**Circulating tumor cells in gynaecological malignancies**

Imrich Kiss¹,²,³, Katarina Kolostova¹, Ireneusz Pawlak⁴, Vladimir Bobek¹,⁴,⁵

¹Department of Laboratory Genetics, Laboratory Diagnostics, University Hospital Kralovske Vinohrady, Srobarova 50, Prague, Czech Republic; ²Department of Gynecology, Military University Hospital and the 3rd Faculty of Medicine, Charles University, U Vojenské nemocnice 1200, Prague, Czech Republic; ³Charles University, 1st Faculty of Medicine, Katerinska 52, Prague, Czech Republic; ⁴Department of Thoracic Surgery, Lower Silesian Oncology Centre, Plac Hirszfelda 12, Wroclaw, Poland; ⁵Department of Thoracic Surgery, Socialni pece 3516/12A, Krajska zdravotni a.s. Hospital, Usti nad Labem, Czech Republic and 3rd Department of Surgery, University Hospital FN Motol and 1st Faculty of Medicine, Charles University, V Uvalu 85, Prague, Czech Republic and Department of Histology and Embryology, Wroclaw Medical University, Wybrzeze Ludwika Pasteura 1, 50-567 Wroclaw, Poland

**Summary**

New non-invasive approaches have developed for diagnosis and treatment of malignant diseases. Cells shed from the primary tumor circulating in the bloodstream with metastasis potential are called Circulating Tumor Cells (CTCs). These cells are easily acquired from the peripheral blood of patients, while several enrichment and isolation methods are available nowadays with different benefits and positive detection rates. A brief characterization of three major categories of detection is described (nucleic acid-based, physical properties-based, antibody-based). In this review we concentrate on gynecological malignancies and how CTCs could be used in the diagnosis of cancer, treatment management and its effective prognosis and early recurrence detection. Presence of CTCs in endometrial cancer patients show worse overall survival, while gene analysis could identify patients in need of systemic therapy after surgical treatment to prevent metastasis and recurrence. Based on the influence of human papillomavirus (HPV) in the etiology of cervical cancer, viral oncogene transcripts could be used as an ideal marker for cervical cancer cells detection. In ovarian cancer, CTCs could help in the differentiation from benign adnexal masses and show a high independence from other biomarkers such as CA125 and HE4. While detection of CTC after complete cytoreductive surgery could indicate invisible lesions, combination of tumor associated genes rises the specificity of CTC detection.

**Key words:** biomarker, cervical cancer, circulating tumor cells, endometrial cancer, liquid biopsy, ovarian cancer

**Introduction**

In the last two decades, big effort and hopes are put into the discovery of new non-invasive methods for diagnosis and understanding the pathophysiology of malignant diseases. Further development of these tools could help in diagnosis, prognosis, personalized therapy and evaluation of its effectiveness or even alert for recurrences in patients in the follow up period. Liquid biopsy which is easily acquired from patients allows to study the molecular architecture and behaviour of tumors in real time [1]. The tumor material is composed most often by circulating tumor cells (CTCs), circulating tumor DNA (ctDNA), circulating tumor miRNA, proteins and exosomes and besides blood they could be present in several body fluids such as saliva, urine, cerebrospinal fluid, uterine aspirates, pleural effusions or even stool [2,3]. This review analyses the momentary state of circulating tumor cells in the malignancies of the female genital system. The studies used in this review are listed in Table 1.

CTCs are shed from the primary tumor into the bloodstream with potential ability of metastasis (Figure 1). Positive isolation and detection of CTCs have been validated as a prognostic factor in metastatic breast cancer and several other solid
<table>
<thead>
<tr>
<th>Type of cancer</th>
<th>Authors</th>
<th>Year</th>
<th>Method</th>
<th>Number of patients</th>
<th>CTC isolated</th>
<th>CTC positivity rate</th>
<th>Notes</th>
</tr>
</thead>
<tbody>
<tr>
<td>Endometrial carcinoma</td>
<td>Kiss et al.</td>
<td>2018</td>
<td>Size based isolation (MetaCell)</td>
<td>92</td>
<td>69</td>
<td>75%</td>
<td>Independent from stage, grade, lymph node involvement</td>
</tr>
<tr>
<td></td>
<td>Bogani et al.</td>
<td>2015</td>
<td>Immunomagnetic selection, immunofluorescence staining (CellSearch)</td>
<td>28</td>
<td>2</td>
<td>7%</td>
<td>Associated with myometrial invasion, lymph node positivity</td>
</tr>
<tr>
<td></td>
<td>Ni et al.</td>
<td>2016</td>
<td>CellSearch</td>
<td>40</td>
<td>6</td>
<td>15%</td>
<td>Stathmin expression as a biomarker for treatment response</td>
</tr>
<tr>
<td></td>
<td>Lemech et al.</td>
<td>2016</td>
<td>Detection of EpCAM (CellSearch)</td>
<td>30</td>
<td>18</td>
<td>60%</td>
<td>Suitable markers: Cytokeratin 19, claudin 4</td>
</tr>
<tr>
<td></td>
<td>Rolbl et al.</td>
<td>2016</td>
<td>RNA isolation, cDNA - qPCR</td>
<td>6 cell lines 10</td>
<td>NA</td>
<td>NA</td>
<td>CNE2, DKK2p762E1, EMP2, MAL2, PFIC, and SLC6A8</td>
</tr>
<tr>
<td></td>
<td>Obermayr et al.</td>
<td>2010</td>
<td>Microarray technology</td>
<td>25 EC, 25 CxGa, 25 GaOv</td>
<td>NA</td>
<td>44% CxGa, 64 EC, CaOv 19%</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Zhang et al.</td>
<td>2016</td>
<td>Flow cytometry</td>
<td>78</td>
<td>NA</td>
<td>NA</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Alonso-Alconada et al.</td>
<td>2014</td>
<td>EpCAM based immunosaturation, RTqPCR</td>
<td>34</td>
<td>NA</td>
<td>NA</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Pfitzer et al.</td>
<td>2014</td>
<td>Digital-Direct-RT-PCR</td>
<td>10</td>
<td>3</td>
<td>30%</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Takakura et al.</td>
<td>2017</td>
<td>Conditionally replicative adenovirus targeting telomerase-positive cells</td>
<td>23</td>
<td>6</td>
<td>26%</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Wen et al.</td>
<td>2018</td>
<td>Magnetic beads separation, CEP8+/DAPI+/CD45-</td>
<td>99</td>
<td>45</td>
<td>45.9%</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Suh et al.</td>
<td>2017</td>
<td>TSF - physical deformability</td>
<td>87</td>
<td>49</td>
<td>56.3% (44.2% benign, 100% early stage, 66.7% advanced stage)</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Chebouti et al.</td>
<td>2017</td>
<td>AdnaTest ovarian cancer</td>
<td>91</td>
<td>NA</td>
<td>NA</td>
<td>Combination of CTC and SCC-Ag a significant predictive marker</td>
</tr>
<tr>
<td></td>
<td>Kuhlmann et al.</td>
<td>2014</td>
<td>AdnaTest ovarian cancer</td>
<td>143</td>
<td>20</td>
<td>14%</td>
<td>Better than other modalities in detecting early stage</td>
</tr>
<tr>
<td></td>
<td>Obermayr et al.</td>
<td>2014</td>
<td>Microarray analysis, RT-qPCR</td>
<td>200</td>
<td>49</td>
<td>24.5%</td>
<td>Emerge of P53Ka and Twist</td>
</tr>
<tr>
<td></td>
<td>Kolostova et al.</td>
<td>2015</td>
<td>Metacell</td>
<td>118</td>
<td>77</td>
<td>65.2%</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Obermayr et al.</td>
<td>2017</td>
<td>Multimarker immunostaining, FISH</td>
<td>102</td>
<td>27</td>
<td>26.5%</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Marth et al.</td>
<td>2001</td>
<td>Microbead coated with MOC-31 antibody</td>
<td>90</td>
<td>11</td>
<td>12%</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Kolostova et al.</td>
<td>2016</td>
<td>MetaCell</td>
<td>56</td>
<td>32</td>
<td>58%</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Judson et al.</td>
<td>2003</td>
<td>Anti-cytokeratin 8, 18, TFS-2, CK-7, CK-20, EGFR rad MiniMACS</td>
<td>64</td>
<td>12</td>
<td>18.7%</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Lee et al.</td>
<td>2017</td>
<td>Electronically conductive chip</td>
<td>54</td>
<td>54</td>
<td>98.1%</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Lou et al</td>
<td>2017</td>
<td>EpCAM+, CK+, DAPI+, CD45 negat</td>
<td>49 (35 malignancy)</td>
<td>9</td>
<td>18.4% (malignant grup)</td>
<td>Only 17.5% positivity in OC, 80% in non-ovarian origin - metastatic tumours to ovary</td>
</tr>
</tbody>
</table>

Table 1. List of studies used in this review
tumors such as prostate, colorectal and lung cancer [4]. The main limitation of CTC is the low quantity of cells in the blood of cancer patients. The quantity of cells detected differs widely also by the method of isolation.

The broad heterogeneity of CTCs in cancer patients may play a dominant role in therapy resistance and recurrence of disease [5]. Disseminated and CTCs may undergo a broad range of biochemical changes and reversibly acquire fibroblastoid or mesenchymal traits described as epithelial-to-mesenchymal-transition (EMT) already published for breast cancer [6]. This mechanism is a key for malignant progression and is referred to as Oncogenic EMT. This allows tumor cells to gain invasive properties, develop metastatic growth characteristics and defend them during dissemination. Metastatic cells can, after reaching the distant organ, change back to their original epithelial phenotype, mesenchymal-epithelial-transition (MET), to support colonization [7].

**CTC detection and isolation: methods and devices**

To fully benefit from CTCs, high purity isolation of viable CTCs and their detection is necessary. Isolation is the process when CTCs are separated from all other cells in the sample, while detection is the direct or indirect identification of tumor cells. The enrichment process may precede when the majority of blood cells are removed from the sample to enhance relative CTC concentration. The most common methods are density gradient centrifugation, red blood cell lysis, positive or negative immunomagnetic separation and sized-based filtration [8]. Based on their working principles, isolation and detection could be classified in three major categories.

1. **Nucleic acid-based methods for CTC detection**

This method directly or indirectly detects the presence of CTCs by identifying specific DNA or mRNA molecules in the sample. Specific primers are enrolled on polymerase chain reaction (PCR) to target DNA or mRNA molecules known to be associated with cancer cells. CTC detection using mRNA is more effective due to short period of presence in the circulation (unstable molecule with rapid degradation) which means capture of living CTCs, while free DNA could deliver false positive result by capturing molecules released by necrotic or apoptotic cancer cells circulating longer period [9]. Nowadays, multiplex reverse transcription followed by primer specific PCR is widely used, in which expression of multiple transcripts

![Figure 1](image-url)  
**Figure 1.** Presentation of potential metastasis: CTCs are shed from the primary tumor into the circulation via EMT process. After intravasation CTCs undergo MET and extravasation with metastasis formation.
could be measured providing improved sensitivity and specificity rated of heterogenic CTCs (commercially available AdnaTest kits – AdnaGen, Germany) [10]. Generally, thanks to the amplification principle of PCR, nucleic acid-based method could effectively pick out the signal from an extremely small amount of marker in a large sample (1 CTC in 5-10×10⁶ nucleated blood cells or more than 5 mL of blood) thus offers the highest sensitivity for CTC detection [11]. The essential factor to reach this high sensitivity is the specificity of selected markers. Common markers of epithelial specific genes, such as cytokeratins or EpCAM, are widely used as they constitute malignancies and normally are absent in peripheral blood. Organ-specific markers, such as PSA, MUC1 or tumor specific markers, such as CAE, HER2, could help specify the correct cancer diagnosis [12]. The downside of this approach is possible false-positive result from tissue- and organ-specific markers originating from non-cancer cells that enter the bloodstream due to inflammation or invasive diagnostic biopsies [13]. Moreover, none of the recent markers used are entirely CTC-specific. The major drawback is the fact that CTCs must be lysed before the PCR process, making impossible for further analysis as observation or enumeration.

2. Physical properties-based methods for CTC isolation

These methods use the physical characteristics of cancer cells like density, size, mechanical plasticity and dielectric properties that could be used to isolate CTCs from samples.

a. Isolation of CTCs based on size and mechanical plasticity

This approach considers that cancer cells are larger than normal blood cell, thus it is selected throughout the filtration [14,15]. The simplest method is using track-edged filters or microfilters which are a porous membrane with 8 µm diameter holes that allow the blood cross but capture the bigger CTCs (ISET – Rarecells, France, ScreenCell systems – ScreenCell, France, MetaCell – MetaCell, Czech Republic). The advantage is that the captured cells remain intact allowing their subsequent morphological and molecular analysis [16-19]. This approach could be performed also in a microfluidic setting, where the separation results in a precisely defined topography of microstructures and the laminar flow in microchannels [20]. Advanced technology, such as CTC chip by Clearbridge Biomedics, enables to isolate single CTCs with automatic vision-based enumeration and analysis. Methods using size-dependent hydrodynamic forces as formation of microscale vortices or Dean-coupled inertial migration has been also published [21,22], as well as active acoustophoresis technique that practices an external acoustic force to separate different cells in the microchannel [23]. The downside of this method is false-positive result in case of leucocytes capture, false negativity in case the cells become more plastic during EMT and altered functions of isolated CTCs due to mechanical stress during isolation [24-26].

b. Electrokinetic isolation of CTCs

Cells are electrically neutral, but in the electric field polarisable and electric dipoles moments are induced in them [27]. The magnitude and direction of these dipole moments depend on the polarity and conductivity of cell membrane and cytoplasm, cells phenotype, physiological state and morphology [28]. Factors affecting this method is the gradual change of dielectric characteristics due to ion leakage, thus the isolation should be completed as fast as possible. Unfortunately, the process is still relatively slow [29].

3. Antibody-based methods for CTC detection and isolation

The most common method for detection as well as isolation of CTCs. The principle is the antibody-antigen specific binding, mainly done by immunochemistry, but other techniques like Raman spectroscopy, photoacoustic flowmetry and nuclear magnetic resonance have been investigated [30-32]. CTCs are captured to the antibody-mediated matrix most often in a form of magnetic particles or microchannels. The performance of this method depends on the antigen it represents. For detection of CTCs most widely EpCAM and different subtypes of CK are used, while more organ- and tumour-specific markers, such as CEA, EGFR, PSA, HER2, MUC1 could be applied. Up to this date, no marker met the high specificity required for the ideal detection and isolation of CTCs.

a. Immunochemistry methods for CTC detection

Although still not achieving ideal performance in practice, it is considered the most reliable and specific method of CTC detection. CTCs are often referred as CK positive/DAPI positive/ CD45 negative cells [33]. While CD-45 negativity rules out white blood cells, DAPI excludes cell fragments and debris. Flow cytometry, including fluorescence-activated cell sorting (FACS) and the more popular image cytometry mainly referring immunofluorescence microscopy is used in this method. The lat-
ter could incorporate several markers and different molecular (FISH) or cytomorphological (N/C ratio) assays which improve the specificity of detection and integrate automated digital microscopy and computerized post-processing for better practical use [54]. CellSearch system (Menarini, Italy) is the only FDA approved assay for CTC detection. About 99% detection sensitivity was reached by the HDCTC array, which without the enrichment process, could detect CTC aggregates with high clinical significance in micrometastasis development as well [35-36]. Living CTCs for prognostic significance for a variety of carcinomas could be detected by a novel approach called EPISPOT [37].

b. Immunomagnetic methods of CTC isolation

Magnetic field can be successfully used to isolate CTCs if their magnetic characteristics are selectively modified. Cancer cells can be tagged by antibody-conjugated magnetic microbeads or nanoparticles that bind to a specific surface antigen [38]. In a non-uniform magnetic field the tagged cells migrate towards areas of higher magnetic flux density where they are captured [59].

c. Adhesion-based methods for CTC isolation

This method focuses on an adhesion surface, whose biochemical and topographical properties have been modified to attract and capture cancer cells. This can be performed in static or in continuous-flow microfluidic modes [40]. In the first mentioned, the sample is left incubated on a collagen-coated surface. During incubation CTCs with invasive characteristics tend to invade the surface and are captured, while the rest non-target cells are washed off [41]. The second is achieved by flowing the sample through a straight microchannel coated with antibody against CTCs so the target cells can effectively interact with the capture surface [42].

Endometrial cancer

Cancer of the corpus uteri (EC) is the 7th most commonly diagnosed cancer in female population worldwide with 382,100 estimated new cases and 89,900 deaths in 2018 [43]. In the developed countries it represents the fourth leading cancer in women and the most common malignancy of the female genital tract. In the United States 63,250 new cases and 11,350 deaths were estimated in 2017 [44]. In Europe, the number of new cases was about 100,000 with an incidence of 13.6 per 100,000 in 2012 [45].

Despite the absence of a reliable screening tool, EC is most often diagnosed in early stage because of symptomatic postmenopausal uterine bleeding. Hematogeneous spread is in correlation with deep myometrial invasion [46]. Surgery is the primary treatment method, in addition with adjuvant radiotherapy and chemotherapy in advanced and high-risk cases.

The largest study was published by Kiss et al, in which blood from 92 patients with various grades and stages of EC was isolated for CTCs. Positivity reached 75% of patients and a method described a successful size-based separation method with high detection rate of viable CTCs with proliferation potential (Metacell®). In addition, there was no significant difference between CTC presence and differentiation level (grade), stage of disease and lymph node involvement [47].

Other studies involved rather a smaller number of high-risk EC patients with EpCAM-positive CTCs isolated by CellSearch. Bogani et al isolated CTCs in 2 EC patients from 28 (7% positivity). Both patients were in stage IIIC and CTCs presence was significantly correlated with myometrial invasion and lymph node positivity [48]. Association in CTCs and cervical involvement was published by Ni et al. From 40 EC patients 6 were positive for CTCs (15% positivity), whereas 3 patients had FIGO stage I and 3 patients had stage III with no significant difference in the quantity of cells. Also, no significant correlation was found between CTCs and serum CA125/human epididymis protein 4 (HE4) levels. One patient with type II stage I had repeated CTC examination after the first dose of adjuvant therapy [49]. Another study was provided by Lemech et al in which demonstrated 18 CTC positive EC patients from 30 (60% positivity). CTC correlated with higher stage disease, worse survival, non-endometroid histology over endometroid and tumour size bigger than 5 cm. In addition, CTCs and FFPE tissue blocks were placed for immunohistochemistry staining of EpCAM and stathmin primary antibodies and put in correlation with CTC status. Stathmin was overexpressed in all CTC-positive patients whose tissue was stained (7 patients). This could mean that stathmin has potential as a marker of PIK3K pathway activity which is one of the most studied pathways in EC with aberrations including oncogenic PIK3CA mutations and PTEN loss of function [50].

Further studies observed the presenting genes in CTCs in patients with high-risk EC. Due to the high expression in the investigated cell lines, Cytokeratin 19 and claudin 4 were identified as a suitable gene marker for CTCs in endometrial adenocarcinoma [51]. Obermayr et al conducted a multimarker analysis of six genes (CCNE2, DK-FZp762E1512, EMP2, MAL2, PPIC and SLC6A8)
which were positively identified in 64% in a group of 25 EC patients [52]. The expression of thyroid transcription factor (TTF-1) in CTCs was strongly correlated with TNM staging, vascular infiltration and lymphatic spread. Progression-free survival rate and median survival time decreased in the TTF-1 positive cohort, while recurrence rate was significantly lower in the negative group [53]. Finally, Alonso-Alconada et al described the association of molecular CTC-phenotype with plasticity, stemness and epithelial-to-mesenchymal transition (EMT) features which promotes CTC dissemination. Markers of EMT show higher expression in ETV5, NOTCH1, SNAI1, TGFβ1, ZEB1 and ZEB2. Expression of ALDH and CD44 pointed to an association with stemness, while the expression of CTNNB1, STS, GDF15, RELA, RUNX1, BRAF and PIK3CA suggests potential therapeutic targets. The significance to clinical practice could be the identification of patients in need of additional systemic therapies after primary surgery to avoid metastasis and to eliminate the risk of recurrence in the future [54].

Cervical cancer

According to a recently published study by the GLOBOCAN, cervical cancer (CxCa) is the third most common cancer after breast and lung cancer worldwide and is also third in cancer-related deaths in female population [43]. Cervical cancer is the most frequently diagnosed cancer in more than half of the countries in Africa and accounting for about 30% of total cancer cases and deaths in the region [55]. In the USA, an estimated 13,240 cases of invasive cervical cancer are expected to be diagnosed with 4,170 deaths in 2018 [44]. In the European Union, there were about 34,000 new cases of cervical cancer and more than 13,000 deaths in 2012 [56].

The etiology of cervical cancer is the infection of cell by Human Papilloma Virus (HPV) and belongs to the so-called virus-induced cancers [57]. The cancers express viral oncogene transcripts specific for infected cells [58]. Over 99% of CxCa are high-risk HPV positive, while the oncogenic properties are mediated by the viral oncogenes E6 and E7 which are responsible for the inactivation of p53 and pRb tumor suppressor proteins [59,60]. The tumour is active only if E6 and E7 are expressed, otherwise cancer cells apoptosis is initiated by the restored p53 and pRb proteins [60]. Therefore, viral oncogene transcripts E6/E7 are the ideal markers for the detection of tumour cells in cancer patients. On this basis it was established a method by Pfitzner et al for detection and quantification of CTCs by digital RT-PCR [61]. She describes a CTC detection rate of 66% in patients with systemic spread and the Digital-Direct-RT-PCR method as a highly sensitive method in separating HPV16/18-E6 expressing cells from a large number of HPV negative cells. This method could be applied in other tumour types where tumour specific transcripts are already discovered.

The presence of the integrated HPV virus in cervical cancer lesions alongside with cancer cell characteristics could be used in additional methods. Telomerase activity is responsible for the restoration of chromosomes length after cell division, which gives the cancer cells their immortality and its expression could be used as a potential biomarker [62]. The expression of hTERT has been identified as a determinant of telomerase activity and is transcriptionally regulated by its promoter [63,64]. Telomerase-specific replication-selective adenoviruses were designed from adenovirus vectors by inserting the hTERT promoter, restricting their proliferation to telomerase activity only, thus could be used in both in vivo and in vitro cancer cell detection and even in oncolytic virotherapy [65-67]. Takura et al used a modified adenoviral vector OBP-1101 which expresses GFP in infected cells. CTCs were identified in 6 of 23 samples (26% positivity), with no correlation with distant metastasis, overall survival or progression-free survival [68].

On the other hand, Wen et al published that elevated CTCs and SCC-Ag levels were associated with poor disease-free survival. They collected blood samples from 99 patients with locally advanced cervical cancer (FIGO stage IIB-IVA) and CTC and SCC were enriched and magnetically separated by anti-CD45 monoclonal antibody coated in magnetic beads and identified by negative enrichment and immune fluorescence in situ hybridization (NeimFISH). The CTC-positive rate was 45.5% and CTC and SCC-Ag alone showed as strong predictors of DFS. The combination of these 2 biomarkers in a new risk model significantly improved their predictive efficiency for survival than CTC or SCC-Ag level alone [69].

Ovarian cancer

Ovarian cancer (OC) is the deadliest gynecological malignancy, with a 5-year survival rate approximately 47% - a number which remained constant over the past two decades. It is the fifth leading cause of cancer death among women in Europe and the United States and the second most common gynecological malignancy [70]. The annual estimates are 295,400 of new ovarian carcinoma cases and 184,800 deaths worldwide [43]. The
highest rates (11.4 per 100,000 and 6.0 per 100,000, respectively) are reported in Eastern and Central Europe [71]. Although China has a relatively low incidence rate of 4.1 per 100,000 due to its large population, the overall estimates are 52,100 new cases and 22,500 related deaths in 2015 [72]. The same year, 21,290 new cases and 14,180 were estimated in the USA [73].

Early diagnosis improves survival, but unfortunately only 15% of ovarian cancers are diagnosed at an early localized stage. Most ovarian cancers are epithelial in origin and treatment prioritizes cytoreductive surgery followed by cytotoxic platinum and taxane chemotherapy. While most tumours initially respond to treatment, unfortunately recurrence is likely to occur within a median of 16 months in advanced-stage disease [74]. Postoperative residual tumour is one of the most important prognostic factors in advanced ovarian cancer [75]. Despite new therapeutic concepts being used as antiangiogenic therapy or PARP inhibitors, more than half of all patients experience recurrence resulting in poor overall prognosis [76].

There are many studies evaluating the possible prognostic significance of CTCs in OC. Despite the early studies in which detection of tumour cells in the bone marrow and/or blood was not associated with poor prognosis [77], just CTC-positive patients had statistically more grade 3 tumours [78], and later studies proved their useful value. In a large systematic review conducted by Cui et al on 10 relevant studies with 1164 patients showed a strong association of CTCs (disseminated tumour cells as well) with advanced staging (stage III-IV), poor prognosis (low OS, shortened PFS, DFS), and treatment response (platinum resistance). On the other hand, no association was found with tumor histology, lymph node metastasis and optimal or suboptimal surgery [79]. In a novel electronically conductive and nanoroughened microfluidic platform-based chip was introduced by Lee et al with 98.1% detection rate of CTCs in 54 OC participants. Additionally, reduced OS in patients with recurrent disease and chemoresistance correlated with CTC-cluster positive samples [80]. High detection rate of CTCs (90%) was published by Zhang et al., when from 109 newly diagnosed OC 98 were CTC-positive. The number of CTCs was significantly lower in stage I patients than in advanced stages. High diagnostic significance could be a 100% detection rate in 7 “occult” patients without epithelial ovarian carcinoma symptoms, while CA-125 was elevated only in 4 patients (57%). Elevated expression of EpCAM and HER2 in CTCs were associated with chemoresistance and shorter overall survival [81].

Not only OC is often diagnosed in later stages, but preoperative differential diagnosis of existing adnexal masses is also a challenge. Many studies have examined various modalities (biomarkers like CA-125 and HE4 levels, imaging studies like ultrasound, CT, MRI, PET and their combinations), while Suh et al studied CTCs as a new platform in the evaluation of findings on the ovaries [82]. From a total number of 87 patients, at least one CTC was found preoperatively in 49 (56.3%); 19/43 (44.2%) were benign, 10/10 (100%) early-stage and 14/21 (66.7%) advanced-stage cancer. Only 1 healthy control from 22 (4.5%) was positive for CTCs. In further analysis, preoperative CTC detection was more sensitive in benign vs. early stage (stage I and II) cancer compared with benign vs all-stage cancer and remained even in benign vs stage I cancer. Other diagnostic modalities showed a reversed pattern: modest performance in early-stage cancer and significant in all-stage cancer including borderline tumours. CTCs showed no association with CA-125 levels or ROMA index and could reflect early hematogeneous metastasis before even peritoneal spread. Another study assessed CTCs in 49 women with newly diagnosed complex pelvic masses. No CTCs were found in benign histology cases (0/14) while malignancy was associated with CTCs in 9/35 (25.7%). CTCs were detected only in 5/29 (17.2%) patients diagnosed with OC (all 5 patients had stage III or IV), and of the rest 5 patients 4 were CTC-positive (80%) and diagnosed with non-ovarian origin tumor that metastasized to the ovaries (2 Krukenberg tumour, 1 metastatic endometrial cancer, 1 abdominal soft-tissue sarcoma with peritoneal carcinomatosis) [83].

Another potential benefit of CTCs is that they could have a role in indicating invisible cancer lesions after complete or minimal-residual cytoreductive operations. CTCs present before surgery or neoadjuvant chemotherapy indicate a higher risk of death even after optimal debulking surgery (R0) [84].

In the majority of human malignancies PIK3K/akt/mTOR signalling pathway is aberrantly activated stimulating proliferation and cell survival [85]. This pathway has also been reported in OC, while EMT is responsible for chemoresistance [86]. Chebouti et al analysed the incidence of epithelial (EpCAM, MUC-1) and EMT-like (PI3Ka, AKT-2, Twist) CTC at primary diagnosis of ovarian cancer (91 patients) and how their detection was altered by platinum-based chemotherapy. Higher number of EMT-like CTCs (30%) were detected than epithelial subtype of CTCs (18%) prior to surgery, which further increased in EMT-like CTCs even after chemotherapy (52%), but decreased in the epithelial subtype of CTCs (14%). Epithelial and EMT-like
CTCs exhibit a low phenotypic overlap as only a minor fraction of CTC-positive patients showed dual positivity for both phenotypes (18% before surgery and 12% after surgery). After chemotherapy a shift towards PIK3Ka and Twist expression was found, which could have a clinical interest as these signaling pathways could be responsible for the recurrence of OC [87].

Further studies of CTC characteristics showed that the presence of ERCC1-positive CTCs at primary diagnosis is an independent predictor of platinum resistance [88]. Auxiliary assessment of ERCC1 transcripts increase the CTC detection rate and presence of ERCC1-positive CTCs reduce progression-free survival and overall survival, while their persistence indicates poor post-therapeutic outcome [89].

Many authors published articles on molecular characterization of CTCs in OC patients. One of the largest studies was conducted by Obermayr et al, in which 11 gene markers (PPIC, GPX8, CDH3, TUSC3, COL3A1, LAMB1, MAM, ESRP2, AGR2, BAIAP2L1, TFF1, EPCAM) were studied in a cohort of 200 patients before therapy (surgery or neoadjuvant chemotherapy) and during follow-up. PPIC gene (Cyclophilin C) was overexpressed in 34 cases (17%) and PPIC positivity during follow up period (13 cases 14% positivity) showed significantly shorter disease-free survival, overall survival and platinum resistance [90]. Another large study of 118 OC patients was conducted by Kolostova et al, successful isolation of CTCs in 77 patients showed 65.2% positivity, from which further 20 patients were tested for gene expression. CTCs overexpressed MUC1 and EPCAM in more than 90% cases, KRT18 and KRT19 was also elevated, while MUC16 (CA125) was detected only in 30% [91]. In another study from the same authors 40 patients with OC were enrolled in a gene expression study. Statistically significant difference was confirmed for the following genes (p<0.02): KRT7, WT1, EPCAM, MUC16, MUC1, KRT18 and KRT19. The results suggest that the combination of the above listed genes could confirm CTCs presence in OC patients with higher specificity than when gene analysis tests are performed for one marker only [92].

Concluding remarks

Cancer cells in gynaecological malignancies are present in the circulation of patients and can be isolated and detected by numerous methods. The presence of CTCs seems to be associated by adverse clinicopathological features and worse clinical outcomes. CTCs have their prognostic value and in times of personal medicine could help in therapy management and its effectiveness control. Recurrences could be detected earlier and reacted more precisely to them.

Conflict of interests

The authors declare no conflict of interests.

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