperative chemotherapy combined with the scheme of cyclophosphamide + adriamycin + vincristine + prednisone (CHOP). Patients in stage II and stage III were treated with hysterectomy + bilateral salpingo oophorectomy + greater omentum resection + pelvic lymph node dissection. Among those patients, two quitted therapy and did not undergo radiotherapy and chemotherapy, and the other patients were treated with postoperative chemotherapy combined with CHOP scheme. Detailed clinical data for all 13 cases are presented in Table I.

**Morphological Classification**

All cases were morphologically classified according to WHO new classification. There were 9 cases (69.2%) of centroblasts, with an average age of (60.3±12.3) years. It was formed by round and oval lymphocytes with medium to big sizes. Cell nucleus showed vacuole-shape with thin chromatin and small cytoplasm, with 2 to 4 basophilia nucleolus. There were 2 cases (15.4%) of immunoblastic, with an average age of (63.5±14.1) years. An apparent basophilic nucleolus lied in central nucleus with abundant cytoplasm. We had 2 cases (15.4%) of anaplastic, with an average age of 30.5±8.1 years. Distributed in large expanse, tumor cells were big in size with different forms and mild nuclear pleomorphism, some of which were similar to R-S cell.

**Immunohistochemistry**

CD20, PAX-5, and CD79a were expressed in all cases, and in 7 cases EMA expression was positive. CD10 was positive in 9 cases, while Bcl-6 was positive in 11 cases. In 9 cases we detected positive expression of MUM-1. Ki-67 positive index was ≤59% in one case, 60% to 89% in 3 cases and >90% in 9 cases (median = 90%). CD10+/bcl-6-/MUM-1-, CD10-/bcl-6+/MUM-1+ was B cell in germinal center (GCB) sample DLBCL, CD10-/bcl-6+/MUM-1+ was B

![Figure 1](image_url). Various imaging data of DLBCL. Ultrasound shows hysterauxesis and intima thickening (A, B); irregular hypoechoic can be seen in uterine cavity (C, D); hysterauxesis can be seen by pelvic cavity CT, enlarged lymph nodes (E); metastatic nodules in lung (F).
cell in non-germinal center (non-GCB) sample DLBCL. Based on this classification standard, there were 10 cases (77%) of non-GCB type, among which 1 case was IB type, the others were CB type. There were 3 cases (23%) of GCB type, among which 1 case was IB type, while the others were ALCL type.

Relationship between Immunohistochemical and Survival Rate

Five-year survival rate of patients who were tested positive for CD10 was 88.9% (8/9), while five-year survival rate for patients who were tested negative for CD10 was 25.0% (1/4). Five-year survival rates were 81.8% (9/11) and 33.3% (1/3) for Bcl-6 positive Bcl-6 negative patients, respectively.

Log-rank analysis results showed that patients who were tested positive for CD10 and Bcl-6 had relatively good prognosis ($p=0.021$, $p=0.042$). Five-year survival rate for patients with positive MUM-1 expression was 33.3% (3/9), while that of patients with negative MUM-1 was 75.0% (3/4). Log-rank analysis results revealed that patients with positive MUM-1 expression had significantly shorter survival time ($p=0.014$).

Discussion

In 6 cases, the primary site was in ovary and in 2 cases it was in endometrium, while the primary site in 5 cases was in cervix. Clinical features of all patients in this group:
Female primary genital system diffuse large B cell lymphoma

The onset age for DLBCL was earlier than that of oopheroma and cervical cancer (average=43.5 years), and the majority of patients were diagnosed at late-stage. The main clinical manifestation and physical sign were fever and painless bony mass, combined with vagina irregular bleeding, vaginal secretion increase or (and) menstruation changes. By b-scan images and/or pelvic CT, we detected solid mass, cystic mass or cystic and solid mass.

As NHL is rarely seen in female genital system, it can be easily confused with uterine fibroid canceration and uterine sarcoma. Therefore, it’s very difficult to do an early diagnosis in most patients and patients are often diagnosed by results obtained from intraoperative or postoperative pathological evaluations. Most of the non-Hodgkin lymphoma of primary female genital system are B lymph cell lymphoma, mainly composed of diffuse large B-cell lymphoma. Tumor morphologies are poly, erosion, nodular type and big fish flesh appearance with minimal bleeding and necrosis. By histological examination, it can be seen that tumor cells are big in size with many nuclear fissions. Based on immunophenotyping results on CD10, BCL-6, and MUM-1, Hans divided DLBCL into two sub-types: GCB and non-GCB cells. All cases in this study were evaluated by antibody detection, such as CD10, BCL-6, and MUM-1. The results showed that the positive expression rate of CD10, BCL-6, and MUM-1 were 40.0%, which was basically consistent with previous literature. The positive expression rate of CD10 and MUM-1 was relatively lower. Among 13 cases of DLBCL, 10 cases were GCB1 and 3 cases were non-GCB. Meanwhile, we found that in all cases tumor cells expressed CD20, PAX-5, and CD79a. CD20, PAX-5, and CD79a are considered good B cell markers.

Metaplasia in this group was CB, IB, ALCL type, of which CB type accounted for more than half of DLBCL (69.2%). Similar results were reported in previous studies. CB is the most common metaplasia of DLBCL, and morphological characteristics of CB and IB type are usually easy to detect. However, ALCL type can be misdiagnosed, therefore the efforts must be concentrated on the differential diagnosis. In ALCL cases, oncocyte is usually sinus or perifollicular distributed and has flaked form. Tumors needed to be identified using cytokeratin of cancer cell in poorly differentiated carcinoma, common leukocyte antigen, malignant melanoma S-100, melanophore melanoma-associated antigen, differentiation antigen and LCA.

Prior studies showed that the survival rate for this type of tumor was only 25%. Other reports revealed that five-year survival rate can be up to 93%. DLBCL has been classified into two types: GCB and non-GCB. The former has good prognosis while the latter has poor prognosis. It was shown that the average index of Ki67 was 90%, which indicated a higher proliferative activity of tumor cells. Survival analysis revealed that the prognosis for CD10 and bcl-6 positive patients was better than that of their negative patients, and the prognosis of MUM-1 positive expression was poorer than its negative expression. Prior studies reported that CHOP scheme produced excellent results in treating both types of DLBCL. Five-year survival rate for GCB and non-GCB were 59% and 30% respectively. Prognosis for patients with non-GCB was apparently better than that of GCB type. However, when rituximab (CD20 monoclonal antibody) was combined with traditional method of CHOP, the difference between the two basically vanished.

Conclusions

The primary diffuse large B-cell lymphoma of primary female genital system can be efficiently diagnosed by pathological examination and immunohistochemistry, and be treated effectively by radical resection combined with chemotherapy.

Conflict of Interest

The Authors declare that they have no conflict of interests.

References


