

## HERA-TEST: A novel precision oncology tool using breast milk for early detection of postpartum breast cancer.

Alejandra Díaz, Regina Peña-Enriquez, Rosalia Caballero, Silvia Guil Luna, Juan De La Haba; Instituto Maimónides de Investigación Biomédica de Córdoba (IMIBIC)-Hospital Universitario Reina Sofía, Universidad de Córdoba, Córdoba, Spain; GEICAM Spanish Breast Cancer Group, Madrid, Spain; Instituto Maimonides de Investigación Biomédica de Córdoba (IMIBIC)-Hospital Universitario Reina Sofía, Universidad de Córdoba, Córdoba, Spain; Hospital Universitario Reina Sofía, Universidad de Córdoba, Córdoba, Spain

**Background:** The incidence of breast cancer (BC) in women under the age of 45 has risen in recent decades, partly due to delayed childbearing. More specifically, postpartum breast cancer (PPBC), defined as BC diagnosed within 10 years of childbirth, accounts for 5 to 7% of these cases and it is recognized as a distinct clinical and molecular entity. PPBC is associated with increased aggressiveness, a higher risk of metastasis, and worse survival outcomes. Previous research has identified observed distinct signature gene expression associated with DNA repair and cell proliferation pathways, as well as T-cell immunity. However, the key molecular drivers of PPBC remain unclear. Currently, invasive procedures such as breast biopsy or ductal lavage are the primary methods to access tumour biomarkers. In this regard, breast milk represents a promising, unique and accessible source of biomarkers—including exfoliated epithelial cells and miRNAs—that directly reflects the breast microenvironment and could provide valuable insights into early molecular changes associated with cancer development. Ultimately, it holds significant potential for identify biomarkers in breast milk for early identification of women at high risk of developing PPBC. **Methods:** This study aims to recruit 2,000 lactating women, requiring a large-scale awareness campaign and collaboration with eight Andalusian hospitals to facilitate donor recruitment and sample collection. To date, breast milk samples (10–30 mL from each breast) have been collected from 3,000 women, exceeding initial recruitment goals. Samples are preserved in two formats: whole milk stored at  $-80^{\circ}\text{C}$  and fractionated components (cells, serum, and lipids). This initiative has led to the establishment of the world's largest breast milk biobank. Simultaneously, dried breast milk samples are being collected parallel to the fresh milk. Additionally, comprehensive clinical, gynaecological and lactation-related data are collected via participant questionnaire, including family history of BC, breastfeeding duration, number of births, weaning patterns, prior breast conditions, and medication history. A multi-omics analysis—including genomic, epigenomic, proteomic, and viromic profiling—will be conducted to identify molecular differences between women who develop PPBC and those who do not. Integrating these findings with clinical and epidemiological data to enhance the understanding of PPBC pathogenesis and improve early detection strategies. Upon identification of robust predictive biomarkers, efforts will focus on adapting the test for use with dried milk samples, facilitating large-scale implementation. This approach aims to address critical gaps in current screening methods for young women, with the ultimate goals of enhancing clinical outcomes, reducing healthcare costs, and advancing precision medicine. Research Sponsor: Instituto de Salud Carlos III; Sociedad Andaluza de Oncología Médica (SAOM).