Immunohistochemical characterization of tumor cells present in non-blood body fluids from patients with metastatic breast cancer in the context of the UPTIDER program

Camille Carette[1,$], Gitte Zels[1,2,$], Anirudh Pabba[1,$], Marion Maetens[1], Kristien Borremans[1,3], Karen Van Baelen[1,3], Josephine Van Cauwenberge[1,3], Tatjana Geukens[4], Maxim De Schepper[2], Ha-Linh Nguyen[1], Madita Nysen[1,5], Hannelore Hoogsteyn[1], Tim van Assche[1,2], Amena Mahdami[1], Sophia Leduc[1], Peter Vermeulen[1,6], Patrick Neven[3], Hans Wildiers[5], Wouter Van Den Bogaert[7], Giuseppe Floris[2], François Richard[1*], Sigrid Hatse[1,5*], Christine Desmedt[1*]

[1]Laboratory for Translational Breast Cancer Research, Department of Oncology, KU Leuven, Belgium; [2] Department of Pathology, UZ Leuven, Belgium; [3]Gynecological Oncology Unit, Department of Oncology, UZ Leuven, Belgium; [4]General Medical Oncology Unit, Department of Oncology, UZ Leuven, Belgium; [5]Laboratory of Experimental Oncology (LEO), Department of Oncology, KU Leuven, Belgium; [6]Translational Cancer Research Unit, Ziekenhuis aan de Stroom Augustinus (ZAS), Antwerp, Belgium; [7]Forensic Biomedical Sciences Unit, Department of Imaging & Pathology, KU Leuven, Belgium; [$]Co-first authors; [*]Co-last authors

BACKGROUND AND AIMS

- Pleural, peritoneal and pericardial effusions are common in metastatic breast cancer (mBC), and together with cerebrospinal fluid, may contain tumor cells, providing a valuable source of liquid biopsies for molecular characterization [1,2,3].
- These body fluids often contain **more tumor cells than blood**, yet standardized collection protocols are lacking [4].
- **Molecular profiling** of these tumor cells can inform about receptor status (ER, PR, HER2) and proliferative (KI67) changes between primary and metastatic sites. **Receptor discordance** occurs frequently and impacts treatment decisions [5,6,7].
- Our post-mortem tissue donation program, **UPTIDER** (NCT04531696)[8], enables collection and comprehensive analysis of body fluids and direct comparison with solid metastases.

Aim 1: Evaluation of the potential association between presence of tumor cells in non-blood fluids and presence of metastases in nearby organ sites from patients with metastatic BC included in UPTIDER.

Aim 2: To assess the heterogeneity of BC biomarkers ER, PR, HER2 and KI67 in these non-blood liquid biopsies and comparison with all solid metastases from patients with metastatic BC included in UPTIDER.

METHODS

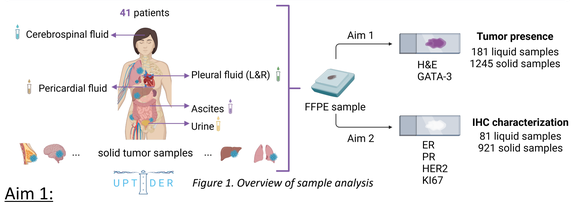


Figure 1. Overview of sample analysis

Aim 1:

181 body fluid samples from 41 patients from UPTIDER were analyzed and scored for the presence of tumor cells via H&E and GATA-3 staining.

Aim 2:

For IHC assessment of ER, PR, HER2 and KI67, a tumor cellularity cutoff of ≥ 5 % (body fluids) and ≥ 10 % (solids) was applied resulting in a final cohort of 81 liquid samples and 921 solid samples from 31 patients. ER and PR were scored per ASCO/CAP 2020 guidelines (1% cutoff). HER2 followed ASCO/CAP 2023 guidelines. KI67 was assessed per International KI67 in Breast Cancer Working Group 2021.

Statistical analysis: Plots were created using R (v.4.3). Associations between body fluids and solids for tumor presence and biomarker expression were assessed using one-tailed Fischer's test and Spearman correlation, respectively.

RESULTS

Patient characteristics and tumor cell detection in four types of body fluids

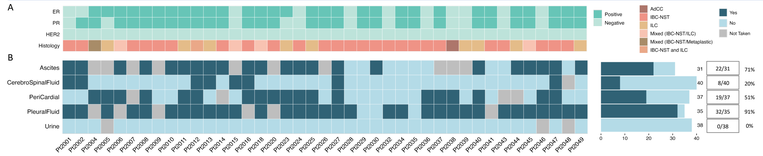


Figure 2: (A) Overview of primary tumor characteristics of the 41 patients included for this study and (B) tumor cell detection in body fluids collected during autopsy. Each column represents a patient; rows (bottom) show fluid types. Dark blue = tumor cells detected, light blue = not detected, gray = sample not taken. Proportion of tumor cell–positive samples per fluid type is shown on the right.

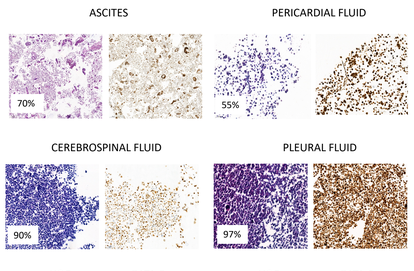


Figure 3: Representative H&E and GATA-3 stainings of body fluids with tumor cellularity.

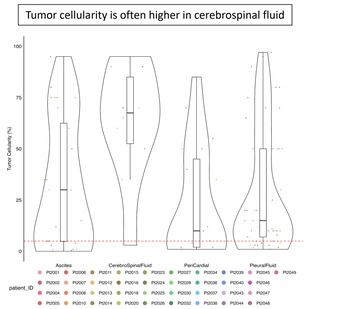


Figure 4: Violin plots showing tumor cellularity distribution in ascites, cerebrospinal, pericardial, and pleural fluids. Each dot represents a patient sample.

Aim 1: Presence of tumor cells in body fluids is not necessarily associated with solid tissue metastases in nearby organ sites

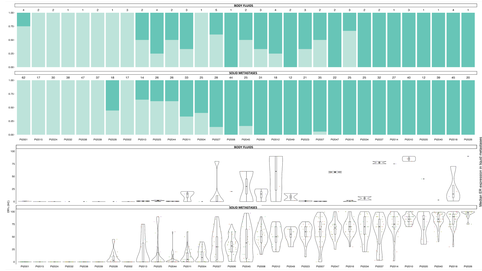
Ascites and pleural fluid showed the highest frequency of concurrent tumor presence in both the fluid and nearby solid tissues

Fluid type	Tumor presence in fluid	Patients without nearby solid metastases	Patients with nearby solid metastases	p-value	Nearby metastatic sites considered
ASCITES	Without tumor	**0 (0%)**	9 (29%)	0.70	Peritoneum/liver/GI /adnexa
	With tumor	1 (3%)	**21 (68%)**		
PLEURAL FLUID	Without tumor	**0 (0%)**	3 (9%)	0.76	Pleura/lung/ mediastinal
	With tumor	3 (9%)	**29 (82%)**		
CEREBROSPINAL FLUID	Without tumor	**16 (43%)**	12 (32%)	0.30	Brain/meninges
	With tumor	4 (11%)	**5 (14%)**		
PERICARDIAL FLUID	Without tumor	**17 (46%)**	1 (3%)	0.10	Pericardium/heart
	With tumor	14 (38%)	**5 (13%)**		
URINE	Without tumor	**32 (84%)**	6 (16%)	1	Bladder/kidney/ ureter
	With tumor	0 (0%)	**0 (0%)**		

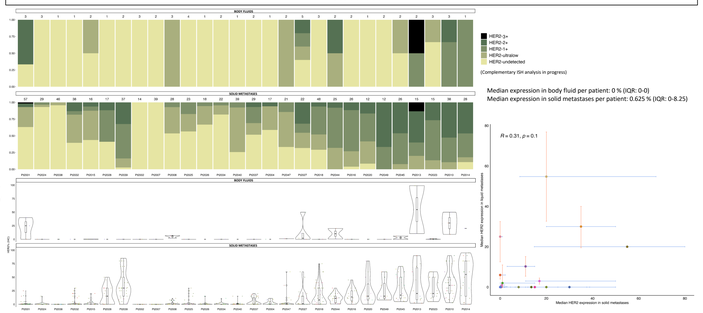
Table 1: Association between tumor cells in body fluids and presence of nearby metastases (p > 0.05)

Aim 2: Characterizing disease heterogeneity using standard BC biomarkers

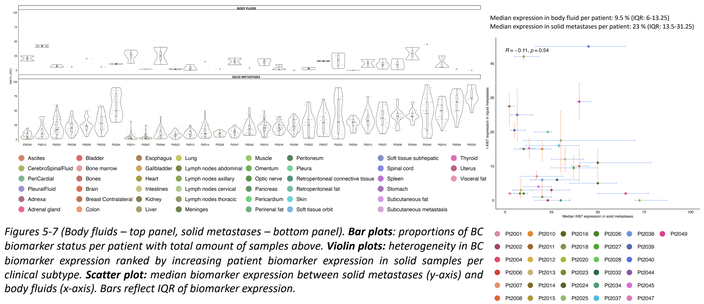
ER expression was significantly correlated between body fluids and solid metastases (p < 0.001)



HER2 expression: absence of HER2 expression was more frequent in body fluids, with a weak correlation with solid metastases



KI67 expression: relatively lower expression of KI67 was observed in body fluids compared to solid metastasis, with a weak negative correlation between both



*Figures 5-7 (Body fluids – top panel, solid metastases – bottom panel). **Bar plots:** proportions of BC biomarker status per patient with total amount of samples above. **Violin plots:** heterogeneity in BC biomarker expression ranked by increasing patient biomarker expression in solid samples per clinical subtype. **Scatter plot:** median biomarker expression between solid metastases (y-axis) and body fluids (x-axis). Bars reflect IQR of biomarker expression.*

CONCLUSIONS

- Tumor cells were detectable across multiple non-blood body fluids collected post-mortem, with the highest prevalence in ascites and pleural fluid.
- Only ER-expression showed a significant correlation between non-blood body fluids and all solid metastases. The other BC biomarkers PR (not shown), HER2, and KI67 showed notable intra-patient heterogeneity between the non-blood body fluid and all solid compartments.
- Non-blood body fluids can provide complementary molecular information to solid metastases, offering unique insights with easier access than tissue biopsies.
- These findings support the feasibility and utility of minimally invasive, multi-fluid sampling for extended tumor profiling.

REFERENCES AND ABBREVIATIONS

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