Evaluating the effectiveness of chemotherapy for thymic epithelial tumors using the CD-DST method

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Abstract

Background: Thymic epithelial tumors (TET) are frequently eligible for curative-intent surgical resection. For locally advanced TETs, chemotherapy has been used to both reduce the tumor burden and achieve prolonged disease control. However, effective therapy for this disease largely remains to be determined. Here, we report the chemosensitivity of 100 patients with TETs determined by the collagen gel droplet embedded culture-drug sensitivity test (CD-DST).

Methods: A total of 100 patients with TETs underwent surgical resection. The efficacy of antitumor agents on TET cells was tested by CD-DST.

Results: Thymic epithelial tumors were pathologically confirmed after surgery: two cases were type A thymoma, 17 were type AB, 12 were type B1, 44 were type B2, 12 were type B3, and there were 13 cases with thymic carcinoma. A total of 36% patients with TETs were sensitive to different types of chemotherapeutic agents. There was no significant differences in age, histological type, clinical staging, or association with autoimmune diseases between sensitive and nonsensitive cases. Type B1 and B2 thymoma were relatively more sensitive to chemotherapeutic agents (6/12 and 18/44, respectively), while sensitivity of type B3 cases to chemotherapeutic agents was much lower (only 2/12). Cases with type A thymoma were not sensitive to any antitumor drugs. Among 11 chemotherapeutic agents tested in our study, the sensitivity of TETs to EPI was the highest (16%). No patients with thymoma were sensitive to Alimta (Pemetrexed).

Conclusions: Our work illuminates the effectiveness of chemotherapy for TETs and provides important clues for choosing antitumor drugs with relatively high drug sensitivity to TETs in advance.

Introduction

Thymic epithelial tumors (TETs) represent the most common anterior mediastinal compartment neoplasm, originating from the epithelial cell population in the thymus. Due to their different histological types, TETs show different clinical characteristics. Furthermore, TETs are often associated with autoimmune disorders,1–3 the most common being myasthenia gravis (MG). Because the tumorigenesis of TETs still remains unknown, there is a lack of effective molecularly targeted therapies to treat thymoma.4,5 TETs are frequently eligible for upfront curative-intent surgical resection. For some cases with a locally advanced TET at the time of diagnosis, with invasion of neighboring organs, dissemination to the pleura, pericardium, or less frequently extra-thoracic organs, chemotherapy has been used both to reduce the tumor burden - possibly allowing subsequent surgery and/or radiotherapy - and to achieve prolonged disease control. However, effective therapy for this disease largely remains to be determined. Some anti-cancer drugs have been reported to be effective in some cases of thymoma or thymic carcinoma.6–9 Unfortunately, there is no single drug which shows a high clinical response. No systematic research has demonstrated the exact thymic tumor responses to chemotherapy. Former studies have indicated that the collagen gel droplet embedded culture-drug sensitivity test (CD-DST)10 could approximate the clinical effect in different types of malignant tumors11,12 and have a high predictive accuracy for
response to chemotherapy, reaching up to 84.1%. In this study, we report on the chemosensitivity of 100 patients with TETs by CD-DST.

Methods

Patients

Between 2015 and 2018, 100 patients with TETs underwent surgical resection at Beijing Tongren hospital. There were 43 (43%) female and 57 (57%) male patients whose mean age was 47 years (ranging from 27 to 82 years) and they had not been treated with chemotherapy before surgery. The clinical profiles of the 100 patients are summarized in Table 1. Among the patients with TETs, 67 had autoimmune diseases (such as MG, primary adrenocortical hypofunction, optic neuritis, optic nerve degeneration, allergic dermatitis, Lupus erythematosus, etc), while 33 had no evidence of autoimmune disease. These patients underwent extended thymectomy using the trans-sternal approach (n = 27) or video-assisted thoracoscopic surgery (VATS) (n = 73). For patients with tumor nodules found on the pleural surface, cytoreductive surgery was performed.

Tumor tissue samples

Samples of TETs were collected from fresh specimens during surgical procedures at Beijing Tongren Hospital. All TETs were reclassified according to the WHO histologic classification and the Masaoka clinical staging system. Antitumor agents included 5-furuolouracil (5FU, 1.0 μg/mL), Epirubicin (EPI, 0.1 μg/mL), adriamycin (ADR, 0.02 μg/mL), cisplatin (CDDP, 0.2 μg/mL), carboplatin (CBDCA, 2.0 μg/mL), Etoposide (VP-16, 1.0 μg/mL), Paclitaxel (PAC, 1.0 μg/mL), Navelbine (VNR, 0.01 μg/mL), Gemcitabine (GEM, 8.0 μg/mL), Docetaxel (DOC, 0.1 μg/mL) and Pemetrexed (Alimta, 0.90 μg/mL).

TETs were pathologically confirmed after surgery. Among these, two cases were type A thymoma, 17 were type AB thymoma, 12 were type B1 thymoma, 44 were type B2 thymoma, 12 were type B3 thymoma, and there were 13 cases with thymic carcinoma. The effect of antitumor agents on cells of TETs was detected by the CD-DST method. As shown in Fig 1, after five to seven days’ growth, the colonies of TET cells were cultured in collagen gel droplets with different antitumor agents and analyzed by the image analysis method (software Primage 1.0.6.3). By measuring the size of colonies, drug sensitivity was tested. The larger the size of the colonies, the higher the growth rates and the lower the drug sensitivities were.

CD-DST methods

Each sample was minced finely using a scalpel or razor blade and digested in a cell dispersion enzyme solution (EZ; Kurabo, Japan) for two hours. The dispersed cancer cells were treated with ethylene glycol tetra-acetic acid (EGTA)-trypsin and filtered through a 200 μm nylon mesh. The cells were then incubated in a collagen gel-coated flask (CG-flask; Kurabo, Japan) containing pre-culture medium (PCM-1; Kurabo, Japan) containing pre-culture medium (PCM-1; Kurabo, Japan) containing 10% fetal bovine serum (FBS) at 37°C in 5% CO₂ overnight. Only

| Table 1 | Patient characteristics |
|---------|-------------------------|
| Total patients | Sensitive cases | Nonsensitive cases | P-value |
| Number | 100 | 36 | 64 | 1.000 |
| Median age (range, years) | 47 (27–82) | 48 (32–72) | 47 (27–82) | 0.536 |
| Male (n) | 57 | 19 | 38 | 0.581 |
| Female (n) | 43 | 17 | 26 | 0.451 |
| WHO histological type | | | | |
| A | 2 | 0 | 2 | |
| AB | 17 | 6 | 11 | |
| B1 | 12 | 6 | 6 | |
| B2 | 44 | 18 | 26 | |
| B3 | 12 | 2 | 10 | |
| Thymic carcinoma | 13 | 4 | 9 | |
| Masaoka’s clinical staging | | | | |
| I | 25 | 10 | 15 | 0.269 |
| II | 41 | 12 | 29 | |
| III | 21 | 10 | 11 | |
| IV | 13 | 4 | 9 | |
| Associated with autoimmune diseases (cases with MG) | 67 (61) | 27 (24) | 40 (37) | |
| Without any autoimmune disease | 33 | 9 | 24 | |
Figure 1  Growth of B2 thymoma cells in collagen gel droplet cultures with different types of chemotherapeutic agents after culture for 5–7 days. By measuring the size of colonies, drug sensitivity was tested. The larger colonies in size were, the less sensitive thymoma cells were to the tested anti-cancer drugs. CBDC, 2.0 μg/mL carboplatin; CDDP, 0.2 μg/mL cisplatin; ADR, 0.02 μg/mL adriamycin; EPI, 0.1 μg/mL Epirubicin; VP-16, 1.0 μg/mL Etoposide; PAC, 1.0 μg/mL Paclitaxel; VNR, 0.01 μg/mL Navelbine; GEM, 8.0 μg/mL Gemcitabine; 5-FU, 1.0 μg/mL 5-fluorouracil; DOC, 0.1 μg/mL Docetaxel; MTA, 0.90 μg/mL Pemetrexed.
the viable cancer cells that adhered to the collagen gel were collected, treated again with EGTA-trypsin and filtered through a 125 μm nylon mesh.

Type I collagen (Cellmatrix Type CD; Kurabo, Japan), 10X F-12 medium and reconstruction buffer were added to ice water at a ratio of 8:1:1. The prepared cancer cell suspension was added to the mixed collagen solution with a final density of 2–5 × 10^5 cells/mL. Three drops of the collagen-cell mixture (30 μL/drop) were placed in each well of six-well plates and in a 35 mm dish and left to set at 37°C in a CO₂ incubator. The final concentration was 3 × 10^5 cells/droplet. Then one hour later, 3 mL of DF medium containing 10% FBS (DF-10) was overlaid on each well and 6 mL on the 35 mm dish and incubated in a CO₂ incubator at 37°C overnight.

At the 0 time control, the drops in the 35 mm dish were stained with neutral red and fixed with 10% formalin and dried. In each well of the six-well plates, the anticancer drugs were added at final concentrations and incubated for 24 hours. Following the removal of the medium containing the anticancer drugs, each well was rinsed twice, overlaid with serum-free culture medium (PCM-2; Kurabo, Japan) and incubated further for seven days. Only 5-fluorouracil (TS-1) and Capecitabine (CAP) was left in the culture media for seven days. Control drops were also cultured for seven days without the drugs under the same condition.

After the seven day culture period, neutral red was added to each well at a final concentration of 50 μg/mL, and viable colonies in the droplets were stained for 1–2 hours. Neutral red staining was used to eliminate the interference of fibroblasts. The tumor cells were dyed red by neutral red, while the fibroblasts were not stained or lightly stained. The fibroblasts could then be removed. After that, each droplet was fixed with 10% formalin, washed in water and dried. A video microscope (Kurabo, Japan), grayscale image digitizer (Kurabo, Japan), personal computer and modification of the NIHImage Macro-program (Primage; Kurabo, Japan) were used to measure and quantify the amount of neutral red dye taken up by the viable cells in the droplets.

When the ratio of control (7-day culture without drug) to the 0-time control was >0.8, the case was regarded as assessable. The growth rate of tumor cells was determined by the T/C ratio (T was the image optical density of the chemo-treated samples on day 7 and C was the image optical density of nontreated controls on day 7). The lower the growth rate of tumor cells was, the greater damage the tested anticancer drugs had on cancer cells, the more sensitive cancer cells were to the tested anticancer drugs.

1 When the growth rate of tumor cells was ≤50%, it showed that individual cancer cells had a high sensitivity to the medicine.

2 When the growth rate of tumor cells was >60%, it showed that individual cancer cells were around the boundary of low and high sensitivity (moderate sensitivity).

3 When the growth rate of tumor cells was >60%, it proved that individual cancer cells had a low sensitivity to the medicine.

**Statistical analysis**

All analyses were performed with IBM SPSS Statistics 19.0. Continuous variables were expressed as mean ± standard deviation (SD). Discrete variables expressed as mean (range) were compared using paired sample t-tests. The χ² test was used to compare frequencies among different groups. P-values less than 0.05 were considered statistically significant. The image analysis method used in this study was the software Primage 1.0.6.3.

**Results**

A total of 36 out of 100 patients with TETs were sensitive to different types of chemotherapeutic agents, while 64 were not sensitive to chemotherapeutic agents as listed in Table 1. There were no significant differences in age, histological type, clinical staging, or association with autoimmune diseases between sensitive and nonsensitive cases. Data information on sensitive cases is listed in Table 2. Among these, six cases were type AB thymoma, six were type B1, 18 were type B2, two were type B3 and there were four cases with thymic carcinoma. One thymoma patient was associated with MG and acute promyelocytic leukemia, one with systemic lupus erythematosus (SLE), one with dermatomyositis, one with MG and mammary cancer, 22 solely with MG, and one with multiautoimmune disorders (including primary adrenocortical hypofunction, optic neuritis, optic nerve degeneration and allergic dermatitis). Among 36 cases sensitive to chemotherapeutic drugs, 18 were solely sensitive to one antitumor agent. WHO histologic subtypes of TET's in relation to the sensitivity to chemotherapeutic agents are shown in Table 3. We found that B1 and B2 types of thymoma were relatively more sensitive to chemotherapeutic agents (6/12 and 18/44, respectively), while sensitivity of B3-type cases to chemotherapeutic agents was much lower (only 2/12). Among six patients with B1 thymoma sensitive to antitumor agents, four were sensitive to EPI; B2 thymoma was sensitive to a broad spectrum of chemotherapeutic agents (including EPI, PAC, DOC, VNR, GEM and 5FU) as shown in Table 3. For sensitive AB thymoma, four out of six cases were sensitive to EPI. Besides EPI, 2/6 cases were sensitive to CBDCA, PAC, DOC, and VNR, respectively. For sensitive thymic carcinoma, two cases were sensitive to EPI, two to VNR, three to VP-16, and one to Alimta. Two...
| Patient Id | Gender | Age | Associated autoimmune disorders/previous malignancy | Size of primary lesion (cm) | Invasion of neighboring organs | Approach of surgery | Clinical resection status | WHO histological type | Chemosensitive agents (survival rate of tumor cells) | Masaoka clinical staging | Metastasis of Lymph Nodes | Metastasis of Distant Metastasis |
|------------|--------|-----|---------------------------------------------------|-----------------------------|-----------------------------|----------------------|-------------------------|-----------------------|--------------------------------------------------|--------------------------|---------------------------|-----------------------------|
| 1          | Male   | 45  | MG (IIb)                                          | 4.7 \times 7.1 \times 8.6 cm | Pericardium                 | Sternotomy           | Complete AB             | Thymoma               | PAC (58.29%), AUMTA (59.39%)                          | III                     | N0                        | M0                          |
| 2          | Male   | 65  | Lupus erythematosus                               | 4.7 \times 4.1 \times 5.3 cm | Left phrenic nerve          | VATS                 | Complete AB             | Thymoma               | EPI (59.27%)                                      | III                     | N0                        | M0                          |
| 3          | Female | 63  | MG (I)                                            | 3.8 \times 2.9 \times 3.7 cm | No                          | VATS                 | Complete AB             | Thymoma               | EPI (48.75%)                                      | I                       | N0                        | M0                          |
| 4          | Male   | 56  | No                                                | 3.5 \times 2.5 \times 1.6 cm | No                          | VATS                 | Complete AB             | Thymoma               | CBDCA (41.22%), VNR (40.75%), DOC (53.17%), EPI (53.22%) | IIa                     | N0                        | M0                          |
| 5          | Female | 72  | No                                                | 6.5 \times 5.0 \times 5.0 cm | No                          | Sternotomy           | Complete AB             | Thymoma               | CBDCA (48.65%), VNR (45.46%), DOC (58.77%)          | I                       | N0                        | M0                          |
| 6          | Female | 47  | No                                                | 4.8 \times 3.9 \times 1.5 cm | No                          | VATS                 | Complete AB             | Thymoma               | PAC (52.38%), EPI (58.42%)                          | I                       | N0                        | M0                          |
| 7          | Female | 44  | MG (IIb)                                          | 3.5 \times 2.0 \times 1.7 cm | Pericardium, innominate vein | Sternotomy           | Complete B1 Thymoma    |                                      | CBDCA (50.13%), CBDC (53.38%), ADR (52.52%), EPI (40.70%), VP-16 (52.37%), PAC (17.75%), VNR (59.16%), SFU (38.45%), GEM (52.53%), DOC (24.62%) | III                     | N0                        | M0                          |
| 8          | Female | 49  | No                                                | 3.3 \times 2.0 \times 1.5 cm | No                          | VATS                 | Complete B1 Thymoma    |                                      | CBDCA (50.13%), CBDC (53.38%), ADR (52.52%), EPI (40.70%), VP-16 (52.37%), PAC (17.75%), VNR (59.16%), SFU (38.45%), GEM (52.53%), DOC (24.62%) | I                       | N0                        | M0                          |
| 9          | Male   | 44  | MG (Ia)                                           | 5.5 \times 3.5 \times 2.7 cm | Right mediastinal pleura    | VATS                 | Complete B1 Thymoma    |                                      | EPI (54.00%)                                     | IVA                     | N0                        | M1a                         |
| 10         | Male   | 46  | MG (I)                                            | 4.0 \times 3.5 \times 1.5 cm | No                          | VATS                 | Complete B1 Thymoma    |                                      | CDDP (53.57%), CBDC (57.43%), ADR (57.21%), VP-16 (57.84%), SFU (58.88%), GEM (53.75%), DOC (56.85%) | I                       | N0                        | M0                          |
| Patient Id | Gender | Age | Associated autoimmune disorders/previous malignancy | Size of primary lesion (cm) | Invasion of neighboring organs | Approach of surgery | Clinical resection status | WHO histological type | Chemosensitive agents (survival rate of tumor cells) | Masaoka clinical staging | Metastasis of Lymph Nodes | Metastasis of Distant Metastasis |
|------------|--------|-----|-----------------------------------------------------|-----------------------------|-------------------------------|---------------------|------------------------|-------------------|---------------------------------------------|-----------------|-------------------|------------------------|
| 11         | Male   | 33  | MG (I)                                              | 6.3 x 4.5 x 4 cm            | No                            | VATS                | Complete              | B1 Thymoma         | EPI (43.41%)                               | Iib             | N0                | M0                     |
| 12         | Male   | 53  | MG (IIb)                                            | 5.5 x 3.6 x 5.3 cm          | No                            | VATS                | Complete              | B1 Thymoma         | GEM (50.45%)                               | I               | N0                | M0                     |
| 13         | Female | 50  | MG (IIIa), Mammary cancer                           | 7.2 x 4.1 x 1.7 cm          | Pericardium, right upper lobe, right mediastinal pleura | Sternotomy          | Complete              | B2 Thymoma         | EPI (47.58%)                               | Iib             | N0                | M0                     |
| 14         | Male   | 65  | No                                                   | 4.5 x 2.1 x 2.1 cm          | No                            | VATS                | Complete              | B2 Thymoma         | EPI (57.54%)                               | I               | N0                | M0                     |
| 15         | Male   | 48  | MG (I)                                              | 6.3 x 3.4 x 2.3 cm          | No                            | VATS                | Complete              | B2 Thymoma         | VNR (56.32%)                               | Iib             | N0                | M0                     |
| 16         | Female | 49  | No                                                   | 7.3 x 6.3 x 2.6 cm          | Left mediastinal pleura       | Sternotomy          | Complete              | B2 Thymoma         | VNR (54.46%)                               | Ila             | N0                | M0                     |
| 17         | Female | 53  | No                                                   | 12.5 x 9.3 x 5.1 cm         | Right phrenic nerve, right mediastinal pleura, pericardium | VATS                | Complete              | B2 Thymoma         | VNR (34.17%)                               | III             | N0                | M0                     |
| 18         | Male   | 64  | MG (I)                                              | 3.6 x 2.6 x 3.3 cm          | Right upper lobe              | VATS                | Complete              | B2 Thymoma         | VNR (19.68%)                               | Iib             | N0                | M0                     |
| 19         | Male   | 56  | MG (IIb)                                            | 2.5 x 1.9 x 2.5 cm          | Innominate vein, pericardium, left upper lobe | Sternotomy          | Complete              | B2 Thymoma         | CDDP (54.69%), CBDCA (55.98%), ADR (37.25%), SFU (33.84%) | III             | N0                | M0                     |
| 20         | Female | 54  | MG (I)                                              | 2.3 x 2.6 x 1.7 cm          | No                            | Sternotomy          | Complete              | B2 Thymoma         | PAC (54.60%), SFU (46.29%), GEM (48.36%), DOC (35.84%) | I               | N0                | M0                     |
| 21         | Male   | 32  | MG (I)                                              | 5.5 x 1.5 x 10 cm           | No                            | VATS                | Complete              | B2 Thymoma         | 5FU (48.71%), PAC (51.34%), DOC (51.75%), EPI (51.75%) | Iib             | N0                | M0                     |
| 22         | Female | 54  | MG (IIb), acute promyelocytic leukemia               | 3.2 x 1.4 x 2.5 cm          | No                            | VATS                | Complete              | B2 Thymoma         | VNR (17.78%), GEM (23.67%), PAC (39.78%) | Ila             | N0                | M0                     |
| 23         | Male   | 38  | MG (IIb)                                            | 6.3 x 1.8 x 1.0 cm          | Pericardium                   | VATS                | Complete              | B2 Thymoma         | PAC (51.59%), SFU (32.49%), GEM (36.47%), DOC (19.70%), ALIMTA (52.68%) | III             | N0                | M0                     |
| 24         | Female | 53  | MG (IIb)                                            | 3.5 x 1.5 x 10 cm           | No                            | VATS                | Complete              | B2 Thymoma         | 5FU (58.59%), PAC (56.87%), EPI (53.68%) | Ila             | N0                | M0                     |
| 25         | Male   | 48  | MG (I)                                              | 3.3 x 2.7 x 2.0 cm          | No                            | VATS                | Complete              | B2 Thymoma         | 5FU (37.72%), PAC (56.87%), EPI (53.68%) | I               | N0                | M0                     |
| Patient Id | Gender | Age | Associated autoimmune disorders/previous malignancy | Size of primary lesion (cm) | Invasion of neighboring organs | Approach of surgery | Clinical resection status | WHO histological type | Chemosensitive agents (survival rate of tumor cells) | Masaoka clinical staging | Metastasis of Lymph Nodes | Metastasis of Distant Sites |
|------------|--------|-----|--------------------------------------------------|-----------------------------|-------------------------------|---------------------|-------------------------|-----------------------|------------------------------------------------|---------------------------|------------------------|---------------------------|
| 26         | Male   | 45  | MG (IIa)                                         | 8.4 × 5.2 × 2.7 cm          | No Pericardium                | VATS                | Complete               | B2 Thymoma            | GEM (55.82%), CDDP (55.53%), CBDCA (48.05%), ADR (21.08%), EPI (27.80%), VNR (38.80%), SFU (47.14%) | IIb                      | N0                     | M0                       |
| 27         | Female | 53  | MG (IIb)                                         | 4.7 × 3.5 × 2.5 cm          | Pericardium                  | Sternotomy          | Complete               | B2 Thymoma            | CDDP (35.53%), CBDCA (48.05%), ADR (21.08%), EPI (27.80%), VNR (38.80%), SFU (47.14%) | III                      | N0                     | M0                       |
| 28         | Male   | 66  | Primary adrenocortical hypofunction, optic neuritis, optic nerve degeneration, allergic dermatitis | 7.5 × 4.5 × 3.6 cm          | Right mediastinal pleura     | Sternotomy          | Complete               | B2 Thymoma            | VNR (48.72%), GEM (32.47%), PAC (55.62%) | IVA                      | N0                     | M1a                      |
| 29         | Male   | 54  | MG (I)                                           | 4.9 × 3.2 × 6.0 cm          | Pericardium, right upper lobe, right middle lobe, right mediastinal pleura | VATS                | Complete               | B2 Thymoma            | DOC (56.49%) | IIb                      | N0                     | M0                       |
| 30         | Female | 66  | No                                               | 5.4 × 4.0 × 5.2 cm          | No Innominate vein, pericardium, left upper lobe | VATS                | Complete               | B2 Thymoma            | PAC (55.56%), GEM (51.52%), DOC (54.47%) | I                        | N0                     | M0                       |
| 31         | Female | 55  | MG (I)                                           | 5.2 × 9.5 × 11.3 cm         | No Innominate vein, pericardium, left upper lobe | Sternotomy          | Incomplete             | B3 Thymoma            | GEM (55.56%), DOC (54.47%) | IVA                      | N0                     | M1a                      |
| 32         | Female | 43  | MG (I)                                           | 11 × 10.5 × 5.5 cm          | Innominate vein, pericardium, right upper lobe, right mediastinal pleura, superior vena cava | Sternotomy          | Complete               | B3 Thymoma            | EPI (50.59%), GEM (55.79%), DOC (53.41%) | IVA                      | N0                     | M1a                      |
| 33         | Female | 54  | No                                               | 3.6 × 2.5 × 2.2 cm          | No Innominate vein, pericardium, left upper lobe, superior vena cava | VATS                | Complete               | Thymic Carcinoma      | EPI (53.01%), VP-16 (59.15%) | I                        | N0                     | M0                       |
| 34         | Male   | 61  | Dermatomyositis                                  | 15.2 × 6.8 × 4.7 cm         | Innominate vein, pericardium, left upper lobe, superior vena cava | Sternotomy          | Complete               | Thymic Carcinoma      | EPI (50.07%), VP-16 (56.65%) | III                      | N0                     | M0                       |
cases with A-type thymoma were not sensitive to any anti-tumor drugs as shown in Table 3.

As shown in Table 4, among 11 chemotherapeutic agents tested in our study, the sensitivity to EPI was the highest (16%), while Alimta had the lowest sensitivity. No patients with thymoma were sensitive to Alimta. Only one patient with thymic carcinoma was moderately sensitive to Alimta. Besides EPI, the sensitivities to PAC, DOC, VNR and GEM were over 10%. Our data demonstrated that CDDP, ADR, and 5-FU were effective medicines for some patients with type B1 or B2 thymoma; CBDCA, PAC, VNR and GEM were effective for some cases with types AB, B1 or B2 thymoma; VP-16 was effective for some patients with B1-type thymoma or thymic carcinoma; EPI could be useful for most subtypes of thymoma and thymic carcinoma except type A thymoma; and DOC could be used for most subtypes of thymoma except type A thymoma.

**Discussion**

TETs, such as thymoma and thymic carcinoma, are peculiar epithelial neoplasms located at the anterior mediastinum. Some may show aggressive clinical behavior, while the majority demonstrate an indolent growth pattern.\(^{15,16}\) Complete resection is generally regarded as the most effective treatment for patients with malignant tumors, provided that the tumors are resectable. The development of targeted therapies has been delayed by the insufficient characterization of the genetic abnormalities of thymic malignant tumors.\(^{17}\) Nonresectable and metastatic thymic malignant tumors are candidates for chemotherapy. Efficacy of chemotherapy varies between reports. Some studies have reported that chemotherapy achieved tumor responses in 60%–80% of patients,\(^{18,19}\) while some clinical data demonstrated that the result of chemotherapy was not fully satisfactory in thymic malignant tumors.\(^{20,21}\)

To the best of our knowledge, our current work is the first systemic evaluation of sensitivity of all pathological types of TET cells to common chemotherapeutics using the CD-DST method. CD-DST has been developed as an evaluation system of effective anticancer drugs. This culture system models the 3-D growth of cancer cells and has been proven to show high predictive accuracy for clinical responses to anticancer drugs.\(^{22,23}\)

A number of anticancer drugs have been reported to treat thymic malignant tumors.\(^ {24,25}\) In our current study, we tested 11 kinds of anticancer drugs, which have been reported in former studies to be possibly effective to treat thymoma or thymic carcinoma. Among 100 cases with TETs, 36% were sensitive towards different chemotherapeutic agents. Our data showed that age, histological type,
clinical staging, or association with autoimmune diseases were not significantly associated with drug responsiveness.

In our study, there was no single drug that showed a high clinical response. Indeed, although the sensitivity of TETs to EPI was the highest, it was only 16%. In addition to EPI, the sensitivities to PAC, DOC, VNR and GEM were over 10%, echoing conclusions in earlier literature that the efficacy of anticancer drugs in the treatment of TETs is not remarkable.20,21 Possibly due to the indolent growth pattern of thymic malignant tumors, the sensitivity of TET cells to common chemotherapeutics is dramatically different from other types of cancer. For example, cisplatin sensitivity was observed in 4% of cases in this study, whereas the response rates to single-agent cisplatin reported in lung cancer are approximately 20%–30%. The combination of anticancer drugs to treat thymoma or thymic carcinoma might produce a relatively higher percentage of clinical responses, as reported in previous studies.24-26

Of note, EPI seemed to be the best choice of B1 thymoma, while for patients with B2 thymoma, EPI, PAC, DOC, VNR, GEM and 5FU might be good choices (as shown in Table 3). For sensitive AB thymoma, four out of six cases were sensitive to EPI. In addition to EPI, 2/6 cases were sensitive to CBDCA, PAC, DOC, and VNR, respectively. These results are worth taking into consideration in setting up clinical guidance for patients with TETs. In our study, it should be noted that Alimta had the lowest sensitivity and no patients with thymoma were sensitive to Alimta, which is in drastic contrast with current dogma. Our work therefore suggests a patient care practice: screening with a CD-DST culture system to determine the drugs for treating patients with non-resectable and metastatic thymic malignant tumors.

In conclusion, the successful selection of chemotherapy is necessary for TETs, and information on in vitro drug sensitivity or resistance may be valuable to guide individual chemotherapy. An important aspect of our work was to shed light on the effectiveness of chemotherapy for TETs and provide important clues for choosing antitumor drugs with relatively high drug sensitivity to certain type of TETs in advance.

Table 3 Drug sensitivities of different World Health Organization histologic subtypes of thymoma and thymic carcinoma

| Number Sensitive cases | Sensitive chemotherapeutic agents                                                                 | Percentage of sensitive cases |
|------------------------|-----------------------------------------------------------------------------------------------------|------------------------------|
| A                      | 2 cases were sensitive to CBDCA; two to EPI; two to PAC; two to DOC; two to VNR                      | 0                            |
| AB                     | 17 cases were sensitive to CBDCA; two to EPI; two to DOC; two to VNR; three to GEM; two to 5FU; two to VP-16 | 35.3%                        |
| B1                     | 12 cases were sensitive to CBDCA; two to EPI; one to PAC; two to DOC; one to VNR; three to GEM; two to 5FU; two to VP-16 | 50%                          |
| B2                     | 44 cases were sensitive to CBDCA; two to EPI; seven to PAC; five to DOC; seven to VNR; five to GEM; six to 5FU | 40.9%                        |
| B3                     | 12 cases were sensitive to CBDCA; two to EPI; two to DOC; two to GEM                                  | 16.7%                        |
| Thymic carcinoma       | 13 cases were sensitive to EPI; two to VNR; three to VP-16; one to Alimta                             | 30.8%                        |

Table 4 Effectiveness of certain chemotherapeutic agents to treat TETs

| Subtypes of thymic malignant tumors | High-sensitivity | Moderate-sensitivity | A | AB | B1 | B2 | B3 | Thymic carcinoma | Number of sensitive cases (%) |
|-------------------------------------|------------------|---------------------|---|----|----|----|----|-------------------|------------------------------|
| CDDP                                | 1                | 3                   | 0 | 0  | 2  | 2  | 0  | 0                 | 4 (4%)                       |
| CBDCA                               | 3                | 3                   | 0 | 2  | 2  | 2  | 0  | 0                 | 6 (6%)                       |
| ADR                                 | 2                | 2                   | 0 | 0  | 2  | 2  | 0  | 0                 | 4 (4%)                       |
| EPI                                 | 5                | 11                  | 0 | 4  | 4  | 5  | 1  | 2                 | 16 (16%)                     |
| VP-16                                | 0                | 5                   | 0 | 0  | 2  | 0  | 0  | 3                 | 5 (5%)                       |
| PAC                                 | 2                | 8                   | 0 | 2  | 1  | 7  | 0  | 0                 | 10 (10%)                     |
| VNR                                 | 7                | 5                   | 0 | 2  | 1  | 7  | 0  | 2                 | 12 (12%)                     |
| 5FU                                 | 7                | 1                   | 0 | 0  | 2  | 6  | 0  | 0                 | 8 (8%)                       |
| GEM                                 | 4                | 6                   | 0 | 3  | 5  | 2  | 0  | 0                 | 10 (10%)                     |
| DOC                                 | 3                | 8                   | 0 | 2  | 2  | 5  | 2  | 0                 | 11 (11%)                     |
| ALIMTA                              | 0                | 1                   | 0 | 0  | 0  | 0  | 0  | 1                 | 1 (1%)                       |
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Disclosure

The authors declare no conflict of interest.

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