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Unmasking Sampling Artefacts in Early Breast Cancer: Molecular Shifts Between Diagnostic and Surgical Specimens in the Absence of Therapy

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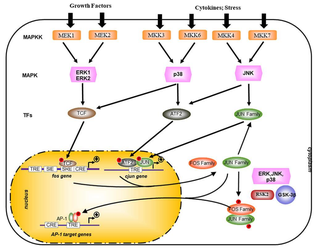
AIMS

SEOM-ARTEFACTO (SEOM/FECMA GRANT 2022) is a prospective study with the objective of evaluating the impact of tumor sampling methodology in the gene expression dinamics (GED) in early breast cancer (eBC) without any perioperative or neoadjuvant treatment

BACKGROUND

- Patient-matched transcriptomic studies using tumour samples collected before and after treatment help control for interpatient heterogeneity.
- However, the lack of untreated control groups often limits the ability to distinguish between true treatment-induced changes and sampling-related artefacts.
- The artefact effect had been observed in the POETIC trial, with an upregulation of cell-stress–related genes even in the absence of treatment.

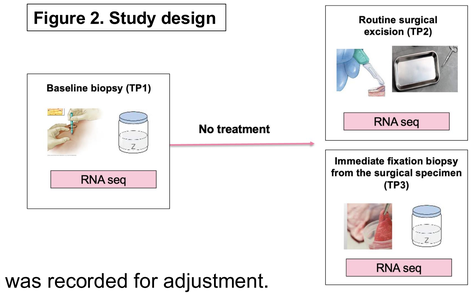
Figure 1. Cell stress pathways



MATERIALS & METHODS

In the SEOM-ARTEFACT Spanish study (2023–2024), we prospectively collected paired tumour samples (diagnostic core biopsy and surgical specimen) from 30 patients with eBC who underwent primary surgery without any neoadjuvant or window-of-opportunity therapy at the Institut Català d'Oncologia – Hospital Germans Trias i Pujol, Badalona.

Figure 2. Study design



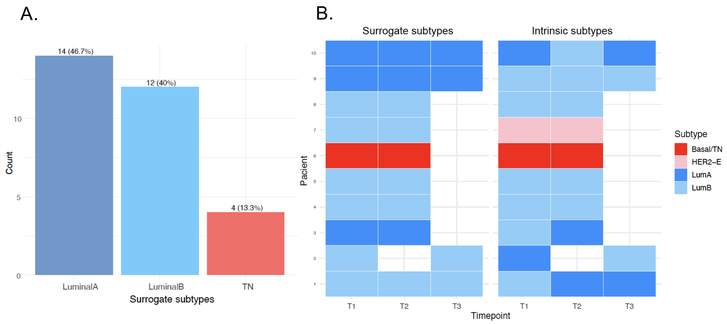
- Time from excision to formalin fixation was recorded for adjustment.
- RNA sequencing was performed to assess intrinsic subtypes with the PAM50 RNAseq-adapted algorithm and GED across three timepoints:
  - Baseline core biopsy (TP1).
  - Routine surgical excision specimen with a fixation delay of 30 minutes to 2 hours (TP2).
  - Immediate fixation biopsy from the surgical specimen (TP3).

RESULTS

Table 1. Summary of the patients' characteristics

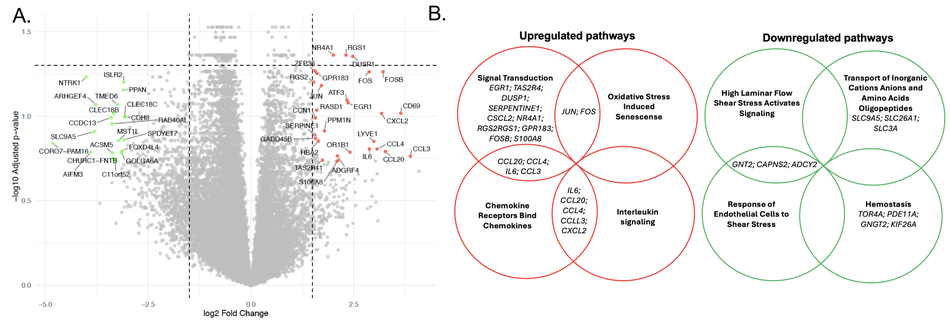
Characteristics	Result / Distribution
Total number of patients	Total of 30 patients (10 with complete data)
Median age (range)	74 years (24.9 – 93.3)
Clinical subtype (surrogate)	Luminal A (40%), Luminal B (47%), Triple negative (13%) – within the entire cohort
Molecular subtype (PAM50)	Luminal A (47%), Luminal B (40%), Basal-Like (10%) – within the available cases
Most frequent histology	Invasive ductal (80%)
Histologic grade (biopsy)	G1: 10%, G2: 53%, G3: 37%
Mean tumor size	23.3 mm (range: 11 – 52 mm)
Mean Ki67 (range)	20.4% (2 – 50%)
Hormone receptors	ER+ 90%, PR+ 70%
HER2 positive	No confirmed HER2+ cases by IHC/FISH
Frozen sample available	5/30 patients (17%)
TP2 sample (intraoperative core biopsy)	21/30 patients (70%).
Adjuvant therapy received	Chemotherapy: 50%, Endocrine therapy: 73%.
Status at end of data collection	1 documented local recurrence (3%)

Figure 3. A Surrogate subtyping distribution across the entire cohort and B. coherence across luminal surrogate and intrinsic subtyping



- Surrogate and intrinsic subtypes matched in 60% of cases (6/10).
- Intrinsic subtype classification shifted across timepoints in four patients.
- However, all of them had borderline Luminal A/B scores, indicating only minimal shifts in proliferation and endocrine-related genes.

Figure 4. A. Differentially expressed genes in paired samples (biopsy and surgery) and B. involved pathways



- Despite no treatment, we observed consistent GED between T1 and T2 (28 upregulated, 20 downregulated genes; FDR<0.05; FC |2.5–4.8|). Upregulated genes were linked to FOS-JUN pathways (stress response, hypoxia) and immune chemokines, while downregulated genes were linked to mechanical stimulus response and glucagon metabolism.
- No significant changes occurred between T1 and T3, suggesting GED alterations were due to sampling methods, not due to temporal evolution.
- GED comparison between T2 and T3 was not feasible due to limited samples.

CONCLUSIONS

- This prospective study demonstrates that tumour sampling methodology significantly influences gene expression, reflecting transient hypoxia and wound-related immune activation.
- These changes are driven by the upregulation of stress-response and pro-inflammatory cytokines underscoring the biological impact of biopsy-induced microenvironmental remodelling.
- These artefactual changes represent a critical confounding factor, particularly in neoadjuvant and window-of-opportunity trial designs and may serve as correction factors to account for sampling-related effects.
- Further analyses in the full ARTEFACT cohort will refine our understanding of these artefacts.

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