# EXPLORATION OF THE SERUM METABOLIC DETERMINANTS OF MAMMOGRAPHY DENSITY, AS A RISK FACTOR FOR BREAST CANCER, IN WOMEN SCREENING IN A REFERENCE HOSPITAL IN BOGOTÁ, 2021

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## INTRODUCTION

### Breast cancer (BC)

It is the most common type of cancer, with more than 2.2 million cases in 2020. It accounted for 24% of all cancers diagnosed in women. Around 685 thousand women died as a result of this disease (1)

**Mammographic density (MD)**

- It´s an important risk factor for CS (2,3). It has been documented that the risk of women with high breast density is 4 to 6 times higher than that of women with low density (4)
- Today, the most widely used method in clinical practice is the Breast Imaging Reporting and Data System (BI-RADS) density score.
- has the potential to improve the quality of risk prediction models, however discriminatory accuracy remains limited at the individual level

**Serum metabolic differences according to the percentage of mammographic density could represent an innovative and useful risk identification tool in clinical practice.**

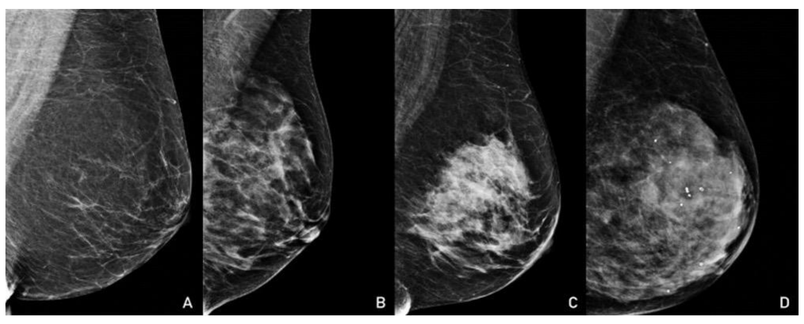


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**Figure 1.** Midlateral oblique mammographic views depicting the 4 BI-RADS density categories:
**(A)** Almost entirely fat (BI-RADS density 1)
**(B)** Scattered fibroglandular densities (BI-RADS density 2)
**(C)** Heterogeneously dense (BI-RADS density 3)
**(D)** Extremely dense (BI-RADS density 4). BI-RADS = Breast Imaging Data and Reporting System.

## OBJECTIVE

To explore the serum metabolic determinants of mammographic density as a risk factor for breast cancer, in women screened at a reference hospital in Bogotá, in the year 2021

## METHODOLOGY

### Study population

Women screened in the breast unit of the Clinica Colombia, in Bogotá, 2021

- Mammography density classification:
  - **Low risk** (LR): < 25%
  - **Moderate risk** (MR): 26-50%
  - **High risk** (HR) : > 50%

Take anthropometric measurements and clinical variables

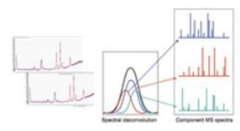
- **Serum sample preparation and metabolite extraction**

The metabolite extracts were derivatized using a two-step protocol: **i)** Methoximation with O-Methoxyamine in pyridine (15 mg/mL, 70oC, 1 h) followed by **(ii)** Silylation with BSTFA with 1% TMCS (70 °C, 1 h).

- **GC-MC**

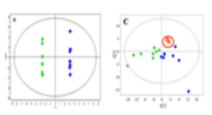
The derivatized samples were analyzed in a Agilent Technologies 7200 gas chromatograph coupled to a mass spectrometer with gas detector Quadrupole and High Resolution Time of Flight (GC-QTOF).

- **Quality control of metabolomic analyses**



- **Data processing and analysis**

SIMCA MATLAB



The deconvolution and identification of the metabolites was made using the Agilent program MassHunter Unknowns Analysis and the Fiehn and NIST libraries.

- **Metabolite identification**

hmdb NIST METLIN

To determine the differences between the metabolic profiles, the groups under study and select the statistically significant metabolites, analysis was carried out univariate and multivariate unsupervised and supervised statistics.

- **Biological interpretation**

MetaboAnalyst 5.0

## RESULTS

**n=60**

**Low risk:** 28
**Moderate risk**: 16
**High risk:** 16

The significant demographic and clinical variables between the risk groups were: **age** *p(<0.001),* **sociodemographic stratum** *p(0.025),* **visceral fat level** *p(0.013),* and **hormonal status** *p(0.001)*

**Characterization**

- **Low risk:** Age 59 ± 4 years, BMI 27.8 ± 3 kg/m2
- **Moderate risk:** Age 56 ± 6 years, BMI 26 ± 5 kg/m2
- **High risk:** Age 52 ± 3 years, BMI 26 ± 5 kg/m2

**Correlation analysis**

**Age**: $r$ = - 0.84
**Degree of obesity**: $r$ = - 0.76
**Visceral fat level**: $r$ = - 0.79
**BMI**: $r$ = - 0.75

In the analysis of metabolic pathways, a total of 19 pathways were detected. However, the metabolic pathway with a significant relationship, even after the FDR correction analysis, was the biosynthesis **pathway of phenylalanine, tyrosine and tryptophan** with significant p-value of FDR

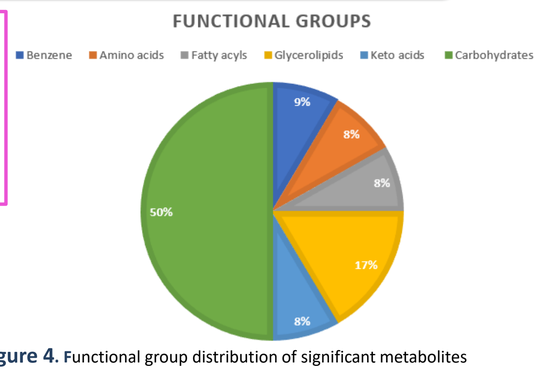
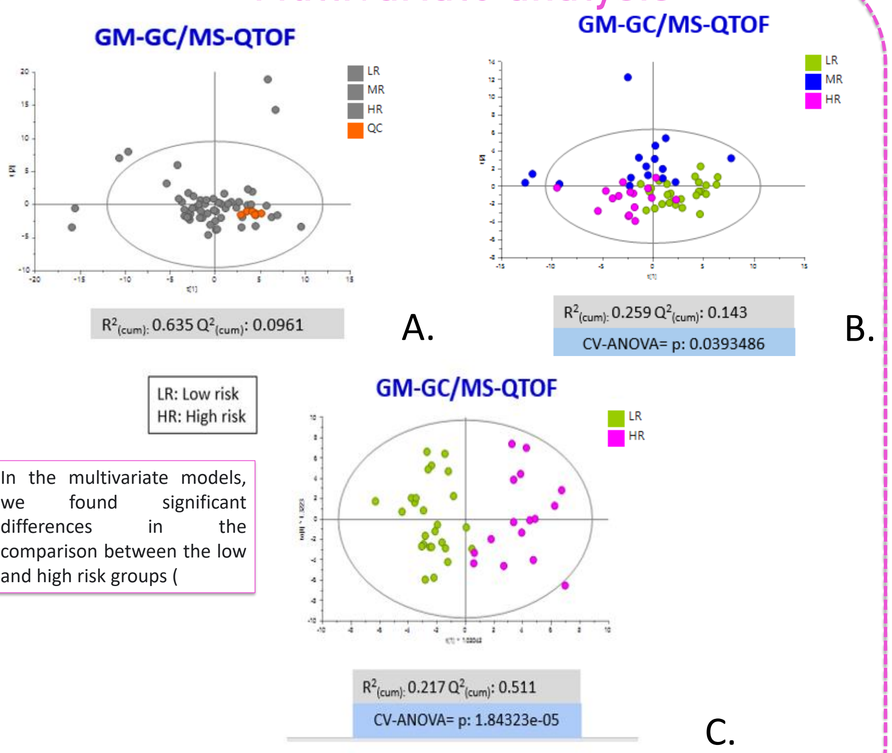



**Figure 4.** Functional group distribution of significant metabolites

### Multivariate analysis




In the multivariate models, we found significant differences in the comparison between the low and high risk groups (

**Figure 2. A.** Quality control of metabolomic analyses PCA **B.** Supervised analysis, partial least squares discriminant analysis (PLS-DA) **C.** Supervised analysis,orthogonal partial least squares regression (OPLS-DA) LR vrs HR

### Univariate analysis

| Name | AUC | T-tests | Log2 FC | |
|---|---|---|---|---|
| Xylitol | 0.96296 | 7.3319E-7 | -0.50256 | ↓ |
| Azelaic acid | 0.84491 | 0.0010325 | -1,0279 | ↓ |
| Alpha Ketoglutaric acid | 0.8287 | 2.7923E-4 | -0,45977 | ↓ |
| Glycerol monostearate | 0.82639 | 3.0332E-4 | 0,45322 | ↑ |
| D-Arabinose | 0.81481 | 0.00927 | -1.0894 | ↓ |
| L-Tyrosine | 0.81481 | 9.3073E-4 | -0.59912 | ↓ |
| L-Threonic acid | 0.7963 | 9.3083E-4 | -0.36204 | ↓ |
| Erythriol | 0.78935 | 5.9481E-4 | -0.38395 | ↓ |
| N-acetyl-D-mannosamine | 0.78704 | 0.0025343 | -0.32856 | ↓ |
| 1- Monopalmitin | 0.77546 | 0.0036462 | 0.41754 | ↑ |
| Glycerol | 0.77315 | 0.0019045 | -0.70909 | ↓ |
| Hippuric acid | 0.76852 | 0.027026 | -0.87262 | ↓ |

**127** metabolites identified, only **12** were significant after FDR corrections

**Lucie Lecuyer et al.** describe that high levels of glycerol are associated with a higher risk of developing breast cancer during the 13 years of follow-up, with an AUC of 0.69, 95% CI for the difference in AUC (0-0.6) *p (0.04).*

**Figure 3.** Discrimination and prediction capacity of metabolites to discriminate between groups LR vrs HR

**Bendinelli et al**. described an inverse association of tyrosine with CS cases with high DM (OR 0.59, 95%CI 0.42–0.82, p value 0.002), highlighting that in models adjusted for confounding variables, only tyrosine continued to have an inverse association with CS cases with high MD (OR 0.51, 95%CI 0.27–0.94, p value 0.03)

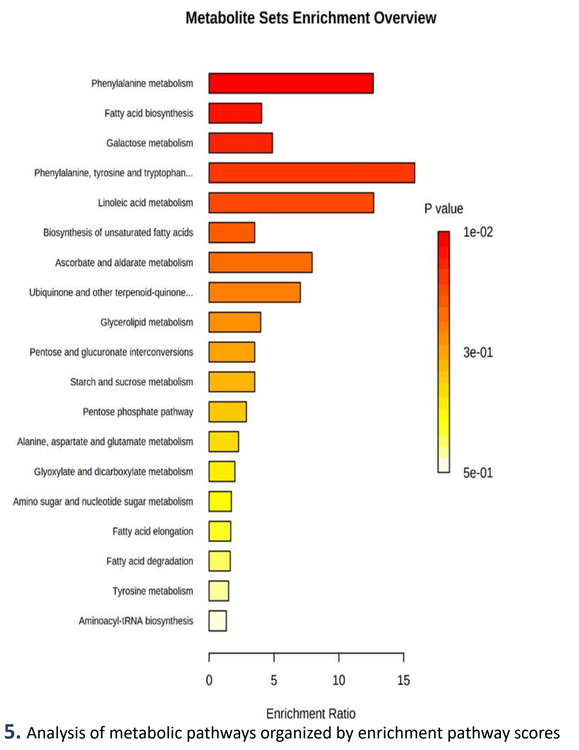



**Figure 5.** Analysis of metabolic pathways organized by enrichment pathway scores

## CONCLUSIONS

1. The significant differentiating metabolites of the risk groups are mainly involved in the pentose phosphate pathway, biosynthesis of phenylalanine, tyrosine and tryptophan, previously reported in the literature.

2. Finding a relationship between the different metabolic profiles with the risk classification by mammographic density will make it possible to open other more specific investigations in the field of metabolomics, considering the identification of a plasmatic marker that will improve the efficacy of the tests currently used test for risk detection and screening of this disease in Colombia and the world.

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