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Detection of circulating tumour cells may add value in endometrial cancer management

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ABSTRACT

Objective: To evaluate the role of circulating tumour cells (CTCs) in patients with endometrial cancer (EC).
Study design: This study included 40 patients with a pre-operative diagnosis of high-risk EC between April 2015 and May 2016. Patients were further divided into high-risk (grade 3, non-endometrioid, myometrial invasion $\geq$1/2 and stage III–IV) and high-intermediate-risk (grade 2–3, endometrioid, myometrial invasion $<$1/2 and stage I–II) groups according to postoperative pathological results. CTCs were detected using the CellSearch system, and CTC results were correlated with standard clinicopathological characteristics and serum tumour marker CA125/HE4 status using Chi-squared test, continuity correction or Fisher's exact test. The pharmacodynamic effect was detected after the first cycle of adjuvant therapy. Patients were followed up for 13 months to assess outcomes.
Results: Fifteen percent of patients had one or more CTCs. The presence of CTCs was found to be significantly associated with cervical involvement (83.33% vs 11.76%, $p = 0.00$). No significant difference in CTC-positive rates was detected between the high-risk and high-intermediate-risk groups, and no significant correlation was found between CTCs and serum CA125/HE4, either by positive rates or exact serum levels of the conventional tumour markers. No more CTCs were detected after the first cycle of standard chemotherapy in this study, and no distant metastases or recurrence were found in the CTC-positive patients during the follow-up period.
Conclusion: The presence of CTCs was correlated with cervical involvement. Early-stage EC patients with CTCs may benefit from additional adjuvant therapies. Assessment of CTCs may be useful in the management of high-risk EC patients.

Introduction

Endometrial cancer (EC) remains the most common cancer of the female reproductive system in the USA, with approximately 60,050 new cases and 10,470 deaths expected in 2016 [1]. Staging is mainly based on the extent of myometrial invasion, lymph node involvement, and presence/absence of distant metastases according to the 2009 International Federation of Gynecology and Obstetrics (FIGO) stage [2]. Lymph node status plays an important role in the prediction of outcomes of patients with operable EC. However, 6–7% of patients with localized disease and without lymph node metastasis have distant metastases after primary surgery [3,4], whereas no evidence was found that lymphadenectomy decreases the risk of disease recurrence or death compared with no lymphadenectomy in stage I patients [5]. These disparate results imply that haematogenous spread is independent of lymph node involvement in some patients with EC. Nevertheless, these early disseminated tumour cells cannot usually be detected by conventional serum tumour markers or imaging technologies. Thus, a more suitable detection method is needed urgently to aid pre-operative diagnosis and ensure optimal management in EC.

The number of circulating tumour cells (CTCs) above a certain threshold has been confirmed to be an independent prognostic predictor of survival in various metastatic cancers [6–8]. When added to full clinicopathological predictive models, the CTC count improves the prognostication of metastatic breast cancer, whereas serum tumour markers do not [9]. Furthermore, the identification of one or more CTCs also predicted a worse outcome in non-metastatic cancer patients [10]. Meanwhile, during systemic

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therapy in metastatic disease, detection of increased CTCs at any time indicated rapid disease progression [11]. These results suggest that detection of CTCs may help to identify early haematogenous metastasis, and therefore may be useful in the management of patients with EC. However, the role of CTCs in EC remains unclear. For example, CTCs have been reported in EC by different types of detection assays, including cytokeratin-20 mRNA amplification [12], CELLection^{TM} Epithelial Enrich kit (Invitrogen, Dynal, Oslo, Norway) [13], and the CellSearch (Veridex, NJ) system [14], and the positive cut-off level varied. While all patients with recurrent disease were CTC positive in one study [12], the result was adverse in the other study [14]. Far less is known about CTCs in early-stage and type II EC patients. Moreover, no comparison of CTCs with standard serum tumour markers for the prediction of distant metastases has been conducted in patients with EC.

This study sought to characterize CTCs in patients with EC, and identify any correlation with other clinical factors. If the presence of CTCs is independent of current prognostic factors, the result may be helpful in EC staging and in patient selection for further adjuvant therapies.

Materials and methods

Participants

Between 29 April, 2015 and 12 May, 2016, high-risk EC patients undergoing primary surgery at the International Peace Maternity and Child Health Hospital, Shanghai Jiao Tong University School of Medicine, China were recruited into the study. Patients were further divided into high-risk (grade 3, non-endometrioid, myometrial invasion $\geq$1/2 and FIGO stage III–IV) and high-intermediate-risk (grade 2–3, endometrioid, myometrial invasion $<$1/2 and FIGO stage I–II) groups according to the postoperative pathological results. Patients diagnosed with any other cancer in the preceding 5 years were ineligible, as were those who declined to participate. Late-stage patients who did not have staging surgery were also excluded from the study. Informed consent was obtained from all patients before blood collection, and this was approved by the Institutional Review Board of the International Peace Maternity and Child Health Hospital.

Detection and definition of CTCs and serum tumour markers

CTCs were quantified in 7.5-ml peripheral blood samples drawn from patients prior to surgery using the CellSearch system (Veridex). The collected peripheral blood samples were preserved in 10-ml CellSave (Veridex) tubes, stored at room temperature, and tested within 72 h of collection. The semi-automated technology enriches cells using ferromagnetic beads coated with epithelial cell adhesion molecules, and defines CTCs as cells with round to oval morphology, nucleic acid dye 4′,6-diamidino-2-phenylindole (DAPI), staining positive for cytokeratins (CK-8, CK-18, and CK-19) and negative for CD45. All CTC evaluations were performed by two qualified laboratory technicians who were blinded to the patients' clinical status. Meanwhile, 3-ml blood samples were obtained pre-operatively for the analysis of CA125 and HE4. The levels were compared with the normal values of 35 U/ml CA125 and 70 pmol/l HE4. All of the patients underwent CA125 and HE4 analysis.

Statistical analyses

All statistical analyses were conducted using Statistical Package for the Social Sciences Version 22.00 (IBM Corp., Armonk, NY, USA). Chi-squared test was used to assess the association between the presence of CTCs and disease characteristics. Continuity correction was used when any one of the expected frequencies was between one and five, and Fisher's exact test was used when any one of the expected frequencies was less than one, or there were less than 40 cases. Statistical significance was set at $p < 0.05$.

Results

Peripheral blood samples of 43 high-risk EC patients were collected pre-operatively. Three late-stage patients did not undergo standard staging surgery and were not included in the study. The mean age was 57 years (range 25–70 years, Table 1).

No adverse complications occurred in patients following blood collection. One or more CTCs were detected in six patients (15%). A cut-off of one CTC was taken to indicate a positive result, and the number of CTCs varied from one to three (Fig. 1).

Among the patients with detectable CTCs, three were FIGO stage I and three were FIGO stage III (Table 2). Half of the CTC-positive patients were non-endometrioid. No significant difference in CTC count was found between stage I and stage III patients. One type II stage I EC patient with CTCs agreed to undergo a repeat CTC examination after the first dose of adjuvant therapy, and the result was negative. No chemotherapy or radiotherapy was practised in the early-stage CTC-positive patients when there was no other standard

Table 1
Patient characteristics.

	Patients	CTC		
		Positive	Negative	*p*-Value
Total	40	6	34	
Age, mean (SD, years)	57 (9)	56 (14)	57 (9)	
Grade (endometrioid = 29)				1.000
G1 or G2	25	3	22	
G3	4	0	4	
FIGO stage				1.000
I or II	23	3	20	
III or IV	17	3	14	
Histological type				0.499
Endometrioid	28	3	25	
Non-endometrioid	12	3	9	
Myometrial invasion				1.000
$<$1/2	23	3	20	
$\geq$1/2	17	3	14	
Positive lymph nodes				0.306
No	30	3	27	
Yes	10	3	7	
Lymphovascular space involvement				0.819
No	25	3	22	
Yes	15	3	12	
Peritoneal cytology				0.667
No	26	4	22	
Yes	10	1	9	
CA125				0.859
Negative	18	2	16	
Positive	22	4	18	
HE4				0.71
Negative	26	3	23	
Positive	14	3	11	
Cervical involvement[a]				0.000*
No	31	1	30	
Yes	9	5	4	

CTC, circulating tumour cell; FIGO, International Federation of Gynecology and Obstetrics; CA125, cancer antigen 125; HE4, human epididymis protein 4.
* *p*-Values $<$0.05 were considered statistically significant.
[a] Including both mucosal and stromal cervical involvement.