Circulating Ensembles of Tumor Associated Cells are Ubiquitous in Breast, Ovarian and Cervical Cancers and Atypical in Asymptomatic Individuals

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BACKGROUND

- Screening tests for breast, ovarian and cervical cancers include mammography, CA125 and PAP smears,
- Mammography is associated with false positives, CA125 tends to be elevated in non-malignant conditions and PAP smears are associated with false negatives,
- Screening tests often necessitate a follow up invasive biopsy for confirmation of malignancy,
- Non-invasive, non-radiological, sensitive and specific screening test is an urgent clinical need.

RATIONALE

- Venous Thromboembolism due to circulating tumor cell emboli in peripheral blood is a risk in many cancers,
- These Circulating Ensembles of Tumor Associated Cells (C-ETACs) are clusters of ≥3 cells of tumorigenic origin,
- C-ETACs can be identified by immunostaining – they are $EpCAM^{+}$, CK^{+} and $CD45^{\pm}$,
- While normal blood cells have functional apoptotic machinery, C-ETACs are apoptosis-resistant
- An epigenetically acting negative selection process was developed where normal cells are selectively eliminated, whereas apoptosis resistant C-ETACs are conferred survival privilege,
- C-ETACs can be further characterized by immunostaining for cancer specific antigens.

APPROACH

- 15 ml blood obtained from female patients with confirmed Ca Breast (n = 1344), Ca Ovary (n = 285) and Ca Cervix (n = 316) as well as asymptomatic females (n = 6727) who were negative for any findings in Mammography, PAP smear and CA125,
- C-ETACs harvested from PBMC fraction by negative enrichment using the epigenetically acting process,
- C-ETACs were immunostained for EpCAM, pan-CK, CD45, GCDFP15 (Breast), CA125 (Ovary), p63 (SCC - Cervical).
- Prevalence of C-ETACs was determined in patients with breast, ovarian and cervical cancers as well as in the cohort of asymptomatic individuals with or without suspicious findings in each of the screening investigations.

STUDY POPULATION

Table 1. Age	Ca Breast	Ca Ovary	Ca Cervix	Asymptomatic
Minimum	18	18	24	40
Maximum	89	82	87	75
Median	52	54	54	53

Table 2. Metastases	Ca Breast	Ca Ovary	Ca Cervix
Metastatic	758 (56.4%)	180 (63.2%)	138 (43.7%)
Non-metastatic	271 (20.2%)	38 (13.3%)	97 (30.7%)
Unavailable	315 (23.4%)	67 923.5%)	81 (25.6%)

Table 3. Therapy Status	Ca Breast	Ca Ovary	Ca Cervix
Pre-treated	939 (69.9%)	219 (76.8%)	192 (60.8%)
Treatment Naive	355 (26.4%)	54 (18.9%)	109 (34.5%)
Unavailable	50 (3.7%)	12 (4.2%)	15 (4.7%)

Table 4. Radiological Status	Ca Breast	Ca Ovary	Ca Cervix
Detectable Disease	1141 (84.9%)	245 (86.0%)	264 (83.5%)
No Evidence of Disease	203 (15.1%)	40 (14.0%)	52 (16.5%)
Unavailable	-	-	-

C-TACS DETECTION AND PREVALENCE

Fig 1. Negative Enrichment of C-ETACs from a known case of Ca Breast. (40x magnification)

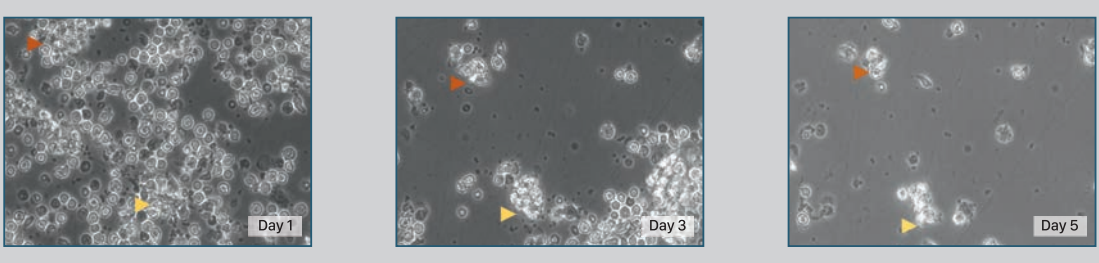


Fig 2. Day 5 images of C-ETACs illustrating varying sizes of cell clusters. (40x magnification)

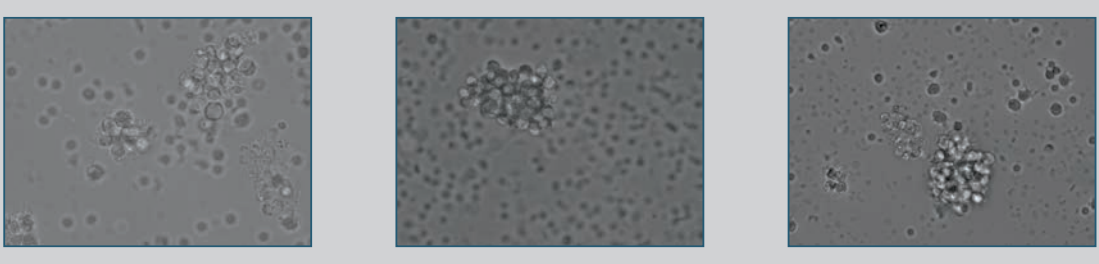


Fig 3. No clusters observed in cell lines and primary tumor cells via the negative enrichment process. Representative images. 1: SiHA Cervical Cancer cells, 2: Primary liver adenocarcinoma cells, 3: Primary ovarian adenocarcinoma. (10x magnification).

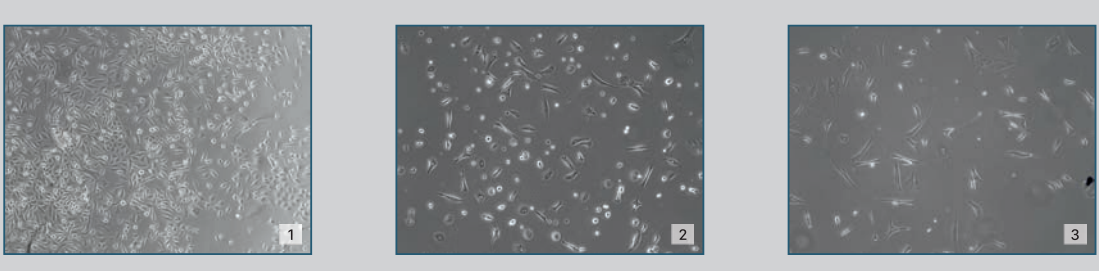
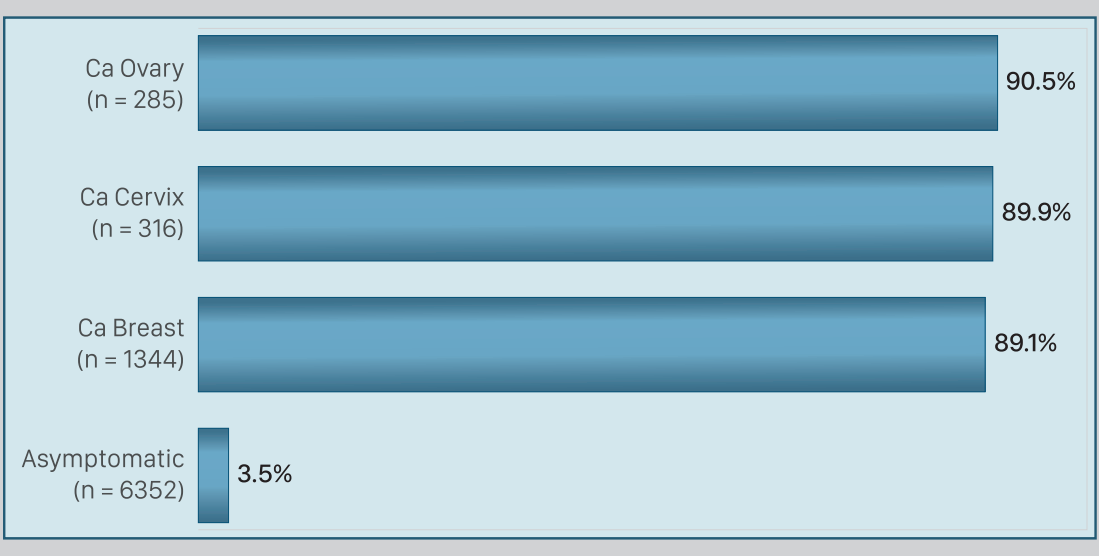


Fig 4. Prevalence of C-ETACs in cancer populations and in asymptomatic populations with or without findings on screening investigations (PAP Smear, Mammography, Ca125).



IMMUNOCYTOCHEMISTRY PROFILING C-TACs

Fig 5. Representative images of cell clusters from a known case of Ca Breast immunostained for detection of C-ETACs based on EpCAM, PanCK and CD45.

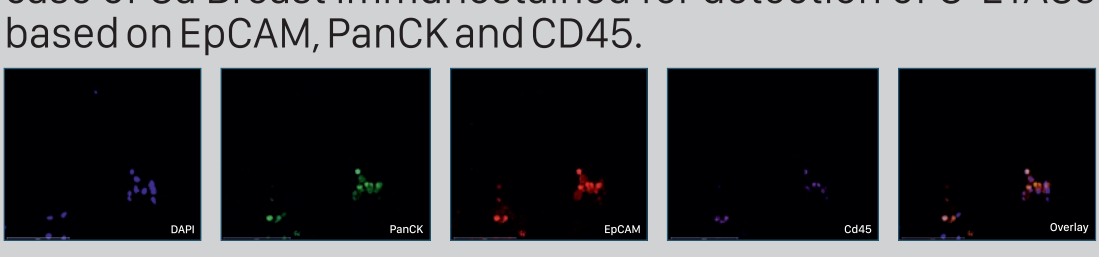


Fig 6. Representative images of C-ETACs from a known case of Ca Breast where GCDFP-15 positivity is observed. GCDFP-15 is a breast tissue specific marker used in routine histopathological analysis.

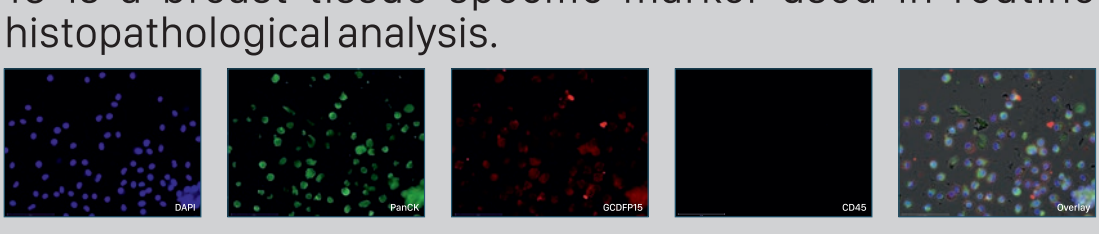


Fig 7. Representative images of C-ETACs from a known case of Ca Cervix where P63 and CK7 positivity are observed. P63 (squamous cell carcinoma specific) and CK7 are markers used in routine histopathological analysis.

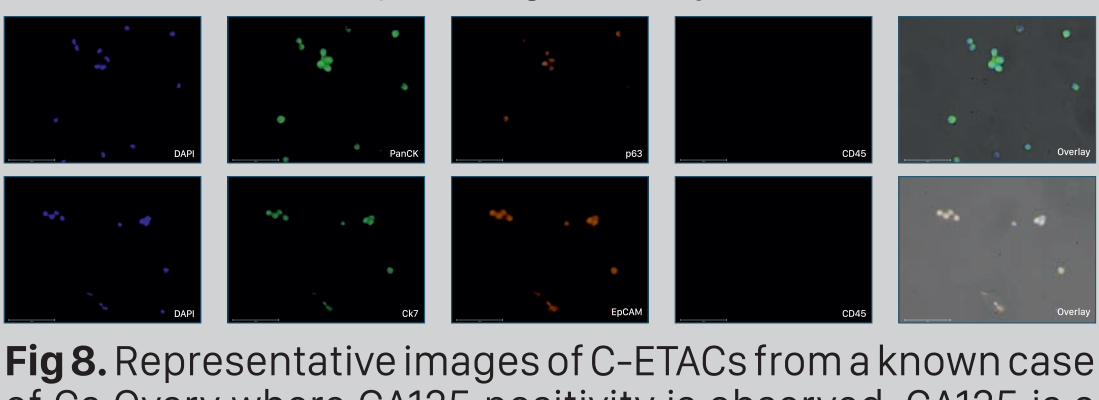
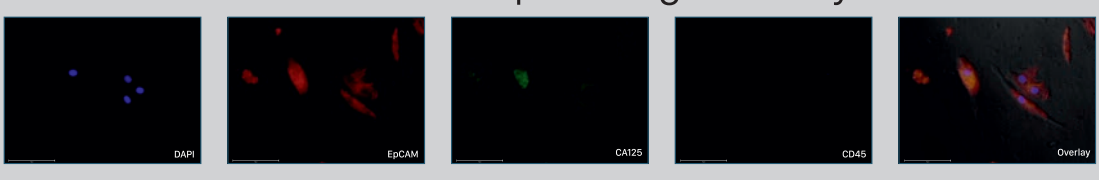


Fig 8. Representative images of C-ETACs from a known case of Ca Ovary where CA125 positivity is observed. CA125 is a marker used in routine histopathological analysis.



FINDINGS AND CONCLUSION

- C-ETACs were detected in ~90% of all known cancer cases and in 3.5% of asymptomatic individuals with no suspicious findings.
- Ubiquity of C-ETACs in Ca Breast, Ca Ovary and Ca Cervix, and rarity in asymptomatic individuals indicates causative connection of C-ETACs with cancers.
- Presence of C-ETACs in asymptomatic individuals can be considered as indicative of cancer.

Conflict of Interest: Datar Cancer Genetics Limited offers commercial services in the domain of oncology.