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Clinical Predictive Value of CD-DST for the Efficacy of Postoperative XELOX Adjuvant Chemotherapy in Advanced Gastric Adenocarcinoma

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Keywords:

CD-DST; gastric adenocarcinoma; drug sensitivity; XELOX adjuvant chemotherapy

1. Abstract

1.1. Introduction: Anticancer drug insensitivity/resistance is a bottleneck in the treatment of gastric cancer. To overcome this challenge, we evaluated the clinical utility of an in-vitro collagen gel droplet embedded culture drug sensitivity test (CD-DST) using patient-derived gastric cancer tissues for personalized treatment guidance.

1.2. Methods: We collected the surgically removed fresh tumor tissue samples from 72 patients with advanced gastric adenocarcinoma who underwent surgical resection between March and December 2018. Overall survival (OS) and disease-free survival (DFS) were analyzed using the Kaplan-Meier method.

1.3. Results: A total of 72 patients with gastric cancer underwent surgical resection. Of the 72 patients, 65 (90.28%) were successfully evaluated for in-vitro sensitivity to 5-FU and L-OHP using the CD-DST. All patients were grouped into high- and low-sensitivity groups based on CD-DST results. Fifteen patients died of gastric cancer during the two-year follow-up period, and 19 relapsed. Of the 15 patients who died, 14 were in the low-sensitivity and one from the high-sensitivity group. Of the 19 who relapsed, 18 were in the low-sensitivity group and one from the high. We observed a significant difference between the high- and low-sensitivity groups in OS (log-rank test, P = .005) and DFS (log-rank test, P = .001).

1.4. Conclusions: Both OS and DFS were remarkably higher for patients in the high-sensitivity group, revealing that the in-vitro CD-DST technique could successfully predict postoperative XELOX adjuvant chemotherapy efficacy in advanced gastric adenocarcinoma. Thus, the present study strongly recommends anticancer drugs' sensitivity screening using the CD-DST for individualizing chemotherapy to avoid ineffective chemotherapy.

2. Introduction

Gastric Cancer (GC) is a common and highly malignant tumor in nature. According to GLOBOCAN 2020 issued estimation of cancer incidence and mortality, GC ranked 5th in incidence with 1.09 million estimated new cases and 4th in cancer-related mortality with 0.77 million mortalities worldwide (Sung et al. 2021). However, the situation in China is much more alarming; there were 0.48 million estimated newly diagnosed cases of GC and 0.37 million mortalities in 2020, contributing 43.9% of the total number of newly diagnosed cases and 48.6% of GC deaths worldwide (Sung et al. 2021). It may be due to genetics, foods, smoking, alcohol, rapid increase in aging, population growth, and socioeconomic status.

Cancer treatment is constantly evolving, with new anticancer agents regularly being approved. The addition of targeted therapies has shown modest improvements in survival (Soularue et al. 2015; Fuchs

et al. 2014; Wilke et al. 2014; Bang et al. 2010). Cancer immunotherapy is the latest addition to the list, which effectively uses the body's immune system against cancer (Salmaninejad et al. 2019). Moreover, in recent years the combinations of anticancer agents have been broadly used, which have improved survival and life quality (Van Cutsem et al. 2015; Shah et al. 2015; Guimbaud et al. 2014; Kang et al. 2009; Okines et al. 2009; Al-Batran et al. 2008; Dank et al. 2008). However, radical surgery is still the only curative treatment for GC despite all these advancements. Unfortunately, the results are still unsatisfactory, owing to the high rate of metastasis and relapse (Jemal et al. 2011; Sant et al. 2009). Besides, radical surgery is curative only in early-stage and non-metastatic advanced-stage cancer. Therefore, systemic chemotherapy is currently the primary treatment option for metastatic cancer, but its use can be limited due to severe side effects or drug resistance (Wang et al. 2017). Moreover, it has been seen that cancer patients are unable to continue chemotherapy due to poor nutritional status. Furthermore, these anticancer agents may not equally respond; even cancer with the same histologic features may have a different efficacy of the same chemotherapy drugs in case of individual differences. Consequently, non-responders may suffer from the cost and adverse reactions without clinical benefit.

According to NCCN guidelines, the XELOX chemotherapy regimen is commonly used as postoperative clinical chemotherapy, which can effectively improve the survival of patients with gastric cancer (Ajani et al. 2016). However, similar to other chemotherapy regimens, the XELOX regimen also has noticeable individualized differences (Zhao et al. 2020). Therefore, to make Gastric cancer patients benefit from XELOX chemotherapy, it is necessary to carry out a sensitivity test to screen the potential beneficiaries of the XELOX regimen and reduce unnecessary ineffective chemotherapy.

The collagen gel droplet embedded culture drug sensitivity test (CD-DST) is a simple three-dimensional (3D) culture method (Kobayashi et al. 1997). CD-DST analyzes the chemosensitivity of cancer cells after distinguishing them from fibroblast cells contaminating the sample at harvesting. It can reproduce a biological tumor microenvironment with cell-cell contact, a vital element of tumor progression (Yasuda et al. 1998; Junttila and de Sauvage 2013). This replication of in vivo environments is advantageous because it analyzes anticancer drugs at physiological concentrations using notably small clinical samples (Kobayashi et al. 1997; Yasuda et al. 1998). Previously, the clinical application value of the CD-DST has been demonstrated for various cancers, including pancreatic cancer (Ariake et al. 2019), lung cancer (Higashiyama et al. 2010; Kawamura et al. 2007), and colorectal cancer (Mekata et al. 2013; Ochiai et al. 2017). However, thus far, there is a lack of research exploring CD-DST clinical feasibility and utility in advanced GC patients. Hence, we conducted this study to evaluate the clinical feasibility and application value of in-vitro CD-DST for postoperative individualized adjuvant chemotherapy. We adopted the postoperative fluorouracil in combination with the L-OHP regimen, which has been extensively practiced as first-line therapy to treat advanced gastric cancer globally.

3. Materials and Methods

3.1. Patient and Tumor Tissue Collection

The study was approved by the ethics committee of First Affiliated Hospital of Jinan University. The written informed consent of all patients was obtained. The inclusion criteria were histologically diagnosed gastric adenocarcinoma patients, regardless of gender, aged between 18-80 years and with no other primary diseases. We collected fresh tumor tissue samples from 72 gastric cancer patients who met the inclusion criteria and underwent surgical resection at the First Affiliated Hospital of Jinan University for CD-DST between March to December 2018. All patients were followed up regularly for two years after surgical resection. Each sample was no less than 0.5g. Cancer tissues rich in tumor cells were collected, and necrotic portions were avoided. After collection, samples were washed, placed into the preservation solution, and transferred to the laboratory at 2 ~ 8℃. The clinico-demographic characteristics of the 72 cases of gastric cancer are listed in Table 1.

3.2. Operational protocol of CD-DST

The entire experimental process of CD-DST is shown in Figure 1.

3.3. Preparation of tumor cell-spheroid suspensions

The fresh gastric tumor tissue samples separated from any outer fascia, fat, blood clots, necrotic tissue, or other attachments were finely grounded using a sterile technique. Each fresh gastric tumor specimen was poured into the sterile plate containing a little DMEM/F12 medium (Gibco, USA), where the tissue was sheared into about 3~5 mm small blocks. Then, the tissue blocks were transferred into another sterile plate, chopped into the mud with sterile razor blades, suspended in DMEM/F12 medium, and treated with a cell dispersion enzyme (EZ; DaruiBiotech, China) at 37℃ for 1-3 hours. Following disintegration, DMEM/F12 containing serum was added to terminate EZ cellular enzyme reaction and centrifuged at 1000 rpm for 5 minutes. The supernatant was cautiously removed and discarded without disturbing the pellet, and the dispersed tumor cells were collected. The recovered cells were filtered with a 300μm nylon membrane, resuspended in PCM-1 media (DaruiBiotech, China), and cultured in a collagen gel (CG)-coated flask (DaruiBiotech, China) in a CO2 incubator (ThermoFisher, USA) at 37℃ for 12-24 hours. After pre-culture, the CG culture flask was gently shaken, and the medium was removed. The cell dispersion enzyme was added to the CG culture flask to dissolve collagen gel on low-speed oscillation at 37℃ for 15~30 minutes. Next, DMEM/F12 containing serum was added to terminate the EZ cellular enzyme reaction after the dissolution. Then, the centrifugation was done at 1000 rpm for 5 minutes, the supernatant was removed and discarded, and the dispersed tumor cells were collected. Next, the collected tumor cells were filtered with a 125μm nylon membrane, resuspended in DMEM/F12, and recentrifuged at 1000 rpm for 5 minutes. Again, the supernatant was sucked and discarded without disturbing the pellet. Next, DMEM/

F12 medium was added to the centrifuge tube to resuspend the cells, and the cell count was performed.

3.4. Collagen gel solution preparation and culturing of isolated cancer cells

In order to prepare the collagen gel solution, the collagen solution, 10×F-12 medium, and collagen reconstitution buffer were mixed at a ratio of 8:1:1 in the ice bath. Once the collagen gel solution was prepared, the suspended primary cancer cells were loaded into the collagen solution at a ratio of 1:10. The final density of the cells in the collagen solution was 2×105/ mL, and the bubbles were avoided in the loading process. Three 30-μl droplets of the cell-collagen mixture were added to each well of a 6-well plate and allowed to gel in a CO2 incubator at 37℃ for 1 hour. Next, 3 ml of DF (10) media containing 10% fetal bovine serum (GIBCO BRL, Grand Island, NY, USA) was overlaid and incubated in a CO2 incubator at 37℃ for 24 hours. Images of collagen gel droplets were taken using Cell imaging microplate detector Cytation 5 (Biotek Instrument Inc., USA).

3.5. Anticancer drugs exposure

After completing the culture, 1% neutral red solution (DaruiBiotech, China) was added to the 0- time plate. Staining, fixation, and natural drying were done per the staining protocols. The anticancer drugs were added at the following final concentrations and incubated for 24 h: 1.0 μg/ml 5-fluorouracil (5-FU) and 1μg/ml Oxaliplatin (L-OHP).

3.6. Serum-free cell culture

Following drug contact, the culture medium from each well was removed, and each well was rinsed twice with DMEM/F12 medium to wash away the anticancer drugs. This cleaning process was carried out in the treatment and control groups synchronously. Then, a serum-free medium (PCM-2; DaruiBiotech, China) was added to each well and incubated in a 5% CO2 incubator at 37℃ for another 5~7 days. During culture, the serum-free medium was changed every 2~3 days.

3.7. Staining and fixation

After serum-free cell culture, 1% neutral red was added to each well. Tumor cell colonies in the collagen gel droplets were stained for 2 hours, fixed with 10% neutral formalin, rinsed with water, and naturally dried.

3.8. Image analysis and evaluation

The total volume of tumor cells was measured using image analysis using differences in the growth morphology of tumor cells and fibroblasts. The quantification procedure was conducted using the cell analysis system DR6690 (DaruiBiotech, China), measuring the absorbance value at 540 nm (OD540). The cell proliferation rate of the control group was calculated according to the following formula: the growth rate (OD540 mean) = the total volume on day 7/total volume at 0- time. The cell proliferation rate of ≥ 0.8 was considered the standard. The sensitivity was represented as the T/C ratio, measured as the ratio of the number of tumor cells in the treatment group (T) to that in the control group (C). A T/C ratio less than or equal to 50% was considered highly sensitive, and a T/C ratio greater than 50% was deemed low.

3.9. Patient treatment and follow-up

All patients enrolled were treated with the XELOX regimen (L-OHP 130mg/m2, ivgtt, d1 and capecitabine 850~1000mg/m2, bid, PO, d1-14), repeated every 3 weeks for 8 cycles. Patients' basic personal details, pathological examination reports, clinical treatment plans, clinical imaging examination data, and other related information were collected during treatment. According to patients' contact information, regular telephone call follow-up was conducted to acquire after treatment survival state or the time of death by speaking to the patient or their family, and follow-up information was recorded truthfully. Follow-ups were arranged once every six months for two years or until the patient died or lost contact.

4. Statistical Analyses

After excluding the patients who failed the CD-DST test, the patients who were highly sensitive to either drug were included in the high-sensitivity group, and the patients who were not sensitive to both drugs were included in the low-sensitivity group. The difference between the two groups was compared using the chi-square test and t-test. Survival curves were calculated according to the Kaplan–Meier method. Log-rank test was used for univariate analysis to compare the differences in DFS and OS between the two groups. $P<0.05$ was considered statistically significant. Software IMB SPSS Statistics for Windows, version 22.0 (IBM Corp., Armonk, N.Y., USA) and GraphPad Prism version 7.0 (GraphPad Software, Inc.) were used for statistical analysis.

5. Results

5.1. Feasibility analysis of drug sensitivity detection in-vitro

From March to December 2018, 72 patients were recruited from the First Affiliated Hospital of Jinan University. At the time of inclusion, it was found that the samples of 3 patients could not be tested for chemosensitivity due to the insufficient number of tumor cells, 2 samples were discarded due to contamination during the experiment, and the cell proliferation rate of 2 samples was less than 0.8. As a result, 65 samples were successfully evaluated, and the overall success rate was 90.28%. Figure 2 shows the results of the anti-tumor sensitivity test in gastric cancer case number 19. There were 11 cases with high sensitivity to 5-FU, the mean T/C% value was 35.53 ± 3.09, 54 cases with low sensitivity, and the mean was 74.18 ± 2.25; a significant statistical difference between the two groups was observed ($P < .001$). Meanwhile, 23 cases were highly sensitive to L-OHP, with a mean T/C% of 35.12±2.44, and 42 low sensitive cases, with a mean T/C% of 69.52±1.99, and also showed significant statistical differences ($P < .001$). See Figure 3.

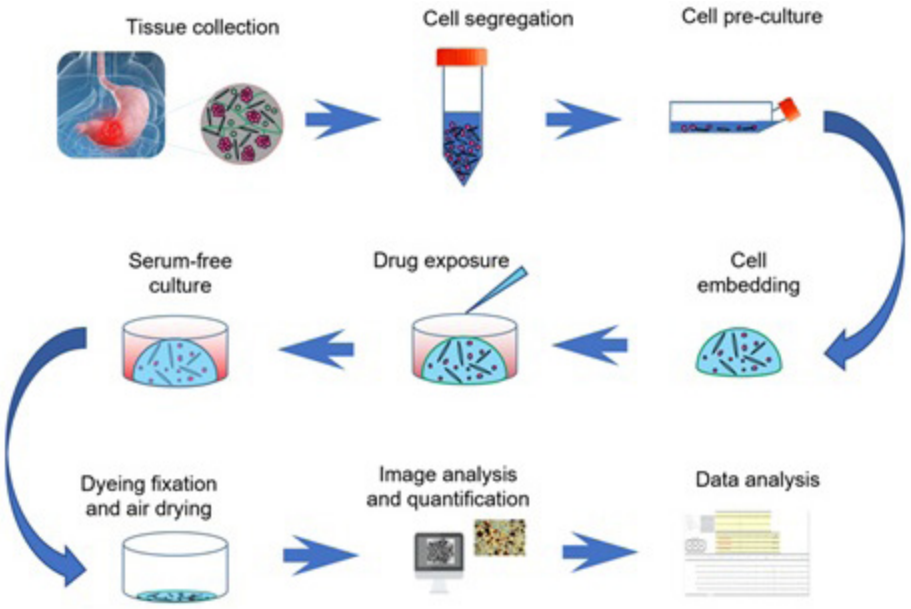


Figure 1: CD-DST experimental process diagram.

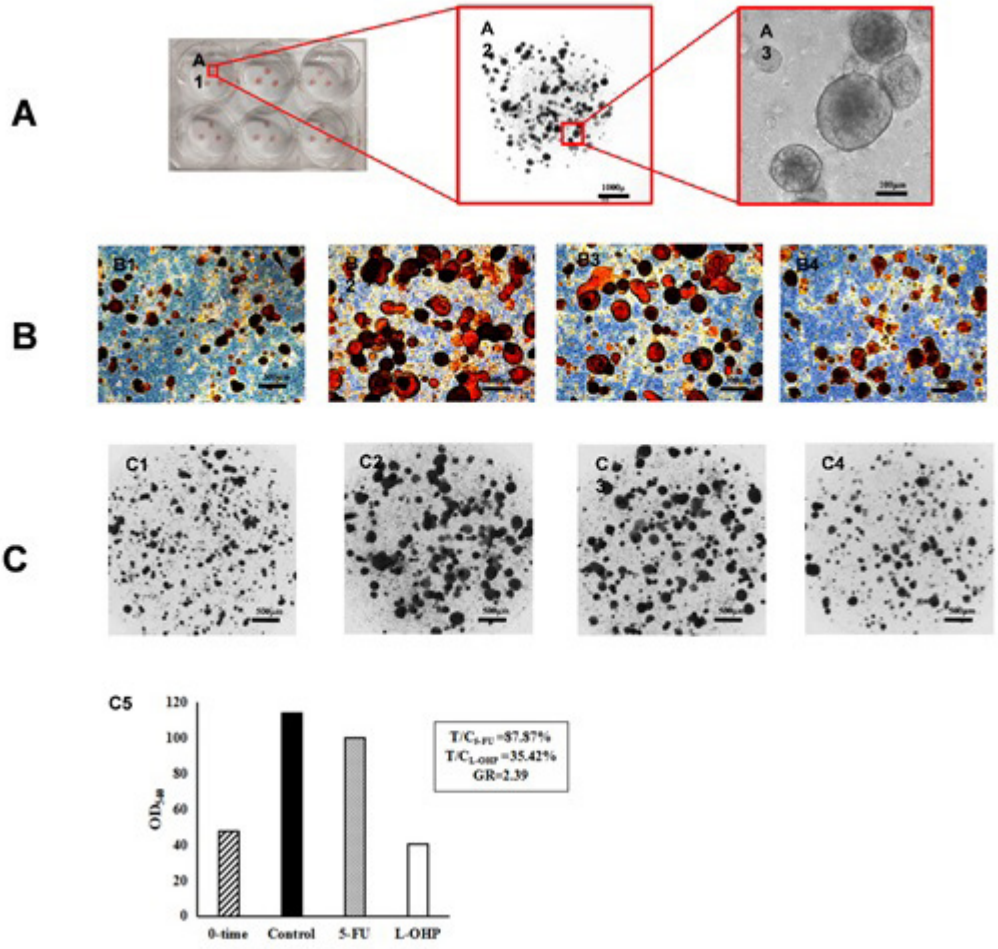
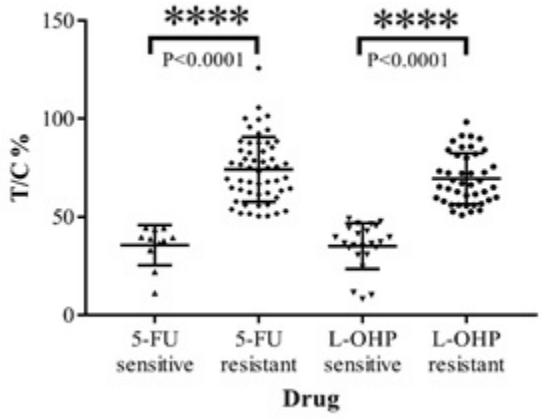


Figure 2: Case 19's result of anticancer agents' sensitivity test in patient-derived gastric cancer tissues. (A) A morphological picture of tumor cells in collagen gel droplets. (A1) Tumor cells in collagen gel droplets in 6 cell culture plates. (A2) A complete picture of the growth of tumor cells in collagen gel droplets (4x). (A3) A partial visual field (20x) for the growth of tumor cells in collagen gel droplets. (B) A picture of tumor cells in collagen gel droplets stained using neutral red. (B1 ~ B4) the pictures of tumor cells in the 0-time group, control group, 5-FU dosing group, and L-OHP dosing group after neutral red staining, respectively. (C) Scanning collagen gel droplet pictures and measuring results using the image analysis system DR6690. (C1~C4) Collagen gel droplets scanning pictures (4x) of the 0-time group, Control group, 5-FU drug group, and L-OHP drug group at the end of tumor cell culture, respectively. (C5) The CD-DST result for case 19.



		T/C (%)	
		Mean ± SEM	95% CI of mean
5-FU	Sensitive	35.53 ± 3.092	(28.64, 42.42)
	Resistant	74.18 ± 2.248	(69.67, 78.69)
L-OHP	Sensitive	35.12 ± 2.437	(30.07, 40.18)
	Resistant	69.52 ± 1.99	(65.5, 73.53)

Figure 3: Summary of CD-DST results of 65 gastric cancer samples.

5.2. Patients' characteristics

A total of 65 gastric cancer patients were compliant with the follow-up strategy, with an average age of 59.02 ± 8.9 years old, ranging from 39 to 77 years old. Of 65 patients, 39 (60%) were female, and 26 (40%) were male. According to the CD-DST results, all the patients were divided into two groups (high- and low-sensitivity) for comparative analysis. The summary of CD-DST results of all 65 gastric cancer samples is demonstrated in Figure 4. The OS and DFS of patients with gastric cancer were analyzed using the Kaplan-Meier method. The overall survival time ended with death, and disease-free survival ended with relapse, metastasis, or death. The demographic and tumor characteristics of enrolled 65 gastric cancer patients are presented in Table 2. There was no significant difference in demographic and tumor characteristics between high- and low-sensitivity groups.

5.3. Drug sensitivity testing and survival

Fifteen patients died of gastric cancer (1 from the high-sensitivity group and 14 from the low-sensitivity group) during a 2-year follow-up. Kaplan-Meier method was used to analyze and compare the 2-year overall survival between the high- and low-sensitivity groups. The mean value of 2-years overall survival time was 23.47±2.64 months in the highly sensitive group and 20.70±5.22 months in the low-sensitivity group. We observed a significant difference in the OS time between the high-sensitivity and low-sensitivity groups (log-rank test, P = .005). See Figure 4.

The number of patients with gastric cancer who relapsed was 19 (1 from the high-sensitivity group and 18 from the low-sensitivity group). Kaplan-Meier method was used to analyze and compare the 2-year disease-free survival between the high- and low-sensitive groups. The mean value of 2-year disease-free survival time in the highly sensitive group was 23.31 ± 3.44 months, while 19.16 ± 6.63 months in the low-sensitivity group. Kaplan-Meier survival curve demonstrated a significant difference in the DFS between the high- and low-sensitivity groups (log-rank test, P = .001). See Figure 5.

Table 1: Clinico-demographic characteristics of advanced gastric cancer patients.

Characteristics	All patients, n	Percentage
Total	72	
Age, years		
Age < 60 years	38	52.78%
Age ≥ 60 years	34	47.22%
Gender		
Male	44	61.11%
Female	28	38.89%
Differentiation		
Poor	36	50.00%
Moderate	30	41.67%
Well	6	8.33%
Pathology staging		
II	36	50.00%
III	32	44.44%
IV	4	5.56%
Lymph node metastasis		
N0	28	38.89%
N1, 2	44	61.11%

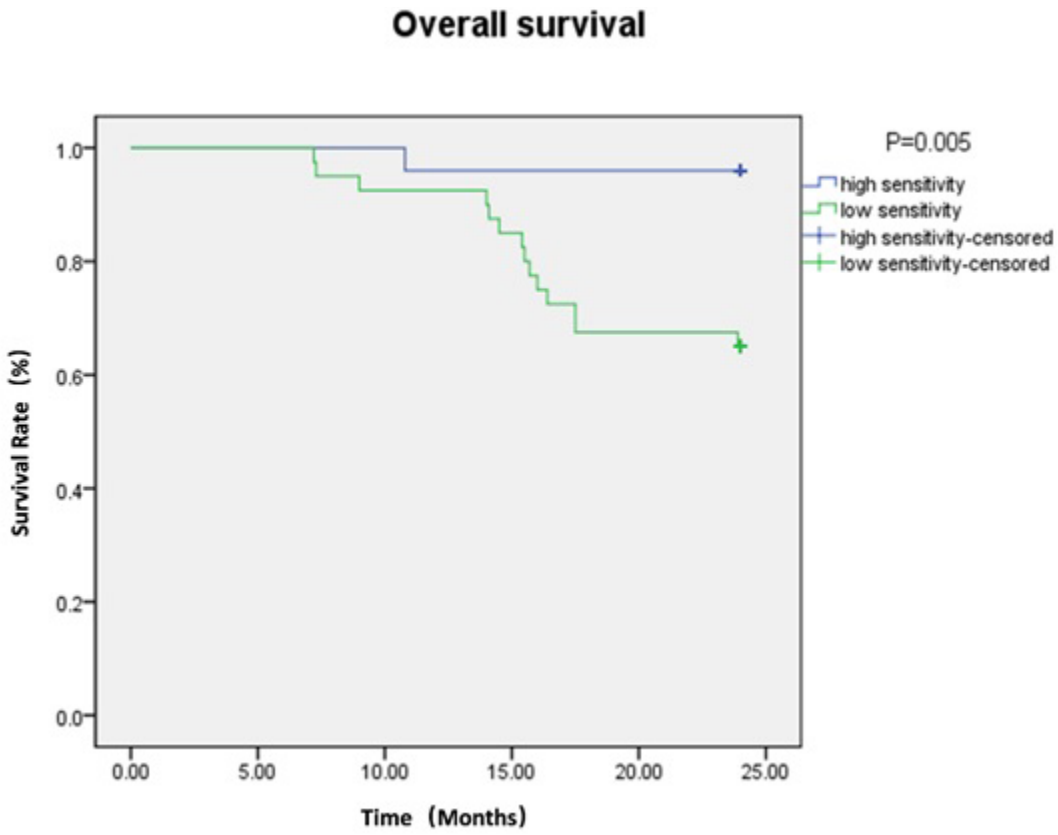


Figure 4: Comparison of overall survival curves among gastric cancer patients with high and low sensitivity. Two curves are depicted, each representing the overall survival of patients treated with in-vitro selected highly sensitive (n = 25) and low sensitive (n = 40) chemotherapeutic agents.

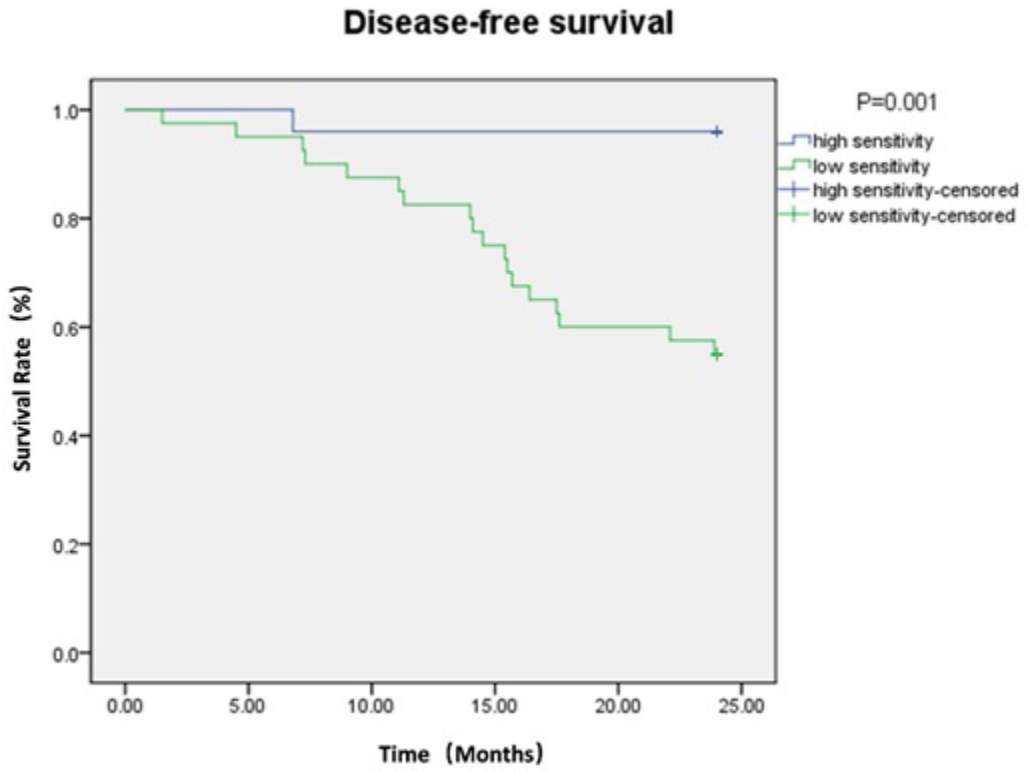


Figure 5: Comparison of disease-free survival curves among gastric cancer patients with high and low sensitivity. Two curves are depicted, each representing the disease-free survival of patients treated with in-vitro selected highly sensitive (n = 25) and low sensitive (n = 40) chemotherapeutic agents.

Table 2: Clinico-demographic characteristics of patients with CD-DST sensitive or resistant results.

Characteristics	Sensitive, %	Resistant, %	P-value
Total	25 (38.5)	40 (61.5)	
Age, years			
Age < 60 years	10 (15.4)	23 (35.4)	0.17
Age ≥ 60 years	15 (23)	17 (26.2)	
Gender			
Male	17 (26.2)	22 (33.8)	0.298
Female	8 (12.3)	18 (27.7)	
Differentiation			
Poor	13 (20)	21 (32.3)	0.736
Moderate	11 (17)	15 (23)	
Other	1 (1.5)	4 (6.2)	
Pathology staging			
II	14 (21.5)	17 (26.1)	0.241
III	11 (17)	19 (29.2)	
IV	0 (0)	4 (6.2)	
Lymph node metastasis			
N0	10 (15.5)	13 (20)	0.538
N1,2	15 (23)	27 (41.5)	

6. Discussion

In the present clinical trial, the overall anticancer agents' sensitivity evaluation success rate was 90.28%, slightly higher than the previous similar reports (Kobayashi et al. 1997; Kobayashi 2003), which may be because, in this study, the primary tumor cells were isolated and cultured in collagen-coated flasks. The tumor cells adhered to the surface of collagen, which could enhance the activity of tumor cells. In addition, the collagen gel could also maintain the survival and proliferation of tumor cells in-vitro. However, contamination is the most common problem in primary cell culture. We lost two samples due to contamination. Therefore, special attention should be paid to the procedure of collecting samples and experimentation. Besides, the difficulty of separating cells from specimens is also related to the origin of organs. Gastric cancer has more interstitial cells, which are dense and challenging to digest. Therefore, it is often difficult to obtain enough primary cells with good activity for CD-DST detection.

Like every other tumor, the risk of local recurrence and distant metastasis is still higher in patients with gastric cancer after the radical operation. The mortality rate of advanced gastric cancer is high, and the 5-year survival rate is less than 20%. More and more attention has been paid to the significance of postoperative chemotherapy but not to the individualization of chemotherapy. XELOX chemotherapy regimen is a promising treatment option for patients with advanced gastric cancer. This regimen destroys existing tumors and stops the spread of cancer to nearby cells. However, it may not be equally efficient for every patient. Consequently, non-responders may suffer from the cost and adverse reactions without clinical benefit. Hence, we investigated the predictive effect of CD-DST in XELOX adjuvant chemotherapy after advanced gastric adenocarcinoma surgery. In this exploratory study, all enrolled patients with gastric cancer were given an adjuvant XELOX regimen after surgical resection and followed up regularly for two years. The overall survival time ended with death, and progression-free survival ended with relapse, metastasis, or death. Fifteen patients died of gastric cancer during the two-year follow-up period, and 19 relapsed. 13 dead and 18 relapsed patients were from the low-sensitivity group. There was no remarkable difference in demographic and tumor characteristics between the two groups. As one of the major findings, the Kaplan-Meier survival curves demonstrated that patients in the sensitive group had better OS (P=0.005) and DFS (P = .001). These findings verify that in-vitro CD-DST is a valuable and reliable technique for predicting the clinical efficacy of the post-operation adjuvant chemotherapy XELOX regimen.

In 2007, the New England Journal published a clinical trial named ACTS-GC (Sakuramoto et al. 2007), which compared the effects of oral S-1 adjuvant chemotherapy and surgery alone in Japan on 1059 patients with stage II or III gastric cancer undergoing D2 radical resection. The 3-years overall survival rate was 80.1% in the S-1 group and 70.1% in the surgery group. Compared with surgical treatment alone, the risk ratio of death in the S-1 group was 0.68 (P < .01), and the recurrence-free risk ratio was 0.62 (P < .01), which confirmed that S-1 adjuvant chemotherapy could prolong the survival of patients with gastric cancer after surgery. The 5-years follow-up data also confirmed the above results. A subsequent study of GACCRO-GC 04 showed that (Tanigawa et al. 2016) CD-DST technology has guiding significance for screening gastric cancer patients sensitive to S-1 drug. In that study, 311 patients from 32 clinical centers across Japan were recruited between 2005 to 2013. The median follow-up time was 3.2 years. According to the statistical analysis of the follow-up information, it was found that there was a significant statistical difference in the recurrence-free survival rate between patients with high sensitivity to S-1 and patients with low sensitivity to CD-DST after three years. The overall survival rate of the highly sensitive group was close to that of the S-1 treatment group. In contrast, the overall survival rate of the low sensitive group was comparable to that of the operation alone group. The findings of these two studies are consistent with the present study, which advocate CD-DST-guided chemotherapy in cancer patients to avoid ineffective chemotherapy.

Altogether, these findings suggest using CD-DST technology to screen the sensitive population and avoid ineffective chemotherapy. However, CD-DST has some disadvantages. It requires fresh, surgically resected tumor tissue samples with sufficient tumor cells, so it needs higher technical requirements for sampling and cell culture. In addition, the CD-DST procedure itself is time-consuming. Fortunately, however, this will not affect the formulation of patient medication plans because the detection process of CD-DST takes 9 days, and patients can get the results before starting adjuvant chemotherapy. Moreover, CD-DST can also be used to develop and promote new drugs and the preclinical study of drugs.

7. Conclusions

The present study observed that both OS and DFS were remarkably higher for patients in the high sensitivity group, revealing that the in-vitro CD-DST technique could successfully predict postoperative XELOX adjuvant chemotherapy efficacy in advanced gastric adenocarcinoma. Hence, the present study strongly suggests the anticancer agents' sensitivity screening using CD-DST for individualizing

chemotherapy to avoid ineffective chemotherapy. However, we have some reservations regarding the practical feasibility of CD-DST in clinical practice. Therefore, more studies are warranted to develop practically more feasible techniques.

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References

1. Ajani JA, D'Amico TA, Almhanna K, Bentrem DJ, Chao J, Das P, et at. Gastric Cancer, Version 3.2016, NCCN Clinical Practice Guidelines in Oncology. J Natl Compr Canc Netw. 2016; 14: 1286-312.
2. Al-Batran SE, Hartmann JT, Probst S, Schmalenberg H, Hollerbach S, Hofheinz R, et al. Phase III trial in metastatic gastroesophageal adenocarcinoma with fluorouracil, leucovorin plus either oxaliplatin or cisplatin: a study of the Arbeitsgemeinschaft Internistische Onkologie. J Clin Oncol. 2008; 26: 1435-42.
3. Ariake K, Motoi F, Mizuma M, Ohtsuka H, Hayashi H, Nakagawa K, et al. Collagen gel droplet-embedded culture drug sensitivity test (CD-DST) predicts the effect of adjuvant chemotherapy on pancreatic cancer. Surg Today. 2019; 49: 1035-43.
4. Bang YJ, Van Cutsem E, Feyereislova A, Chung HC, Shen L, Sawaki A, et al. Trastuzumab in combination with chemotherapy versus chemotherapy alone for treatment of HER2-positive advanced gastric or gastro-oesophageal junction cancer (ToGA): a phase 3, open-label, randomised controlled trial. Lancet. 2010; 376: 687-97.
5. Dank M, Zaluski J, Barone C, Valvere V, Yalcin S, Peschel C, et al. Randomized phase III study comparing irinotecan combined with 5-fluorouracil and folinic acid to cisplatin combined with 5-fluorouracil in chemotherapy naive patients with advanced adenocarcinoma of the stomach or esophagogastric junction. Ann Oncol. 2008; 19: 1450-57.
6. Fuchs CS, Tomasek J, Yong CJ, Dumitru F, Passalacqua R, Goswami C, et al. Ramucirumab monotherapy for previously treated advanced gastric or gastro-oesophageal junction adenocarcinoma (REGARD): an international, randomised, multicentre, placebo-controlled, phase 3 trial. Lancet. 2014; 383: 31-39.
7. Guimbaud R, Louvet C, Ries P, Ychou M, Maillard E, Andre T, et al. Prospective, randomized, multicenter, phase III study of fluorouracil, leucovorin, and irinotecan versus epirubicin, cisplatin, and capecitabine in advanced gastric adenocarcinoma: a French intergroup (Federation Francophone de Cancerologie Digestive, Federation Nationale des Centres de Lutte Contre le Cancer, and Groupe Cooperateur Multidisciplinaire en Oncologie) study. J Clin Oncol. 32: 3520-6.
8. Higashiyama M, Oda K, Okami J, Maeda J, Kodama K, Imamura F, et al. Prediction of chemotherapeutic effect on postoperative recurrence by in vitro anticancer drug sensitivity testing in non-small cell lung cancer patients. Lung Cancer. 2010; 68: 472-7.
9. Jemal A, Bray F, Center MM, Ferlay J, Ward E, Forman D. Global cancer statistics. CA Cancer J Clin. 2011; 61: 69-90.
10. Junttila MR, de Sauvage FJ. Influence of tumour micro-environment heterogeneity on therapeutic response. Nature. 2013; 501: 346-54.
11. Kang YK, Kang WK, Shin DB, Chen J, Xiong J, Wang J, et al. Capecitabine/cisplatin versus 5-fluorouracil/cisplatin as first-line therapy in patients with advanced gastric cancer: a randomised phase III noninferiority trial. Ann Oncol. 2009; 20: 666-73.
12. Kawamura M, Gika M, Abiko T, Inoue Y, Oyama T, Izumi Y, et al. Clinical evaluation of chemosensitivity testing for patients with unresectable non-small cell lung cancer (NSCLC) using collagen gel droplet embedded culture drug sensitivity test (CD-DST). Cancer Chemother Pharmacol. 2007; 59: 507-13.
13. Kobayashi H. Development of a new in vitro chemosensitivity test using collagen gel droplet embedded culture and image analysis for clinical usefulness', Recent Results Cancer Res. 2003; 161: 48-61.
14. Kobayashi H, Tanisaka K, Doi O, Kodama K, Higashiyama M, Nakagawa H, et al. An in vitro chemosensitivity test for solid human tumors using collagen gel droplet embedded cultures. Int J Oncol. 1997; 11: 449-55.
15. Mekata E, Sonoda H, Shimizu T, Tatsuta T, Yamaguchi T, Endo Y, et al. Clinical predictive value of in vitro anticancer drug sensitivity test for the therapeutic effect of adjuvant chemotherapy in patients with stage II-III colorectal cancer. Mol Clin Oncol. 2013; 1: 763-67.
16. Ochiai T, Nishimura K, Watanabe T, Kitajima M, Nakatani A, Nagayasu K, et al. Impact of the individualization of the first-line chemotherapy for advanced colorectal cancer based on collagen gel droplet-embedded drug sensitivity test. Oncol Lett. 2017; 14: 6045-52.
17. Okines AFC, Norman AR, McCloud P, Kang YK, Cunningham D. Meta-analysis of the REAL-2 and ML17032 trials: evaluating capecitabine-based combination chemotherapy and infused 5-fluorouracil-based combination chemotherapy for the treatment of advanced oesophago-gastric cancer. Ann Oncol. 2009; 20: 1529-34.
18. Sakuramoto S, Sasako M, Yamaguchi T, Kinoshita T, Fujii M, Nashimoto A, et al. Adjuvant chemotherapy for gastric cancer with S-1, an oral fluoropyrimidine. N Engl J Med. 2007; 357: 1810-20.
19. Salmaninejad A, Valilou SF, Shabgah AG, Aslani S, Alimardani M, Pasdar A, et al. PD-1/PD-L1 pathway: Basic biology and role in cancer immunotherapy. J Cell Physiol. 2019; 234: 16824-37.
20. Sant M, Allemani C, Santaquilani M, Knijn A, Marchesi F, Capocaccia R, et al. EUROCARE-4. Survival of cancer patients diagnosed in 1995-1999. Results and commentary. Eur J Cancer. 2009; 45: 931-91.
21. Shah MA, Janjigian YY, Stoller R, Shibata S, Kemeny M, Krishnamurthi S, et al. Randomized Multicenter Phase II Study of Modified Docetaxel, Cisplatin, and Fluorouracil (DCF) Versus DCF Plus Growth Factor Support in Patients With Metastatic Gastric Adenocarcinoma: A Study of the US Gastric Cancer Consortium. J Clin Oncol. 2015; 33: 3874-9.
22. Soularue E, Cohen R, Tournigand C, Zaanan A, Louvet C, Bachet JB, et al. Efficacy and safety of trastuzumab in combination with oxaliplatin and fluorouracil-based chemotherapy for patients with HER2-positive metastatic gastric and gastro-oesophageal junction adenocarcinoma patients: a retrospective study. Bull Cancer. 2015; 102: 324-31.
23. Sung H, Ferlay J, Siegel RJ, Laversanne M, Soerjomataram I, Jemal A, et al. Global Cancer Statistics 2020: GLOBOCAN Estimates of Inci-

dence and Mortality Worldwide for 36 Cancers in 185 Countries. CA Cancer J Clin. 2021; 71: 209-49.

24. Tanigawa N, Yamaue H, Ohyama S, Sakuramoto S, Inada T, Kodera Y, et al. Nakajima. Exploratory phase II trial in a multicenter setting to evaluate the clinical value of a chemosensitivity test in patients with gastric cancer (JACCRO-GC 04, Kubota memorial trial). Gastric Cancer. 2016; 19: 350-60.

25. Cutsem E, Boni C, Tabernero J, Massuti B, Middleton G, Dane F, et al. Docetaxel plus oxaliplatin with or without fluorouracil or capecitabine in metastatic or locally recurrent gastric cancer: a randomized phase II study. Ann Oncol. 2015; 26: 149-56.

26. Wang J, Seebacher N, Shi H, Kan Q, Duan Z. Novel strategies to prevent the development of multidrug resistance (MDR) in cancer. Oncotarget. 2017; 8: 84559-71.

27. Wilke H, Muro K, Cutsem EV, Oh SC, Bodoky G, Shimada Y, et al. Ramucirumab plus paclitaxel versus placebo plus paclitaxel in patients with previously treated advanced gastric or gastro-oesophageal junction adenocarcinoma (RAINBOW): a double-blind, randomised phase 3 trial. Lancet Oncol. 2014; 15: 1224-35.

28. Yasuda H, Takada T, Wada K, Amano H, Isaka T, Yoshida M, et al. A new in-vitro drug sensitivity test (collagen-gel droplet embedded-culture drug sensitivity test) in carcinomas of pancreas and biliary tract: possible clinical utility. J Hepatobiliary Pancreat Surg. 1998; 5: 261-8.

29. Zhao Q, Lian C, Huo Z, Li M, Liu Y, Fan L, et al. The efficacy and safety of neoadjuvant chemotherapy on patients with advanced gastric cancer: A multicenter randomized clinical trial. Cancer Med. 2020; 9: 5731-45.