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A pilot study evaluating the feasibility of enriching and detecting circulating tumour cells from peripheral and ovarian veins in rare epithelial ovarian carcinomas

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ABSTRACT

Introduction: Studies on circulating tumour cells (CTCs) in rare epithelial ovarian carcinomas (EOC) are limited, despite their potential as a minimally invasive biomarker for monitoring cancer progression and predicting outcomes. This pilot study aimed to assess the feasibility of enriching and detecting CTCs from both peripheral and ovarian vein blood samples in rare EOC subtypes.

Materials and methods: Blood samples were collected from the peripheral and ovarian veins of 20 patients with rare EOC. Among the 20 patients, 12 had early-stage disease (I-II), while 8 had advanced disease (III-IV). CTCs were enriched using the Parsortix® system and immunophenotyped via immunofluorescence targeting epithelial markers (EpCAM/pan-cytokeratin) and Hoechst for positive selection, and CD45 for negative selection. CTC status (positive versus negative) was correlated with clinicopathological data.

Results: CTCs were successfully detected in 45 % (1–19 CTCs) of baseline peripheral samples and 55 % (1–4776 CTCs) of ovarian vein samples. CTC doublets and clusters were detected in ovarian vein samples (3/11), but not in peripheral samples (0/20). A higher proportion of deaths were observed in CTC+ patients compared to CTC- patients ($p = 0.0088$).

Conclusion: Here we demonstrate the feasibility of enriching and detecting CTCs from both peripheral and ovarian vein blood in patients with rare EOC. The higher CTC yield in ovarian vein blood suggests that tumour-draining blood may play a role in improving CTC detection. This pilot study paves the way for larger studies to investigate the prognostic utility of CTCs and refine their clinical value in these rare understudied EOC.

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1. Introduction

Epithelial ovarian carcinomas (EOC) are the eighth most commonly diagnosed cancer in women in Europe and have the highest mortality rate of all gynaecological malignancies [1]. This high mortality is attributed to the absence of an effective screening tool, late-stage diagnosis, and frequent recurrence. The prognosis for EOC is closely tied to the stage at diagnosis, with the 5-year survival rate dropping from 93 % at stage I, when the disease is localised, to 31 % at stage IV disease [2]. Identifying biomarkers for disease monitoring and predicting therapeutic responses are crucial for improving patient survival and wellbeing.

One promising avenue of research is circulating tumour cells (CTCs), a component of liquid biopsies. These rare cells, shed from primary or metastatic tumours, play a pivotal role in the metastatic cascade by migrating through the circulation with the potential to form disseminated disease [3]. As such, CTCs could serve as a minimally invasive biomarker for real-time monitoring of disease burden and provide insights into the metastatic processes in EOC. While CTCs have gained significant interest in recent years as valuable biomarkers for prognostic evaluations in metastatic breast, prostate, and colorectal cancers [4–6], the unique behaviour of EOC, which primarily involves transcoelomic spread rather than haematogenous, presents distinct research opportunities [7]. One area of interest is the ovarian vein, which directly drains the ovaries and may provide a more enriched source of CTCs compared to peripheral blood, offering a window into tumour dissemination at its origin [8]. Studies investigating CTCs from the pulmonary vein in lung cancer have demonstrated their prognostic value, supporting the idea that blood sampling from the tumour-draining vein may be more valuable both in research and clinical practice [9]. The typically higher CTC yield from tumour-draining veins compared to peripheral veins as seen in several cancer types, may also enhance the feasibility of CTC analysis in EOC [10,11].

Most studies on CTCs in EOC either group all histological subtypes together or focus exclusively on high-grade serous carcinoma (HGSC), which accounts for 70 % of cases [12–17]. Few studies have addressed the heterogeneity of the rarer EOC subtypes, including clear-cell, endometrioid, mucinous, and low-grade serous carcinoma (LGSC) [18]. In addition, it is important to analyse HGSC and rarer subtypes distinctly in research as their biologic behaviour including site predilection and potential response to treatment are distinct [19]. The aim of this study was therefore to assess the feasibility of CTC enrichment and detection from peripheral and ovarian vein samples in a cohort of patients with rarer EOC subtypes. We hypothesise that ovarian vein sampling may yield a higher concentration of CTCs because of its proximity to the primary tumour site, providing more accurate insights into patterns and disease burden in these rare EOC subtypes.

2. Materials and methods

2.1. Patient recruitment

Peripheral blood samples were collected from 20 treatment-naïve patients with rare EOC subtypes at St. James's Hospital, Dublin 8, Ireland between April 2021 and July 2024. Intraoperative sampling from the ovarian vein was conducted by trained clinicians during primary cytoreductive surgery (PCRS), when appropriate, in a subset of 11 patients from this cohort. Written informed consent was obtained from all patients prior to participation, and the study was approved by the St. James's Hospital (SJH)/Tallaght University Hospital (TUH) Joint Research Ethics Committee (ID:2095) in compliance with the General Data Protection Regulation (GDPR) and the Data Protection Act 2018. Clinical characteristics including treatment trajectory, age, histological subtype, tumour stage (FIGO, Federation of Gynaecology and Obstetrics), CA19-9 levels, CA-125 levels, radiological primary tumour size (PTS), peritoneal cancer index (PCI) score, tumour laterality, progression-free survival (PFS), overall survival (OS), recurrence status, and mortality status were collected for each patient. Only patients with a minimum follow-up of one year from the date of diagnosis were included in the recurrence and mortality chi-squared correlation analysis. PTS was determined by extracting the largest dimension of the pelvic mass reported in radiology reports, using the single dimension provided or the maximum of the three dimensions (length, width, height) when available. PCI was recorded from surgical reports to correlate peritoneal spread with CTC status. A PCI score of 0 indicates the absence of peritoneal disease, while any score greater than 0 denotes the presence of peritoneal disease. Exclusion criteria included individuals under 18 years of age or those with a prior history of treatment for EOC. Each patient was designated a pseudonymised number.

2.2. Ovarian vein sampling

Prior to any substantial manipulation of the ovarian tumour, the infundibulopelvic ligament and gonadal vessels are isolated and clamped proximally above the pelvic brim prior to sampling. This technique ensures surgical safety, preventing uncontrolled bleeding post-sampling. The ovarian vein is differentiated from the artery, and a 23-gauge needle attached to a 10 mL syringe is used to aspirate 4–10 mL of blood from the right and/or left ovarian vein(s).

2.3. CTC enrichment and detection

Peripheral and ovarian vein blood samples (7.5 mL) were collected in Vacutainer® K2E K2EDTA tubes (Greiner Bio-One, Austria) and processed within 24 h on the Parsortix® PR1 (ANGLE PLC, UK) microfluidic CTC enrichment system. Parsortix® separation cassettes with a 6.5 μm gap size were used and 99 mbar pressure was set on the system to subject the whole blood to enrichment. Following the enrichment step, the cells were fixed with 4 % paraformaldehyde (Sigma Aldrich, Ireland) and stained with an antibody cocktail containing anti-epithelial cell adhesion molecule (EpCAM) (9C4, Alexa Fluor 488, BioLegend, USA), anti-pan-cytokeratin (panCK) (CK3-6H5, FITC, Miltenyi Biotec, Germany), and 20 mM Hoechst 33342 nuclear dye (815-968-0747, Thermo Scientific) for positive selection, and anti-CD45 (HI30, Alexa Fluor 647, BioLegend, USA) to exclude leukocytes. All antibodies were diluted in permeabilisation buffer Inside Perm (#130-090-477, Inside stain Kit, MACS Miltenyi Biotec, Germany) at 1:120 dilution. Parsortix® cassettes were viewed using EVOS® FL digital inverted fluorescence microscope (Invitrogen™) and CTCs were enumerated as described previously [20]. To visualise the 3D structure of the CTCs, z-stacks were captured using the Leica SP8 scanning confocal microscope with Leica LAS X software. Downstream image analysis was performed using FIJI ImageJ2 V2.14.0. CTC positivity was defined as having ≥1 EpCAM/panCK+, Hoechst+, CD45- round intact cell with a large nucleus-to-cytoplasm ratio.

CTCs were enumerated in baseline peripheral samples and intraoperative samples from the ovarian vein. CTCs were defined as single CTCs, doublets (2 CTCs), and clusters (>2 CTCs). CTC doublets and clusters were enumerated as single events, without counting the individual cells within these clusters toward the total CTC count, as it was not always feasible to determine the number of cells in large clusters.

2.4. Statistical analysis

All data were analysed using GraphPad Prism (GraphPad Software, San Diego, CA, 10.1.1) for Macintosh (GraphPad Software Inc., USA). The associations between CTCs and clinicopathological variables were evaluated using chi-squared tests with significance set at $p \leq 0.05$. Kaplan-Meier survival curves were used to estimate survival outcomes in different groups. The log-rank test was used to statistically compare the curves for different groups.

3. Results

3.1. Clinicopathological data

20 patients with rare EOC subtypes were recruited. Of the 20 patients, 4 had mixed histology, 7 clear-cell, 5 endometrioid, 2 mucinous, and 2 LGSC. To note, in the 2 patients with mucinous histology, metastasis from a primary gastrointestinal cancer was ruled out. 17 patients underwent PCRS followed by adjuvant chemotherapy (ACT), two had neoadjuvant chemotherapy (NACT) followed by interval cytoreductive surgery (ICRS), and one patient had chemotherapy only. Ovarian vein sampling was not conducted during ICRS in patients who had received NACT. It was conducted during PCRS when deemed suitable by the surgeon, in 11 patients in our cohort. Treatment trajectory (PCRS-ACT/NACT-ICRS/Chemo only), age, histological subtype, FIGO stage, CA19-9 levels, CA-125, PTS, PCI score, laterality, PFS, OS, recurrence status, mortality status, and peripheral baseline and ovarian vein CTC counts are displayed in Table 1.

3.2. CTC detection and enumeration

CTCs were detected in 45 % (9/20) (range 1–19 CTCs) of baseline peripheral samples and 55 % (6/11) (range 1–4776 CTCs) of ovarian vein samples. CTCs were detected in 5 of the 12 early-stage patients at baseline and/or in their ovarian vein sample. All CTCs detected in baseline peripheral samples were single CTCs. Among the six patients with CTCs in the ovarian vein, three (one with mixed histology, two with clear-cell carcinoma) had single CTCs, one patient with mixed histology had a CTC doublet, and two patients (one endometrioid, one mucinous) had CTC clusters. The number of cells within a CTC cluster ranged from 3 to approximately 30 cells; the exact number was difficult to evaluate in large clusters (>15 CTCs) (Example shown in Fig. 1).

3.3. Association between CTCs and clinicopathological data

The association between clinical characteristics and CTC status (positive or negative) in peripheral baseline and ovarian vein samples is displayed in Table 2. Median age was 54.5 (36–79) years and median PTS was 12.95 [3–35] cm. Four patients (patients 8, 9, 16, 17) were excluded from the recurrence and mortality chi-squared analysis due to not meeting the minimum 1-year follow-up period. No significant association was identified between CTC+ and CTC- samples in either peripheral or ovarian vein samples with respect to age, histological subtype, FIGO stage, CA 19-9 levels, CA-125 levels, PTS, laterality, or recurrence status. A trend towards elevated CA19-9 and CA-125 was observed in CTC+ patients, with higher CA19-9 levels in patients with ovarian vein CTCs ($p = 0.0578$), and higher CA-125 in patients with peripheral CTCs ($p = 0.0893$). Patients with peritoneal disease (PCI score $\geq$1) were more likely to be CTC+, while patients with no peritoneal disease (PCI = 0) were more likely to be CTC- ($p = 0.0117$). Moreover, of the 7 patients who were CTC+ in baseline peripheral samples, 4 (57 %) had recurred, while 3 (43 %) had no evidence of disease (NED). In contrast, 7 (78 %) of the 9 patients who were CTC- at baseline did not recur, with 2 (22 %) experiencing recurrence. A higher proportion of deaths were observed in the CTC+ cohort compared to the CTC- cohort ($p = 0.0088$). Out of the 7 patients who were CTC+, 4 (57 %) of them were deceased, while 3 of them (43 %) were alive. All 9 patients who were CTC- were alive (100 %). This data suggests that mortality status at 1-year may differ depending on peripheral CTC status at baseline (whether the patient is CTC+ or CTC-), however, larger studies are needed to validate this. A Kaplan-Meier survival curve was constructed to assess OS in all 20 patients. While no statistical difference ($p = 0.0936$) was identified between patients who were CTC+ or CTC- in peripheral baseline samples, a trend toward reduced OS in CTC+ patients was observed (Supplementary Fig. 1). Median OS for CTC+ patients was 32 months, and median OS for CTC- patients was not reached. Median follow-up time was 14 months (range 1–42 months). There was no statistical association found between the presence of ovarian vein CTCs and mortality status ($p = 0.2123$). Notably, ovarian vein sampling was not performed in three of the four patients who died. In the fourth patient (patient 6), 45 CTCs were detected in their ovarian vein sample.

4. Discussion

The detection of CTCs in peripheral and ovarian vein blood samples in patients with rare EOC subtypes offers a promising perspective on their potential as clinical biomarkers for disease monitoring and predicting outcomes in rarer EOC subtypes. In this pilot study using the Parsortix® microfluidic system, CTCs were detected in 45 % of peripheral samples, ranging from 1 to 19 CTCs. This is lower than the 63 % detection rate reported in another study using the Parsortix® in EOC by Asante and colleagues [12]. Asante's study, however, included predominantly HGSC (15 HGSC, 1 mucinous borderline), with only 2/16 patients with early-stage disease, while 12/20 in our cohort had early-stage disease. This is expected, as early-stage disease typically shows a more even distribution of rarer histological subtypes, whereas in advanced stage disease, HGSC predominates [21]. The small sample size as well as the difference in CTC positivity rates in both studies may be attributed to the histological subtype analysed (HGSC versus rare EOC subtypes), as each subtype of EOC differs markedly morphologically, clinically, and at a molecular level [22]. The lower peripheral CTC positivity rate in our study may also be related to stage distribution, since CTC detection using cell adhesion matrix enrichment techniques has been shown to correlate with tumour stage in EOC patients [23]. However, our positivity rate was higher than studies using CellSearch®-based approaches for detecting EOC CTCs (CTC positivity also defined as $\geq$ 1 CTC), where rates ranged from 3.6 % to 44 % [16,24,25]. As CellSearch® relies on the immunomagnetic attraction of EpCAM, it fails to capture EpCAM-low or EpCAM-negative CTCs, which have been reported in EOC [26]. Nonetheless, it was the first system to receive FDA approval for CTC-based prognostic monitoring in metastatic breast, prostate, and colorectal cancers [4–6]. Compared to these cancers, haematogenous metastasis is less prevalent in EOC due to its unique mode of transcoelomic spread [27]. This likely explains the low frequency of CTCs detected in EOC compared to these cancers, which underpins the importance of utilising CTC enrichment and detection approaches with higher sensitivities. As CTCs are such a rare event in EOC (approx. 1 CTC surrounded by 1 million white blood cells and 1 billion red blood cells), maximising the capture rate of enrichment systems is paramount [28,29]. It is important to note that the limited representation of rarer EOC subtypes in existing studies makes it challenging to compare the findings in this study to the broader EOC studies in the literature, as these studies often included few or no cases of these rarer subtypes [24,30,31]. However, the detection of CTCs during early-stage EOC as documented here suggests that tumour cells may enter the blood circulation earlier than previously thought, providing an intriguing window into the biology of EOC. Similarly, Lawrence's comprehensive review on CTCs in early-stage cancers supports the idea that early detection of CTCs could signal aggressive disease, potentially expediting diagnosis and treatment initiation for such patients, while minimising overdiagnosis and overtreatment of less aggressive tumours [32]. This highlights the need for longer follow-up of our cohort to gain further insight into these findings.

Furthermore, the presence of single CTCs, doublets, and clusters in ovarian vein samples in this study highlights CTC heterogeneity. CTCs were detected in 55 % of ovarian vein samples, ranging from 1 to 4776 CTCs, including the presence of CTC doublets in one patient and CTC clusters in two patients. The identification of CTC clusters is notable, as strong evidence in other cancers suggests that CTC clusters are associated with greater metastatic potential and worse clinical outcomes than single CTCs (see studies cited in Ref. [8]). Despite the association between CTC clusters and worse outcomes in other cancers, three of the

Table 1
Demographics and clinical characteristics of patient cohort. Peripheral baseline and ovarian vein CTC counts per 7.5 mL of blood are listed. #Patient received chemotherapy only, as they were not suitable for surgery so were likely never disease free. Abbreviations: HGSC, high-grade serous carcinoma; LGSC, low-grade serous carcinoma; PCRS, primary cytoreductive surgery; NACT, neoadjuvant chemotherapy; ICRS, interval cytoreductive surgery; ACT, adjuvant chemotherapy, FIGO, Federation of Gynaecology and Obstetrics; CA19-9, cancer antigen 19-9; CA-125, cancer antigen 125; PTS, primary tumour size; PCI, peritoneal cancer index; PFS, progression-free survival; OS, overall survival; NA, not applicable; ND, not documented.

Patient ID	Treatment	Age	Histological subtype	FIGO stage	CA19-9 (U/mL)	CA-125 (U/mL)	PTS (cm)	PCI score	Laterality	PFS	OS	Recurrence status	Mortality status	Peripheral baseline CTC	Ovarian vein CTC
1	PCRS-ACT	41–50	Mixed (clear-cell & endometrioid)	IC2	4661	4230	7.5	5	Left	25	25	No recurrence	Alive	5	25 (doublet)
2	Chemo only	71–80	Mixed (mucinous & HGSC)	IVA	ND	778	ND	NA	ND	9	12	Recurrence#	Deceased	1	Not sampled
3	PCRS-ACT	61–70	Mixed (clear-cell & endometrioid)	IIB	ND	22	7	0	Left	7	23	Recurrence	Alive	0	Not sampled
4	PCRS-ACT	51–60	Mixed (clear-cell & endometrioid)	IIB	ND	80	7.5	6	Left	16	16	No recurrence	Alive	4	1
5	PCRS-ACT	61–70	Clear-cell	IC2	4	113	4	0	Left	18	32	Recurrence	Alive	0	0
6	PCRS-ACT	41–50	Clear-cell	IIIA2	87	10224	18	4	Bilateral	8	22	Recurrence	Deceased	6	45
7	PCRS-ACT	51–60	Clear-cell	IIB	250	295	25	0	Bilateral	14	14	No recurrence	Alive	0	Not sampled
8	PCRS-ACT	51–60	Clear-cell	IIIB	23	793	20	7	Bilateral	3	3	No recurrence	Alive	0	0
9	PCRS-ACT	51–60	Clear-cell	IIIA1	2546	684	15	7	Right	1	1	No recurrence	Alive	1	1
10	PCRS-ACT	61–70	Clear-cell	IB	ND	4273	21	0	Bilateral	9	14	Recurrence	Deceased	2	Not sampled
11	PCRS-ACT	61–70	Clear-cell	IC1	16	17	19	0	Right	7	7	No recurrence	Alive	0	Not sampled
12	PCRS-ACT	41–50	Endometrioid (low-grade)	IIA	23	177	3	8	Left	42	42	No recurrence	Alive	19	0
13	NACT-ICRS	51–60	Endometrioid (high-grade)	IVB	233	302	11	NA	Bilateral	18	32	Recurrence	Deceased	1	NA
14	PCRS-ACT	61–70	Endometrioid (low-grade)	IIB	ND	265	11.4	16	Bilateral	9	9	No recurrence	Alive	0	Not sampled
15	PCRS-ACT	71–80	Endometrioid (low-grade)	IIB	1865	1033	13.9	0	Left	16	16	No recurrence	Alive	0	Not sampled
16	PCRS-ACT	51–60	Endometrioid (low-grade)	IVB	ND	549	15.3	9	Bilateral	3	3	No recurrence	Alive	1	19 (clusters)
17	PCRS-ACT	51–60	Mucinous	IA	90	21	35	0	Left	9	9	No recurrence	Alive	0	4776 (clusters)
18	PCRS-ACT	31–40	Mucinous	IC2	56600	215	10	0	Left	16	16	No recurrence	Alive	0	0
19	PCRS-ACT	41–50	LGSC	IIIB	66	1532	12	ND	Right	8	8	No recurrence	Alive	0	0
20	NACT-ICRS	61–70	LGSC	IVB	ND	1623	ND	NA	Bilateral	14	14	No recurrence	Alive	0	NA

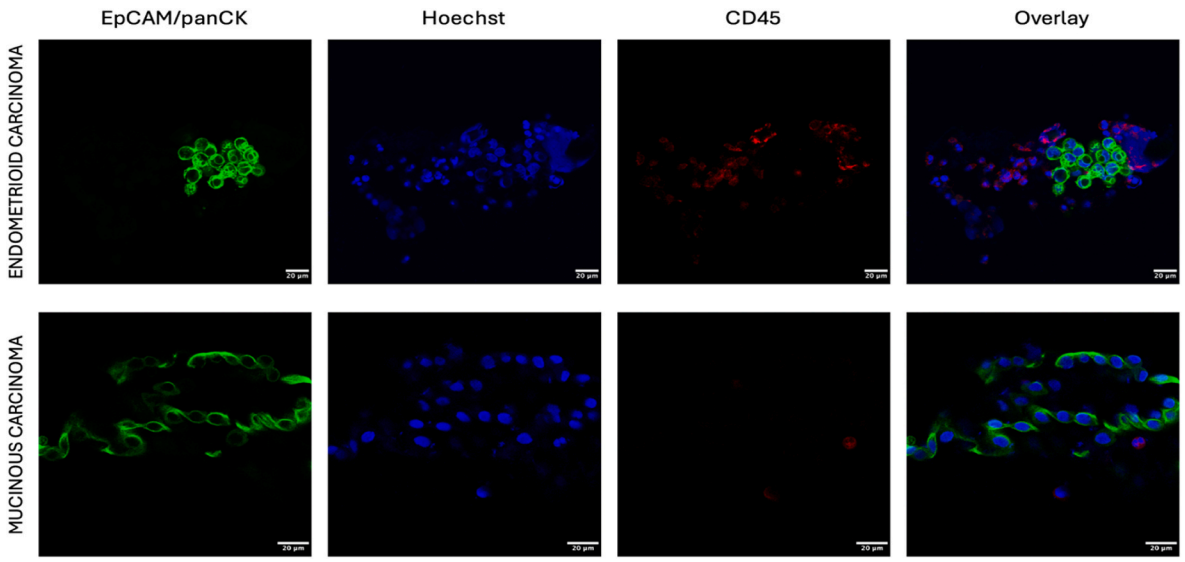

Fig. 1. CTC clusters enriched from the ovarian vein in patients with rarer EOC subtypes. EpCAM/panCK were used as CTC detection markers, Hoechst as a nuclear stain marker, and CD45 as a negative selection marker. Image taken using Leica SP8 scanning confocal microscope and processed using FIJI. Scale bar = 20 μm. Abbreviations: EpCAM, epithelial cell adhesion molecule; panCK, pan-cytokeratin.

Table 2
Association between clinical characteristics and CTC status in peripheral and ovarian veins. ⊗Only patients with a minimum follow-up of one year from the date of diagnosis were included in this analysis.

Clinical characteristics	Patients *n* (%)	Peripheral CTC^{pos} *n* (%)	Peripheral CTC^{neg} *n* (%)	*p* value	Ovarian vein CTC^{pos} *n* (%)	Ovarian vein CTC^{neg} *n* (%)	*p* value
Age (median 54.5 years)	20 (100 %)						
<54	9 (45 %)	6 (30 %)	3 (15 %)	*0.0781*	4 (36 %)	3 (27 %)	*0.8190*
≥54	11 (55 %)	3 (15 %)	8 (40 %)		2 (18 %)	2 (18 %)	
Histological subtype							
Mixed	4 (20 %)	3 (15 %)	1 (5 %)	*0.2679*	2 (18 %)	0	*0.5690*
Clear-cell	7 (35 %)	3 (15 %)	4 (20 %)		2 (18 %)	2 (18 %)	
Endometrioid	5 (25 %)	3 (15 %)	2 (10 %)		1 (9 %)	1 (9 %)	
Mucinous	2 (10 %)	0	2 (10 %)		1 (9 %)	1 (9 %)	
LGSC	2 (10 %)	0	2 (10 %)		0	1 (9 %)	
FIGO stage							
I	6 (30 %)	2 (10 %)	4 (20 %)	*0.8996*	2 (18 %)	2 (18 %)	*0.8214*
II	6 (30 %)	2 (10 %)	4 (20 %)		1 (9 %)	1 (9 %)	
III	4 (20 %)	2 (10 %)	2 (10 %)		2 (18 %)	2 (18 %)	
IV	4 (20 %)	1 (5 %)	3 (15 %)		1 (9 %)	0	
CA19-9 (U/mL)							
≤37	4 (31 %)	1 (8 %)	3 (23 %)	*0.5060*	0	3 (33 %)	*0.0578*
>37	9 (69 %)	4 (31 %)	5 (38 %)		4 (44 %)	2 (22 %)	
CA-125 (U/mL)							
≤35	3 (15 %)	0	3 (15 %)	*0.0893*	1 (9 %)	0	*0.3384*
>35	17 (85 %)	9 (45 %)	8 (40 %)		5 (45 %)	5 (45 %)	
PTS (median 12.95 cm)							
<13 cm	9 (50 %)	4 (22 %)	5 (28 %)	*>0.9999*	2 (18 %)	4 (36 %)	*0.1217*
≥13 cm	9 (50 %)	4 (22 %)	5 (28 %)		4 (36 %)	1 (9 %)	
Peritoneal disease							
No (PCI = 0)	8 (50 %)	1 (6 %)	7 (44 %)	*0.0117*[a]	1 (10 %)	2 (20 %)	*0.2598*
Yes (PCI ≥1)	8 (50 %)	6 (38 %)	2 (13 %)		5 (50 %)	2 (20 %)	
Laterality							
Right	3 (16 %)	1 (5 %)	2 (11 %)	*0.8594*	1 (9 %)	1 (9 %)	*0.8850*
Left	8 (42 %)	3 (16 %)	5 (26 %)		3 (27 %)	3 (27 %)	
Bilateral	8 (42 %)	4 (21 %)	4 (21 %)		2 (18 %)	1 (9 %)	
Recurrence status ⊗							
Recurrence	6 (37 %)	4 (25 %)	2 (13 %)	*0.1523*	1 (14 %)	1 (14 %)	*0.8091*
No recurrence	10 (62 %)	3 (19 %)	7 (44 %)		2 (29 %)	3 (43 %)	
Mortality status ⊗							
Deceased	4 (25 %)	4 (25 %)	0	*0.0088*[b]	1 (14 %)	0	*0.2123*
Alive	12 (75 %)	3 (19 %)	9 (56 %)		2 (29 %)	4 (57 %)	

[a] *P* value ≤ 0.05.
[b] *P* value ≤ 0.01.

four patients who died had only 1–2 single CTCs. The absence of ovarian vein sampling in these three patients represents a missed opportunity to explore if CTC doublets or clusters were present in the tumour-draining blood. The fourth patient, however, had 6 CTCs in their peripheral baseline sample and 45 CTCs in their ovarian vein sample, with no doublets or clusters present. These data underscore the importance of CTC enumeration on the Parsortix® across all patients, as even single CTCs may have prognostic significance.

Additionally, the higher CTC yield observed in ovarian vein samples compared to peripheral samples may reflect the phenomenon of CTCs being more concentrated in tumour-draining blood than peripheral blood, as previously shown in other cancers [10,11,33]. We did not assess the prognostic value of ovarian vein CTCs or CTC clusters in this study, as the sample size and proof-of-concept nature of the study were intended to guide future larger studies. However, to note, the patient with 4776 CTCs, including numerous clusters, in her ovarian vein sample (patient 17) did not meet the minimum 1-year follow-up period for inclusion in the outcome analysis; although, at the time of follow-up the patient was alive with NED. The prognostic value of pulmonary vein CTCs in lung cancer has already been established, however, to the best of the authors' knowledge, there are no reports on the prognostic and therapeutic values of CTCs isolated from ovarian vein samples in patients with rare EOC subtypes [9,34]. Assessing CTC counts from ovarian vein samples could constitute a novel strategy, considering over half of the ovarian vein samples in this study had detectable CTCs. A recent pilot study in endometrial cancer hypothesised that the chances of CTC detection may be higher close to the tumour, particularly from ovarian vein blood, rather than peripheral blood [35]. The study detected CTCs in 8/10 ovarian vein samples, with CTC clusters detected in 4 samples, but found no CTCs in peripheral samples. The same study found no significant correlation between CTC count and clinicopathological characteristics, though the small sample size was a limitation. Nonetheless, ovarian vein sampling is an exciting frontier in liquid biopsy research and its value adjunct to peripheral sampling may further refine prognostication.

We also explored the clinical correlations of CTCs with patient clinicopathological characteristics, although no significant associations were observed between CTC positivity in either peripheral or ovarian vein samples with respect to age, histological subtype, FIGO stage, CA19-9 levels, CA-125 levels, PTS, laterality, or recurrence status. In addition to the small sample size, the lack of association between CTCs and tumour markers in this study could be due to the fact that 12 out of 20 patients had early-stage disease, where blood markers like CA-125 have known limitations [36]. Elevated CA-125 levels are observed in only 50 %–62 % of early-stage EOC patients [37], while CTCs have been detected in more than 90 % of EOC patients with early-stage disease [38]. Larger sample sizes and consideration of confounding factors are needed to explore this relationship further in rare EOC subtypes. However, we found that baseline peripheral CTC+ patients tended to have higher PCI scores compared to CTC- patients, suggesting that CTCs may be associated with peritoneal disease. PCI has previously demonstrated prognostic value in advanced serous EOC, highlighting the importance of disease distribution in outcomes [39]. Our PCI score cut-off was lower than those used in most studies, where cutoffs range from 0 to 20 (see studies cited in Ref. [40]), likely due to the predominance of early-stage patients and the biology of non-HGSC EOC subtypes, which typically present as localised masses, whereas HGSC is often diagnosed at advanced stages with extensive peritoneal involvement [19,41,42]. We did not see the same association between PCI and ovarian vein CTCs, which may be due to the fact that ovarian vein CTCs may have not yet undergone the selective pressures of the systemic circulation, unlike peripheral CTCs, which have already demonstrated their capacity to survive and may be more likely to contribute to disease progression. Moreover, a statistically significant association was found between baseline peripheral CTC status and mortality, suggesting that treatment-naïve patients with rare EOC who were CTC- in peripheral blood samples appear to have better survival outcomes compared to those who were CTC+. This would be in agreement with other studies which have reported on the poorer prognosis of detectable baseline EOC CTCs [13–15,24,31,43]. However, no statistical difference in OS between groups was observed using Kaplan-Meier analysis, possibly due to the small sample size. Additionally, only one CTC- patient had at least 32 months of follow-up, which corresponds to the median OS observed in the CTC+ group. Nonetheless, larger studies are needed to explore these findings further.

It must be noted that the current dataset has several other limitations. Ideally, the data should be stratified and analysed with respect to each histological subtype, as opposed to grouped together, and multivariate factors should be considered, though our small sample size limited these analyses. Given the lack of studies focusing on CTCs in rarer EOC subtypes in the literature, it is also important to consider this when interpreting findings from the broader EOC studies discussed herein, as their results may not fully represent the biological and clinical differences of the rarer subtypes. Baseline carcinoembryonic antigen (CEA) levels, which can be useful for distinguishing mucinous tumours among EOC cases, were also not documented for any patient [44]. Our follow-up time is also limited, especially for the early-stage group, which accounted for 12 out of the 20 patients in our cohort. Additionally, without the inclusion of a mesenchymal CTC detection arm alongside the epithelial arm in our study, these subpopulations may have gone undetected, potentially limiting the integrity of the analysis and data interpretation. However, current mesenchymal markers such as vimentin and N-Cadherin are ubiquitous in the blood, which can limit their specificity for CTC detection, making their application challenging. The low CTC positivity rate from both peripheral and ovarian vein samples is another limitation and this preliminary data requires further validation in a larger cohort. Furthermore, the absence of ovarian vein samples from three out of the four patients who died represents missed opportunities to evaluate their CTC status and identify potential CTC doublets or clusters, which could have provided further insights into their disease progression.

5. Conclusion

This study demonstrates the feasibility of enriching and detecting CTCs in both peripheral and ovarian vein blood samples in patients with rare EOC subtypes, which could be of prognostic interest. The Parsortix® microfluidic system was capable of enriching CTC singlets, doublets, and clusters. The current dataset suggests a potential difference in outcomes between CTC+ and CTC- patients, though this data is preliminary so further research and additional analysis are needed to clarify the nature of the relationship. Additionally, the detection of CTCs in 5 of the 12 early-stage patients at baseline and/or their ovarian vein samples highlights the potential for early dissemination of tumour cells even in the initial stages of disease. This proof-of-concept pilot study paves the way for larger studies to fully understand the prognostic utility of CTCs across the rarer EOC subtypes. Further interrogation of CTCs in patients with EOC, such as marker expression analysis, may facilitate a more holistic understanding of the disease landscape and further refine their clinical significance, progressing beyond simple enumeration.

CRediT authorship contribution statement

Faye Lewis: Conceptualization, Writing – original draft, Writing – review & editing. **Mark P. Ward:** Conceptualization, Writing – original draft, Writing – review & editing. **Feras Abu Saadeh:** Writing – review & editing. **Catherine O'Gorman:** Writing – review & editing. **Patrick J. Maguire:** Writing – review & editing. **James P. Beirne:** Funding acquisition, Writing – review & editing. **Waseem Kamran:** Writing – review & editing. **Elzahra Ibrahim:** Writing – review & editing. **Lucy Norris:** Writing – review & editing. **Tanya Kelly:** Writing – review & editing. **Sinéad Hurley:** Writing – review & editing. **Brian Henderson:**

Writing – review & editing. **Marika Kanjuga:** Writing – review & editing. **Lorraine O'Driscoll:** Funding acquisition, Writing – review & editing. **Kathy Gately:** Funding acquisition, Writing – review & editing. **Ezgi Oner:** Writing – review & editing. **Volga M. Saini:** Writing – review & editing. **Karen Cadoo:** Writing – review & editing. **Cara Martin:** Writing – review & editing. **John J. O'Leary:** Conceptualization, Funding acquisition, Writing – original draft, Writing – review & editing. **Sharon A. O'Toole:** Conceptualization, Funding acquisition, Writing – original draft, Writing – review & editing.

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Declaration of competing interest

The authors declare no conflict of interest.

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Appendix A. Supplementary data

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