Circulating tumor cells enumerated by a centrifugal microfluidic device as a predictive marker for treatment monitoring of ovarian cancer patients

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Abstract
Background: We investigated the size-based isolation and enumeration of circulating tumor cells (CTCs) using a centrifugal microfluidic device equipped with fluid-assisted separation technology (FAST disc) and demonstrated the correlation among CTC count, CA125, and the clinical course.
Methods: We prospectively recruited 13 women with ovarian cancer between December 2016 and August 2018 at Keimyung University Dongsan Hospital. We collected 49 serial blood samples at multiple time-points. CTCs were isolated from whole blood using the FAST disc and were defined as EpCAM+, cytokeratin+, CD45−, and DAPI+.
Results: We successfully achieved the high-throughput, efficient, and label-free isolation of CTCs from the blood of ovarian cancer patients using FAST discs. The mean and median CTC count were 20.2 and 6.0, respectively, and 84.62% patients (11/13) had more than 1 CTC at baseline and a decreased CTC count after surgery and chemotherapy, except for 2 patients who had no CTCs at baseline. The median follow-up duration was 22.7 months. At the time of complete response, CTC count in 8 patients was <3. CTC count was correlated with CA125 in 3 patients with no recurrence but elevated in 3 patients with recurrence and normal range of CA125. CTC count and CA125 had high concordance with directional change (increasing 71.4% and decreasing 75.0%). CTC count showed higher association with the clinical status and higher sensitivity (100.0% vs. 60.0%), positive predictive value (55.6% vs. 42.9%), and negative predictive value (100.0% vs. 87.5%) than CA125.
Conclusions: CTC count was better associated with treatment response and recurrence than CA125.
Introduction
Ovarian cancer is the eighth major cause of death due to malignancies in Korean women and has a relatively poor prognosis compared with other gynecologic cancers [1]. Most patients are diagnosed in the late stages of the disease owing to the lack of symptoms and a useful screening tool. CA125 is the biomarker mainly used for initial diagnosis and treatment monitoring in ovarian cancer [2]. CA125 > 30 U/mL is generally considered to indicate recurrence in ovarian cancer. However, CA125 has poor sensitivity and specificity. Moss et al. [3] reported 88.6% sensitivity and 72.0% specificity in 799 epithelial ovarian cancer patients. In addition, some non-malignant conditions such as endometriosis,
pregnancy, and infection are well-known causes of elevated serum CA125 [4]. Therefore, CA125 is not useful for predicting prognosis and early recurrence after surgery and chemotherapy. Identifying prognostic indicators reflecting current disease activity is crucial for patients with ovarian cancer. Circulating tumor cells (CTCs) are tumor cells that are shed into the bloodstream from the primary tumors, recurrences, or metastases, and possess antigenic and genetic tumor-specific characteristics [5]. In previous studies, CTCs were demonstrated to have prognostic and predictive value in patients with breast, colorectal, gastric, lung, and pancreatic cancers [6, 7]. Additionally, the prognostic value of CTCs in ovarian cancer was investigated in previous studies, but the results were controversial [8]. Although similar immunocytochemistry methods were used for CTC quantification, Judson et al. reported no significant association of CTCs with overall survival (OS) and progression-free survival (PFS) [9]; Fan et al. reported significant association between CTCs and PFS [10]; and Pearl et al. reported a significant relationship between CTCs and both OS and PFS [11]. Optimal strategies can be established using CTC count to predict disease activity, treatment response, or recurrence for effective management of ovarian cancer.

The optimal methods for CTC enumeration still remain a challenge for clinical use. Because CTCs are rarely found in the peripheral blood, and the status of the tumor changes continuously as the disease progresses [12]. Currently, the CellSearch® system (Menarini Silicon Biosystems, Inc., Pennsylvania(PA), US) which utilizes ferrofluids loaded with an EpCAM antibody to capture CTCs [13], is the only US Food and Drug Administration (FDA) approved system for clinical use in metastatic breast cancer, metastatic prostate cancer, and metastatic colorectal cancer [14]. However, its clinical use in ovarian cancer is not established. Poveda et al. obtained a 14.4% positivity rate with a cut-off value of 2 CTCs/7.5 ml, and no significant relationship was found between CTC count and both OS and PFS [15]. Behbakht et al. obtained a 44.0% positivity rate with a cut-off value of 1 CTC/7.5 ml, and no significant association was found between CTC count and PFS [16]. Moreover, Liu et al. reported no significant relationship between CTC count and both OS and PFS with a cut-off value of 2 CTCs/7.5 ml [17].

We have developed a centrifugal microfluidic device equipped with fluid-assisted separation
technology (FAST disc) which is based on label-free, size-based CTC isolation without any sample pre-treatment using a tangential flow filtration (TFF). This system enables clog-free, ultrafast (> 3 ml/min), viable CTC enrichment with gentle pressure drops (~ 1 kPa). This device has been tested in 142 patients suffering from 7 different types of cancers including breast, lung, and stomach [18]. The sensitivity and specificity of CTC count in gastric cancer (sensitivity 85.3% and specificity 90.3% with a cut-off value of 2 CTCs/7.5 ml in 116 patients) [19], esophageal squamous cell carcinoma (sensitivity 85.3% and specificity 90.0% with a cut-off value of 2 CTCs/7.5 ml in 116 patients) [20], and colorectal cancer (sensitivity 75.0% and specificity 100.0% with a cut-off value of 5 CTCs/7.5 ml in 74 patients) [21] was demonstrated. Moreover, Baek et al. found that patients with ≥ 5 CTCs showed a trend toward poor OS and PFS and had frequent vascular invasion (P = 0.035) in colorectal cancer [21]. However, this disc was not evaluated for clinical validation of predictive factors in ovarian cancer.

In this prospective study of 13 patients with ovarian cancer, we aimed to investigate a strategy for the enumeration and detection of CTCs and to demonstrate the correlation between CTC count, CA125, and clinical course.

Methods

Patients and study design

In this study, we prospectively recruited 13 women between December 2016 and August 2018. The patients were pathologically confirmed to have primary ovarian cancer. They were scheduled to undergo a staging debulking surgery and perioperative chemotherapy. They were treated at Keimyung University Dongsan Hospital and followed up until the date of death or last visit. Written informed consent was obtained from all patients before enrollment. We collected 49 serial blood samples from 13 patients at various time-points including at diagnosis, before and after surgery or chemotherapy, and at radiological or clinical evaluation. A staging workup was performed before recruitment according to the 2014 International Federation of Gynecology and Obstetrics (FIGO) staging system. Patient information was obtained from the medical records for the following characteristics: age at diagnosis, type of treatment including surgery and chemotherapy, date of
treatment and follow-up for evaluation, date of recurrence, initial FIGO stage, histology, response after surgery and chemotherapy based on Response Evaluation Criteria in Solid Tumours (RECIST version 1.1), and serum CA125 levels. The study was approved by the Institutional Review Board of the Keimyung University Dongsan Hospital (IRB 2016-03-014-001) and was conducted according to the precepts of the Helsinki Declaration.

Enumeration of CTCs using FAST disc
We used a commercial version of the FAST disc called CD-PRIME™ (Clinomics, Ulsan, Korea), which consisted of two parts, CD-CTC™ Duo (disc) and CD-OPR-1000™ (disc operating machine), to isolate CTCs from the whole blood of ovarian cancer patients. The CD-CTC™ Duo is a label-free, size-selective CTCs isolation device that can be operated by the table-top sized, stand-alone spinning system CD-OPR-1000TM. In the case of CD-CTC™ Duo, the FAST and TFF-enabled rapid (> 3 ml of whole blood/min) and clog-free isolation of CTCs from whole blood without any pre-treatment steps. Immunostaining was conducted to identify the isolated cells on the membrane in the filtration chamber of the FAST disc. Isolated cells were fixed with 4% formaldehyde for 20 min at room temperature and permeabilized with 0.1% Triton-X 100 for 5 min. Subsequently, washing was followed by blocking with 10 µg/ml IgG in PBS. Cells were stained with commonly used fluorescence conjugated antibodies in CTC study including anti-cytokeratin (CK) (CAM5.2; BD, Franklin Lakes, NJ, USA mixed with AE1/AE3; eBioscience, San Diego, CA, USA) and anti-EpCAM (9C4; BioLegend, San Diego, CA, USA) for CTCs, anti-CD45 antibody (H130; Life Technologies, Carlsbad, CA, USA) for white blood cells (WBCs), and 4,6-diamidino-2-phenylindole (DAPI) for nuclear staining. Merged images of three different colors were used to distinguish tumor cells from WBCs. Cells that showed CK + or EpCAM+ (FITC channel), CD45- (PE channel), and DAPI+ (DAPI channel) and were morphologically intact were identified as CTCs while cells expressing high CD45 were identified as WBCs. The fluorescence images of isolated cells on the membrane were automatically scanned through a Bioview workstation (BioView, Inc.).

Cell culture and spike experiment
SKOV3 ovarian cancer cell line was purchased from American Type Culture Collection (Manassas, VA).
The cells were cultured with RPMI medium including 5% FBS and 1% antibiotics/antimycotics at 37 °C, under 5% CO₂ atmosphere set incubators. For the spiking experiments, the cells were labeled with a fluorescent dye called CellTracker CMFDA (Life Technologies, Carlsbad, CA) before the experiment according to the manufacturer recommended protocol.

Results
Patient characteristics
A total of 13 women with ovarian cancer were enrolled in this study. Patient characteristics are presented in Table 1. The median age at diagnosis was 56 years (range 40–75). The histology of ovarian cancer was high-grade, serous carcinoma in 9 patients. The initial FIGO stage in 4, 7, and 2 patients was stage I-II, stage III, and stage IV, respectively. All patients underwent surgery and chemotherapy, and a complete response was observed in 11 patients. Six patients had a recurrence in lung, liver, or peritoneal seeding. Initial CA125 was high in all patients, and CTCs were detected in initial samples of 84.62% patients (11/13). All patients underwent debulking surgery including total abdominal hysterectomy, bilateral Salpingo-oophorectomy, bilateral pelvic lymph node dissection, para-aortic nodal dissection, appendectomy, and omentectomy. Thirteen patients received platinum doublet-based adjuvant chemotherapy such as paclitaxel plus carboplatin with or without bevacizumab for 4–6 cycles, and 4 patients received neoadjuvant chemotherapy before surgery because of the unresectable status. After the therapy, samples from 10 patients were collected and tested. In 90% patients, CA125 levels and CTC counts were lower than those in baseline samples. Only patient 6 had increased CTC counts after therapy. In addition, this atypical patient had a normal range of CA125 (< 30 U/ml) in the baseline sample.

Table 1. Clinical characteristics of patients with ovarian cancer

| FIGO, International Federation of Gynecology and Obstetrics; CTCs, Circulating Tumor Cells; CR, complete response; PR, partial response. |
| From each patient sample, isolated cells were stained and discriminated based on the conventional criteria for CTC identification that is EpCAM/CK positive and DAPI positive cells without CD45 expression. Figure 2 shows the representative images of CTCs from patient samples. Detection of CTCs and correlation with CA125 |
| No. | Age at diagnosis (years) | FIGO Stage | Histology                                      | Initial CA125 (U/mL) | Initial CTCs (/3 mL) | Treatment response | CA125 after therapy (U/mL) | CTCs after therapy (/3 mL) | Recurrence | No. of blood samples |
|-----|--------------------------|------------|-----------------------------------------------|----------------------|----------------------|---------------------|---------------------------|-----------------------------|-------------|---------------------|
| 1   | 65                       | IIIC       | High-grade serous carcinoma                   | 3186.5               | 76                   | CR                  | 5.8                       | 0                           | No          | 8                   |
| 2   | 67                       | IIIC       | High-grade serous carcinoma                   | 76                   | 26                   | CR                  | 8.1                       | 0                           | Yes         | 7                   |
| 3   | 55                       | IIIC       | High-grade serous carcinoma                   | 4278.5               | 5                    | CR                  | 11.3                      | 0                           | Yes         | 6                   |
| 4   | 53                       | IC         | Mucinous carcinoma                             | 18.2                 | 64                   | CR                  | 8.5                       | 1                           | No          | 5                   |
| 5   | 49                       | IIIA2      | High-grade serous carcinoma                   | 631.9                | 55                   | CR                  | 9.3                       | 2                           | No          | 4                   |
| 6   | 56                       | IC         | High-grade serous carcinoma                   | 25.7                 | 0                    | CR                  | 7.7                       | 3                           | No          | 4                   |
| 7   | 75                       | IV         | Adenocarcinoma with serous carcinoma          | 10000                | 0                    | PR                  | 244.5                     | 0                           | Yes         | 4                   |
| 8   | 40                       | IIB        | Clear cell carcinoma                           | 34.6                 | 13                   | CR                  | 41.6                      | 3                           | Yes         | 3                   |
| 9   | 59                       | IIB        | High-grade serous carcinoma                   | 696.4                | 1                    | CR                  | 65.1                      | 0                           | Yes         | 3                   |
| 10  | 46                       | IIIC       | Clear cell carcinoma                           | 2432.8               | 6                    | CR                  | 153.1                     | 0                           | No          | 2                   |
| 11  | 47                       | IV         | High-grade serous carcinoma                   | 553.3                | 2                    | CR                  | NA                        | NA                          | Yes         | 1                   |
| 12  | 66                       | IIIC       | High-grade serous carcinoma                   | 3399.8               | 10                   | CR                  | NA                        | NA                          | No          | 1                   |
| 13  | 65                       | IIIC       | High-grade serous carcinoma                   | 8767.4               | 4                    | PR                  | NA                        | NA                          | No          | 1                   |

At baseline, 84.62% of patients had a positive CTC count with 1 or more CTCs in 3 ml of blood (range 1–76). The mean and median CTC count of all patients were 20.2 and 6.0 at baseline. The median follow-up duration was 22.7 months (range 5.2–28.7). Most of the patients presented a decreased CTC count after surgery and chemotherapy, except for 2 patients who had no CTC at baseline. At the time of complete response, CTC count evaluated in 8 patients was < 3.

For the correlation analysis between CTC count and CA125 according to clinical course, patients were selected according to the following criteria: 1) patients who gave blood samples > 3 times, 2) patients who had higher than normal (30 U/ml) initial CA125 level, and 3) patients diagnosed with complete response based on the imaging result after surgery. Based on these criteria, 6 patients were selected, and the patients were divided into two groups: no recurrence group and recurrence group.
For the 3 patients in the no recurrence group, the CTC count and CA125 had similar patterns and were correlated with the clinical course. Patient 1 was diagnosed with ovarian cancer, high-grade serous carcinoma, and initial FIGO stage IIIC. The pre-treatment CTC count and CA125 were 76 and 3186.5, respectively. After perioperative chemotherapy and surgery, both parameters decreased to baseline levels, and a complete response was observed in the computed tomography (CT) scan at the end of chemotherapy (Fig. 3A). Patient 5 was diagnosed with ovarian cancer, high-grade serous carcinoma, and initial FIGO stage IIIA2. The pre-treatment CTC count and CA125 were 55 and 631.9, respectively. After surgery and chemotherapy, a durable complete response was observed in the CT scan, and CTC count and CA125 were 2 and 9.3, respectively, which further decreased to 0 and 10.0, respectively after 14 months (Fig. 3D). Patient 8 was diagnosed with ovarian cancer, clear cell carcinoma, and initial FIGO stage IIB. The pre-treatment CTC count and CA125 were 13 and 67.8, respectively. Both parameters were similar during treatment, and a complete response was observed after 2 months (Fig. 3E).

For the 3 patients in the recurrence group, the CTC count and CA125 showed similar patterns. However, CTC count was elevated before or after recurrence while CA125 remained in the normal range. For example, patient 2 was diagnosed with ovarian cancer, high-grade serous carcinoma, and initial FIGO stage IIIC. The pre-treatment CTC count and CA125 were 26 and 76.0, respectively. After chemotherapy and surgery, CTC count and CA125 were 0 and 8.1, respectively, and a complete response was observed in the CT scan. Recurrence occurred 5 months later with the appearance of new lung nodules. The increase in CTC count was followed by a recurrence, although CA125 remained within the normal range (Fig. 3B). Patient 3 was diagnosed with ovarian cancer, high-grade serous carcinoma, and initial FIGO stage IIIC. The pre-treatment CTC count and CA125 were 5 and 4278.5, respectively. After surgery and chemotherapy, CTC count and CA125 decreased to 0 and 11.3, respectively, and a complete response was observed in the CT scan. Five months later, an increase in CTC count with CA125 in the normal range was observed followed by a recurrence of peritoneal seeding (Fig. 3C). Patient 9 was diagnosed with ovarian cancer, high-grade serous carcinoma, and initial FIGO stage IIB. After 20 months from diagnosis, CTC count was slightly increased with a normal
range of CA125, and recurrence was detected in the peritoneum (Fig. 3F).

All the changes in CTCs and CA125 were analyzed to check concordance and association with the corresponding clinical assessments. First, to confirm the concordance between CA125 and CTC count, each point of change in the 6 cases was classified into 4 categories: both increased, only CTC increased, only CA125 increased, and no change in both (no change or decrease). Concordance in increasing change was calculated in cases where both increased, and it was 71.4%. On the other hand, concordance in no change was calculated in cases where there was no change in both, and it was 75.0%. According to the directional concordance analysis, CTC count had a high concordance with a change in CA125 (Fig. 4A). To analyze the association with the corresponding clinical status, each point of change in the 6 cases was classified according to recurrence. Both CA125 and CTCs had the same specificity of 77.8%. However, CTCs had higher values in other aspects including sensitivity (CTCs 100.0% vs. CA125 60.0%), positive predictive value (PPV, CTCs 55.6% vs. CA125 42.9%), and negative predictive value (NPV, CTCs, 100.0% vs. CA125 87.5%) than CA125 (Fig. 4B).

Discussion
In this study, we confirmed the performance of the FAST disc for CTCs in ovarian cancer patients. Before the clinical sample test, FAST disc was verified using whole blood spiked with SKOV3 ovarian cancer cells, and it had a capture efficiency of 87.5 ± 4.2% (Fig. 1F). It was slightly lower than the performance reported in a previous work (95.9 ± 3.1% of capture efficiency) which used other cancer cell lines; however, it was acceptable owing to the advantages of FAST disc including clog-free, highly sensitive, and rapid CTC isolation without any pre-treatment steps. In the tests with clinical samples obtained from 13 ovarian cancer patients, the detection rate at baseline was 84.62%. Furthermore, we demonstrated that the CTC count and CA125 were associated with the clinical course in 6 patients. There was > 70% concordance between change in CTC count and CA125, and the change in CTC count showed much higher sensitivity (100.0%), PPV (55.6%), and NPV (100.0%) than the change in CA125 (sensitivity 60.0%, PPV 42.9%, and NPV 87.5%) (Fig. 4). Therefore, the CTC count was more predictive for recurrence than CA125. We reviewed the clinical course of patients with ovarian cancer over a long period and compared the level and changes in CTC count and CA125 according to the
treatment response. Previously, Pearl et al. monitored the treatment response of 6 patients who had > 6 measurements of CTCs and CA125. They found that CTCs, but not CA125, antedated a change in clinical response from progression to complete remission during and after chemotherapy and relapse [11], which was in line with the results in our study.

CTCs are considered a liquid biopsy tool and a non-invasive method for diagnosis and prognosis. It can be easily collected at multiple time-points throughout the treatment which allows the real-time monitoring of treatment response and drug resistance for all metastatic cancers [22]. Moreover, CTC analysis enables the detection of multiple mutations within a single cell which contributes to the understanding of tumor heterogeneity and clonal evolution, and this might establish a potential connection between mutational status and pathway activation by combining the genomics and transcriptomics of CTCs [23]. However, some limitations have to be overcome such as the lack of large scale studies with standardized approaches to enrich and detect CTCs [24].

Most of the studies on ovarian CTCs using the FDA-approved CellSearch system had negative results because of a comparatively low detecting accuracy of CTCs and the availability of patients with ovarian cancer. A multicenter, randomized, phase III study that included 216 ovarian cancer patients demonstrated that only 14.4% had 2 or more CTCs before the start of therapy using this system [15]. Liu et al. revealed that the CTC count did not significantly correlate with survival outcomes in 78 patients with ovarian cancer using this system [newly-diagnosed patients, median time to progression (TTP) 14.0 vs. 16.5 months, \( p = 0.88 \); recurrent disease, median TTP 3.8 vs. 6.4 months, \( p = 0.13 \)] [17].

Many studies tried to enhance the yield and purity of CTCs using biophysical properties such as size, deformability, or dielectric susceptibility. By using RT-qPCR to detect the presence of CTCs based on overexpression of the cyclophilin C gene (PPIC) in CTCs, Obermayr et al. showed that the presence of CTCs was significantly higher in the platinum-resistant than in the platinum-sensitive patient group and was related to poor prognosis in 93 follow-up patients [25]. Pear et al. revealed that serial measurements using CAM + selection of CTCs could predict therapeutic responsiveness in 31 patients with ovarian cancer who received standard taxol plus carboplatin therapy [11]. Kim et al. found that
the positive detection of postoperative CTCs using the tapered-slit filter platform was associated with inferior PFS rates in 39 patients with stage III/IV ovarian cancer (18.8% vs. 57.1%, p = 0.077) [26]. Moreover, CTC count measured by CAM + selection was associated with the stage of cancer and CA125 level [10] and had a better monitoring value than CA125 [11]. These results were consistent with our findings.

This study has several limitations. First, this was conducted with a small sample size at a single center. Second, the numbers and timing of blood sampling were irregular, and therefore, it was hard to compare the samples from different patients to make a statistically significant comparison during the time course of the therapy. Third, we only quantified EpCAM or CK positive CTCs although the enumeration method was not EpCAM marker sensitive. Fourth, we only measured the number of CTCs per sample and did not compare with other enumeration methods or analyze other molecular characteristics of the CTCs. Therefore, further studies with large sample size and in-depth characterization should be performed.

Conclusions
We successfully isolated sufficient CTCs from the blood of ovarian cancer patients using a FAST disc. We demonstrated that the CTC count was better associated with treatment response and recurrence throughout the clinical course than CA125. Further large-scale prospective studies are needed for the validation of the FAST disc as a diagnostic tool in ovarian cancer.

Abbreviations
CTCs: circulating tumor cells; FAST: fluid-assisted separation technology; OS: overall survival; PFS: progression-free survival; TFF: tangential flow filtration; FIGO: International Federation of Gynecology and Obstetrics; CK: anti-cytokeratin; WBCs: white blood cells; DAPI: 4,6-diamidino-2-phenylindole; PPV: positive predictive value; NPV: negative predictive value

Declarations

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Author contributions
Y.K.C., C.H.C. contributed to the study concept and design. M.L., Y.K.C. contributed to execution of experiments. H.K., J.Y.K., S.J.S., C.H.C. contributed patients to the study and to data collection. H.K., M.L., J.Y.K., S.J.S., Y.K.C., C.H.C. contributed to the data analysis, data interpretation, drafting, and revising the manuscript.

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**Availability of data and materials**

All data generated or analysed during this study are included in this published article.

**Ethics approval**

The study was approved by the Institutional Review Board of the Keimyung University Dongsan Hospital.

**Consent for publication**

Not applicable.

**Conflicts of interest**

Patents on FAST-disc are licensed to Clinomics (Ulsan, Korea). Y.K.C. receives compensation for the sale of the FAST disc. All other authors have no conflicts of interest to declare.

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Figures
Figure 1

Mechanism and workflow of CD-PRIMETM (A) Image of CD-CTCTM Duo; (B) Schematic illustration of CTC isolation in the filtration chamber; (C) Image of CD-OPR-1000TM; (D) and (E) Separate images of disc operation before and after washing. (F) Capture efficiency test with SKOV3 cell line spiked blood sample using FAST disc: capture efficiency was 87.5 ± 4.2 % (mean ± SD).
Examples of fluorescence images of patient-driven CTCs. CTCs are defined as DAPI+, EpCAM+ or CK+, and CD45- cells. (Scale bar: 10 µm).
Figure 3

Correlation between CTC count, CA125, and clinical course in 6 patients (A) Patient 1: after perioperative chemotherapy and surgery, CTC count and CA125 were decreased to almost baseline, and a complete response was observed in a CT scan at the end of chemotherapy. (B) Patient 2: at the end of chemotherapy and surgery, CTC count and CA125 were 0 and 8.1, respectively with a complete response. Five months later, the increase in CTC count was followed by a recurrence, although CA125 remained within the normal range. (C) Patient 3: following surgery and chemotherapy, CTCs count and CA125 were 0 and 11.3,
respectively with a complete response. Five months later, an increase in CTC count with a normal range of CA125 was observed followed by a recurrence of peritoneal seeding. (D) Patient 5: following surgery and chemotherapy, a durable complete response was observed in the CT scan, and CTC count and CA125 were 2 and 9.3, respectively, followed by 0 and 10.0, respectively. (E) Patient 8; CTC count and CA125 were similar throughout the treatment. (F) Patient 9; after 20 months from diagnosis, CTC count was slightly increased with a normal range of CA125, and recurrence was detected in the peritoneum.

![Figure 4](chart.png)

**Correlation analysis between CTC count and CA125 according to the clinical status (A)**

Concordance between the change in CTC count and change in CA125: >70% concordance in both increasing and decreasing directions (B) **Association with clinical status:** clinical status was divided into the two groups PD and CR. CTC showed a much better association with the clinical status than CA125. PD, Progressive disease; CR, complete response; PPV, positive predictive value; NPV, negative predictive value.