DUAL BIOMARKER APPROACH FOR MINIMAL RESIDUAL DISEASE DETECTION IN EARLY BREAST CANCER USING HIGH-VOLUME BLOOD SAMPLES

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Iñaki Comino-Méndez[1,2,3], **Javier Pascual**[1,2,3], Jesús Velasco-Suelto[1,2,3], María Elena Quirós-Ortega[1,2,3], Ana Godoy-Ortiz[1,2,3], Martina Álvarez[1,2,3,4,5], Emilio Alba[1,2,3,4,5].

[1] Servicio de Oncología Médica, Hospital Universitario Virgen de la Victoria, Málaga, Spain; [2] Instituto de Investigación Biomédica de Málaga (IBIMA), Málaga, Spain; [3] Centro de Investigación Biomédica en Red de Cáncer (CIBERONC - CB16/12/00481), Madrid, Spain; [4] Universidad de Málaga, Facultad de Medicina, Málaga, Spain; [5] Laboratorio de Biología Molecular del Cáncer (LBMC), Centro de Investigaciones Médico-Sanitarias (CIMES-UMA), Málaga, Spain.

INTRODUCTION

Breast cancer (BC) is the most common malignancy in women and a major cause of cancer-related death. Recurrence due to minimal residual disease (MRD) remains a key clinical challenge. Circulating tumor DNA (ctDNA) enables early MRD detection but is limited by low abundance in early disease. Additionally, circulating tumor cells (CTCs), often overlooked, may provide complementary information. This study uses droplet digital PCR (ddPCR) on high-volume blood samples to enable dual ctDNA and CTCs detection, aiming to improve MRD assessment, risk stratification, and post-treatment monitoring in early-stage BC.

OBJECTIVES

- To assess the clinical utility of a dual biomarker approach combining ctDNA and CTCs for MRD detection in early-stage BC.
- To determine the sensitivity and lead time of this strategy for anticipating clinical relapse using high-volume blood sampling and ddPCR-based assays.
- To explore the complementary value of both biomarkers across treatment settings and timepoints (pre-treatment, post-surgery, and follow-up).

METHODOLOGY

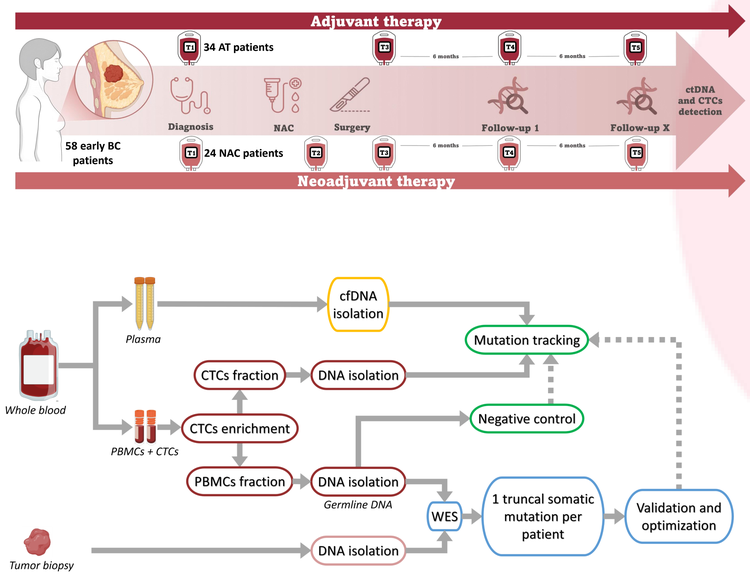


FIGURE 1. Schematic overview of the study design. Upon breast cancer (BC) diagnosis, a baseline blood sample is collected prior to any treatment. For patients receiving neoadjuvant chemotherapy (NAC), an additional sample is collected post-NAC and prior to surgery. One month after surgery, a post-operative blood sample is obtained. During follow-up, blood samples are collected every six months in patients considered at high risk.

FIGURE 2. Schematic overview of the study workflow. From each high-volume blood sample (up to ~300 mLs), plasma and PBMCs were isolated. Cell-free DNA (cfDNA) was extracted from plasma using a protocol optimized for large volumes. PBMCs underwent immunonegative selection to enrich for CTCs. Discarded PBMCs fractions were used as controls for whole-exome sequencing (WES) and ddPCR assays. One truncal somatic mutation per patient was selected for longitudinal detection of both ctDNA and CTCs, previous validation of WES data.

RESULTS

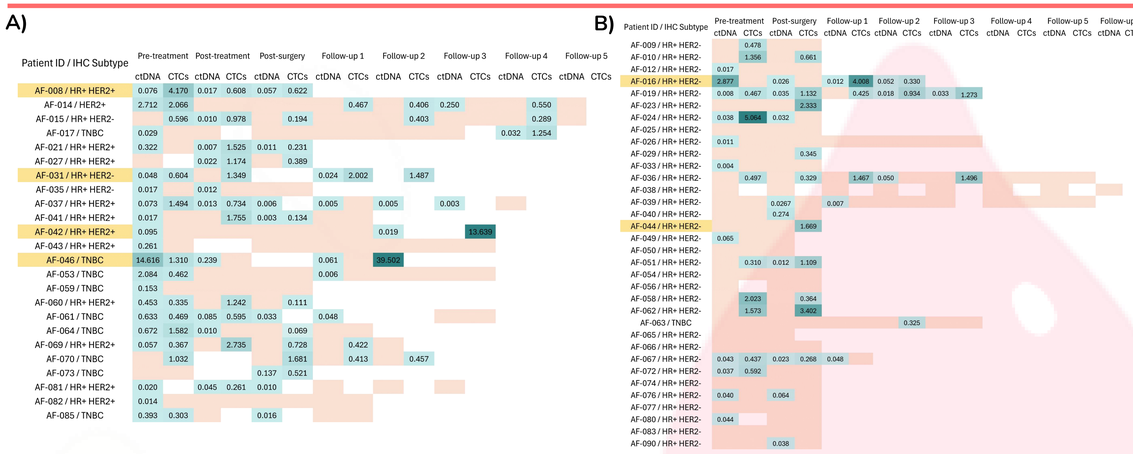
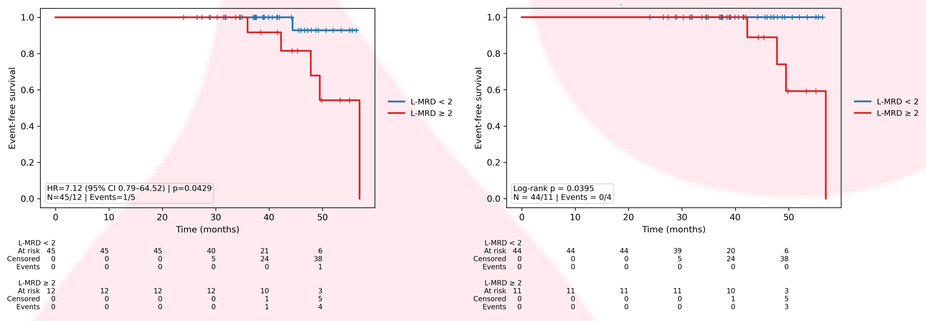


FIGURE 3. Heatmaps showing ctDNA and CTCs detection. ctDNA levels are represented as variant allele frequencies (VAFs, %) and CTCs levels as CTCs per millilitre of blood for **A)** the NAC cohort and **B)** the adjuvant therapy (AT) cohort. Confirmed clinical recurrences are highlighted in yellow. Negative tests are indicated by red boxes.



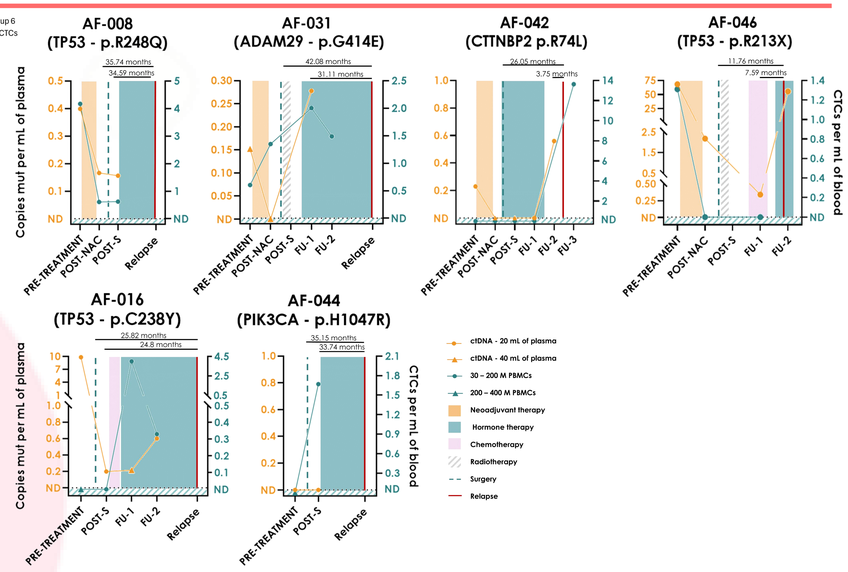


FIGURE 4. Individual graphs of relapsed patients showing the tracked mutation and corresponding ctDNA and CTCs detection across all timepoints. Treatments and the timing of clinical relapse are also annotated.

FIGURE 5. The L-MRD score stratifies early BC patients by risk of recurrence with high prognostic resolution. (A) Kaplan–Meier estimates of relapse-free survival according to the MRD score (low risk: 0–1 points; intermediate/high risk: ≥2 points), including both locoregional and distant recurrence events. (B) Kaplan–Meier estimates of distant relapse-free survival based on the same score but excluding locoregional events. Tick marks indicate censored observations.

CONCLUSIONS

- All 6 patients who experienced recurrence had prior MRD positivity, resulting in 100% sensitivity for relapse prediction.
- The median lead time between MRD detection and clinical relapse was 27.95 months (range 7.6–34.7 months), exceeding previously reported lead times using commercial assays.
- The assay enabled detection of low-abundance tumor signals with a median ctDNA VAF of 0.057% at baseline and 0.028% post-surgery.
- The L-MRD score demonstrates strong prognostic value by effectively stratifying early BC patients by recurrence risk across both all-event and distant-only survival analyses.
- **This approach offers a cost-effective, minimally invasive solution for long-term MRD surveillance and may support personalized follow-up in early BC.**

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Contact: inaki.comino@ibima.eu