UNIVERSIDAD AUTÓNOMA DE SAN LUIS POTOSÍ

FACULTAD DE MEDICINA

Centro de Investigación en Ciencias de la Salud y Biomedicina (CICSaB)

“IDENTIFICACIÓN DE BIOMARCADORES DE RIESGO EN EL DESARROLLO DE CÁNCER DE MAMA MEDIANTE EL USO DE HERRAMIENTAS METABOLÓMICAS”

TESIS QUE PRESENTA

M. en C. MARÍA JOSÉ SANTOYO TREVIÑO

PARA OBTENER EL GRADO DE DOCTORA EN CIENCIAS BIOMÉDICAS BÁSICAS

CO-DIRECTORES DE TESIS
DR. FRANCISCO JAVIER PÉREZ VÁZQUEZ
DR. SERGIO ZARAZÚA GUZMÁN

Septiembre 2023

CRÉDITOS INSTITUCIONALES

Esta tesis se llevó a cabo en el Centro de Investigación Aplicada en Ambiente y Salud (CIAAS) de la Coordinación para la Innovación y Aplicación de la Ciencia y la Tecnología (CIACyT), bajo la tutoría del Dr. Francisco Javier Pérez Vázquez y Dr. Sergio Zarazúa Guzmán, gracias al Hospital Centra “Dr Ignacio Morones Prieto” y gracias al apoyo del Consejo Nacional de Ciencia y Tecnología que otorgó la beca con el número 70586.

Tesis que presenta:

M. en C. MARÍA JOSÉ SANTOYO TREVIÑO

PARA OBTENER EL GRADO DE DOCTORA

EN CIENCIAS BIOMÉDICAS BÁSICAS

CO-DIRECTORES DE TESIS

DR. FRANCISCO JAVIER PÉREZ VÁZQUEZ

DR. SERGIO ZARAZÚA GUZMÁN

ASESORES INTERNOS

DR. FERNANDO DÍAZ-BARRIGA MARTÍNEZ

DR. ROGELIO FLORES RAMÍREZ

ASESOR EXTERNO

DR. JORGE ALEJANDRO ALEGRÍA TORRES

ASESOR CLÍNICO

DR. JOSÉ DE JESÚS ZERMEÑO NAVA

JURADO

Presidente de sinodales: Dra. Leticia G. Yáñez Estrada
Sinodal: Dra. Patricia Cossío Torres
Sinodal: Dr. José de Jesús Zermeño Nava
Sinodal externo: Dr. Jorge Alegría Torres
Sinodal suplente: Dra. Saray Aranda Romo

Septiembre 2023

Índice

RESUMEN GENERAL DEL PROYECTO

Introducción.

El cáncer de mama es un proceso oncológico en el que las células sanas de la glándula mamaria se degeneran y se transforman en tumorales, proliferando y multiplicándose hasta la formación del tumor, el cual puede diseminarse a otros órganos a través de los nódulos linfáticos (1).

Actualmente, de acuerdo con la Organización Mundial de Salud (OMS), cada año se registran 2.3 millones de nuevos casos en todo el mundo, de los cuales más del 90% son detectados en mujeres (2). Además, según estimaciones del GLOBOCAN en 2020, el cáncer de mama causó la muerte de más de 500 000 mujeres a nivel mundial, de las cuales, el 70% ocurrió en países en vías de desarrollo, donde el diagnóstico de este tipo de cáncer se realiza en etapas avanzadas (3). En México, el cáncer de mama es considerado la segunda causa de muerte más frecuente en mujeres de 30 a 54 años de edad (4).

El cáncer de mama es una enfermedad silenciosa ya que se desarrolla de manera asintomática, el diagnóstico se basa principalmente en la autoexploración y la realización de estudios de imagen, sin embargo, las técnicas de exploración mas eficaces para la detección temprana del cáncer de mama son las pruebas de imagen como la mastografía y el ultrasonido, ambas, requieren equipos de calidad y de profesionales expertos en oncología (4,5).

De lo anterior, surge la importancia de desarrollar nuevas técnicas para el tamizaje de esta enfermedad, desde esta perspectiva, han surgido nuevas estrategias analíticas basadas en las ciencias “ómicas” (genómica, proteómica, transcriptómica y

metabolómica), como herramientas potenciales, que permiten una comprensión integral de los procesos biológicos (6). Los marcadores “ómicos” pueden mejorar la predicción del riesgo de la aparición del cáncer de mama en sus etapas tempranas, así como su diagnóstico más allá de los marcadores tradicionales e inclusive implementar una terapia personalizada (7,8).

Objetivo General.

Identificar patrones de metabolitos de riesgo en el desarrollo de cáncer de mama mediante el uso de herramientas metabolómicas.

Hipótesis.

Los patrones metabolómicos de mujeres con diagnóstico de cáncer de mama son diferentes a los de las mujeres sanas y entre los distintos estadios de la enfermedad.

Objetivos específicos.

1. Estandarizar las técnicas de nariz electrónica y cromatografía de gases-masas para identificar patrones de metabolitos en muestras de aliento exhalado y orina.
2. Calcular la sensibilidad y especificidad del método estadístico empleado en el análisis de los patrones obtenidos de la nariz electrónica, a través de la elaboración de curvas ROC.
3. Identificar patrones de metabolitos en orina y aliento exhalado mediante metabolómica no dirigida, empleando cromatografía de gases-masas y nariz electrónica.

4. Comparar los patrones metabolómicos de compuestos orgánicos volátiles globales entre mujeres sanas, pacientes con cáncer de mama y pacientes con cáncer de mama que cursan los estadios I-II y III-IV.

Material y métodos.

Este es un estudio piloto y transversal el cual fue aprobado por el Comité de Ética e Investigación del Hospital Central “Dr. Ignacio Morones Prieto”, con número de registro CONBIOETICA-24-CEI-001-20160427. Se realizó un estudio de metabolómica no dirigida en la cual se analizaron aliento exhalado de mujeres clínicamente sanas (n = 37) y mujeres con cáncer de mama estadios I a IV (n = 35) y muestras de orina de (n = 46) pacientes con cáncer de mama, que acudían a consulta de revisión o diagnóstico al Hospital Central “Dr. Ignacio Morones Prieto”. El diagnóstico de cáncer lo realizaba el médico adscrito o el residente mediante distintas pruebas de imagen (mastografía, ultrasonido) e histopatología. Al término de su consulta se les explicaba a las pacientes el objetivo del estudio y los beneficios que recibirían, aquellas mujeres que aceptaban participar lo hacían mediante la firma del consentimiento informado. En la presente investigación se incluyeron mujeres mayores de 18 años con diagnóstico de cáncer de mamá en estadios (0-IV), que contaran con estudios confirmatorios (mastografía/ultrasonido/diagnóstico histopatológico). Los criterios de no inclusión fueron, mujeres que se encontraran bajo algún tratamiento de quimioterapia, que hubiesen padecido otro tipo de cáncer o fumadoras. Mientras que los criterios de eliminación fueron, aquellas pacientes que decidieran retirarse del estudio y pacientes en las cuales no fuera posible la obtención de muestras de aliento y/u orina. El protocolo del proyecto se dividió en dos etapas, en la primera se recolectaron las muestras de aliento exhalado y en la segunda etapa

las muestras de orina. Debido a dificultades metodológicas y de logística, no fue posible obtener en la misma paciente y al mismo tiempo, la muestra de aliento exhalado y la de orina.

El aliento exhalado fue colectado en bolsas de obtención de aliento (BCB), previamente purgadas con nitrógeno ultrapuro, se transportaron en una hielera del Hospital Central al laboratorio “Salud Total” de la CIACYT de la Facultad de Medicina de la UASLP e inmediatamente fueron analizadas en la E-Nose Cyranose 320®. Los resultados obtenidos de los sensores de la nariz electrónica fueron analizados en un modelo PCA (Análisis de Componentes Principales), un análisis de varianzas por permutaciones (PERMANOVA), para evaluar si existían diferencias significativas entre las pacientes y un análisis predictivo canónico de coordenadas principales (CAP), con el software PRIMER v7.0. Posteriormente se elaboró una Reciever Operating Characteristics curve o curva ROC para identificar y evaluar la sensibilidad y especificidad de la prueba estadística empleada.

Con respecto a la colecta de las muestras de orina, una vez que las pacientes finalizaban su consulta en el Hospital Central “Dr. Ignacio Morones Prieto”, si éstas cumplían con los criterios de inclusión, se les explicaba el protocolo de investigación, las que aceptaban participar lo hacían mediante la firma del consentimiento informado y en el momento se colectó una muestra de orina espontánea. Éstas fueron transportadas en una hielera al laboratorio de “Salud Total” de la CIACYT de la Facultad de Medicina de la UASLP y almacenadas a -70ºC hasta su análisis por CG-MS. Para el análisis estadístico de estas muestras, se conformaron diferentes grupos de estudio: BI-RADS 0-2 (n=25) y BI-RADS 3-6 (n=21) y estadios I-II (n=11) y III-IV (n=35). Se identificaron los metabolitos presentes en cada muestra mediante la

comparación de similitudes estructurales de compuestos reportados en la biblioteca de la base de datos de NIST. Posteriormente, se realizó un análisis de varianza por permutaciones (PERMANOVA), para evaluar si existían diferencias significativas considerando como variables el diagnóstico BI-RADS y el estadio, a continuación se realizó un análisis canónico de coordenadas principales (CAP) y para determinar el porcentaje de contribución de cada metabolito identificado se realizó un análisis SIMPER (Similarity Percentage).

Resultados.

No se observaron diferencias estadísticamente significativas entre ambos grupos de estudio en relación con los parámetros antropométricos y la edad. Los valores registrados de peso, talla, IMC y edad de la población clínicamente sana son: 69.5±15.67 Kg, 1.55±0.32 m, 28.73±5.5 y 54±12.18 años, respectivamente, mientras que los del grupo de mujeres con cáncer de mama son: 66.13±14.63 Kg, 1.56±0.05 m, 27.12±4.60 y 49±11.22 años, respectivamente.

Con base a los expedientes clínicos de las pacientes con cáncer de mama, a quienes se les colectó aliento exhalado, el 50% fue diagnosticada con cáncer tipo luminal, siendo el 32 y el 18% luminal A y B respectivamente. En relación a los datos obtenidos del análisis de componentes principales (PCA) y la prueba estadística PERMANOVA, se observó una separación entre las pacientes con cáncer de mama y las clínicamente sanas, que explica el 95.9% de la variabilidad de los datos, con una diferencia estadísticamente significativa entre ambos grupos ($p<0.05$). Posteriormente, se realizó un análisis canónico de coordenadas principales (CAP),

con una clasificación correcta del 95%. Con los resultados del eje CAP1, se realizó una curva ROC, que arrojó una sensibilidad y especificidad del 100%.

Para las muestras de orina, el PCA, explicó el 31.5% de la variabilidad natural de los datos entre las pacientes con cáncer de mama del grupo 0-2 del sistema BI-RADS y el grupo 3-4, de los cuales, el PC1 explicó el 19.3% y el PC2 el 12.2% de esta variabilidad. El análisis PERMANOVA mostró una diferencia significativa entre ambos grupos ($p<0.01$). Posteriormente, se efectuó el CAP, con una clasificación correcta del 61.9%.

Finalmente, de acuerdo con el resultado del análisis SIMPER, el compuesto limoneno fue el que contribuyó en mayor proporción (8.49 %), a la variabilidad observada.

Discusión y conclusiones.

Con base en los resultados obtenidos de las muestras de aliento exhalado, se sugiere que la separación observada entre los grupos de estudio es indicativa de que ambas poblaciones presentan diferentes patrones de los compuestos orgánicos volátiles, probablemente porque las mujeres con cáncer de mama tienen distintos procesos bioquímicos durante la progresión de la enfermedad. Estos resultados son comparables con el estudio realizado por Rodríguez-Aguilar et al., quienes evaluaron muestras de aliento exhalado de pacientes con cáncer de mama o cáncer de pulmón, los autores reportan una diferencia estadísticamente significativa entre el grupo control y los grupos de pacientes con cáncer de mama o cáncer de pulmón (9). Sin embargo, con el diseño de nuestro estudio no es posible elucidar las causas de la separación observada entre las poblaciones evaluadas dada la técnica analítica empleada ya que fue un análisis de metabolómica no dirigido. No obstante lo anterior,

la presente investigación sienta las bases para estudios futuros para el desarrollo de metodologías más sensibles que puedan emplearse como herramientas y/o biomarcadores de riesgo de cáncer de mama.

Con respecto a los resultados obtenidos a partir de las muestras de orina, no se observaron diferencias estadísticamente significativas (PCA y CAP), entre los grupos de estudio. El porcentaje de correcta clasificación obtenido fue menor al 70%, por lo que se infiere que el patrón de metabolitos detectados es el mismo entre las mujeres clínicamente sanas y las que fueron diagnosticadas con cáncer de mama. En un estudio realizado por Ruiz B, Spinosa en el cual se comparó un grupo de mujeres sanas contra uno de mujeres con cáncer, se encontró una diferencia significativa entre ambos grupos en un análisis metabolómico no dirigido (10).

Dado a que la identificación de los metabolitos se realizó por comparación de compuestos de la biblioteca del NIST y que el análisis fue del tipo “no dirigido” (únicamente es posible separar los grupos de estudio de acuerdo con los patrones de los metabolitos detectados) (11), por lo tanto el haber identificado al limoneno como al compuesto que contribuyó en la mayor proporción (8.49 %), a la variabilidad observada, no tiene una relevancia individual, sin embargo reportes en la literatura relacionan a este compuesto con cáncer de colon (12).

No obstante el que mediante la metabolómica no dirigida no es posible elucidar ni las concentraciones ni las rutas metabólicas de los compuestos referidos, en las muestras analizadas se lograron identificar compuestos principalmente pertenecientes a los grupos de los alcanos alifáticos, alquenos y en menor proporción al de los alcoholes. La literatura menciona que compuestos derivados de estos grupos

químicos, principalmente de los alcanos, tienen papeles relevantes en el inicio del desarrollo de cáncer, debido a su estrecha relación con el efecto de Warburg (13,14) (aumento de la tasa metabólica de la glucólisis) y de la peroxidación de ácidos grasos poliinsaturados (PUFA) y productos de la síntesis de aminoácidos (15,16).

Una de las limitantes de realizar estudios de metabolómica no dirigida empleando la nariz electrónica y CG-MS, es la identificación precisa de los compuestos químicos detectados y por ende sus concentraciones, lo que limita también la elucidación de las rutas metabólicas; sin embargo, con esta metodología es posible identificar los metabolitos más representativos de cada grupo, información indispensable para realizar estudios de metabolómica dirigida, con la cual es factible la identificación de metabolitos específicos, asi como sus rutas metabólicas. La utilidad clínica de este tipo de técnicas contribuye al desarrollo de programas de intervención temprana.

Bibliografía.

1. Smolarz B, Zadrożna Nowak A, Romanowicz H. Breast Cancer—Epidemiology, Classification, Pathogenesis and Treatment (Review of Literature). Cancers (Basel). 2022;14(10):1–27.
2. World Health Organization. Estimated number of new cases in 2020, breast, females, all ages. 2020;774:1. Available from: https://gco.iarc.fr/today/online-analysis-table?v=2020&mode=population&mode_population=continents&population=900&populations=&key=asr&sex=2&cancer=20&type=0&statistic=5&prevalence=0&population_group=0&ages_group%5B%5D=0&ages_group%5B%5D=17&group_cancer=0&

3. OPS. Epidemiología: Cáncer de mama en las Américas. 2018;1–2.
4. Knaul FM, Nigenda G, Lozano R, Arreola-Ornelas H, Langer A, Frenk J. Cáncer de mama en México: Una prioridad apremiante. Salud Publica Mex. 2009;51(SUPPL.2):335–44.
5. Rodríguez-Cuevas S, Guisa-Hohenstein F, Labastida-Almendaro S. First breast cancer mammography screening program in Mexico: Initial results 2005-2006. Breast J. 2009;15(6):623–31.
6. Maggi N, Gazzarata R, Ruggiero C, Lombardo C, Giacomini M. Cancer precision medicine today: Towards omic information in healthcare systems. Tumori. 2019;105(1):38–46.
7. Zeki ÖC, Eylem CC, Reçber T, Kır S, Nemutlu E. Integration of GC–MS and LC–MS for untargeted metabolomics profiling. J Pharm Biomed Anal. 2020;190.
8. Patti GJ, Yanes O, Siuzdak G. Metabolomics: the apogee of the omics trilogy. Nat Rev Mol Cell Biol [Internet]. 2012 Apr 22;13(4):263–9. Available from: http://www.nature.com/articles/nrm3314
9. Gorocica-rosete P, Domínguez-reyes CA, Tenorio-torres JA, Ornelas-rebolledo O, Mehta G, Zamora-mendoza BN. Clinica Chimica Acta Application of chemoresistive gas sensors and chemometric analysis to differentiate the fingerprints of global volatile organic compounds from diseases . Preliminary results of COPD , lung cancer and breast cancer. 2021;518(January):83–92.
10. Ruiz B, Spinosa B, Martinis D. Clinical Mass Spectrometry Analysis of urinary VOCs using mass spectrometric methods to diagnose cancer: A review. 2020;18:27–37.
11. Nam H, Chung BC, Kim Y, Lee K, Lee D. Combining tissue transcriptomics

and urine metabolomics for breast cancer biomarker identification. 2009;25(23):3151–7.

12. Coleman MP, Quaresma M, Berrino F, Lutz JM, De Angelis R, Capocaccia R, et al. Cancer survival in five continents: a worldwide population-based study (CONCORD). Lancet Oncol. 2008;9(8):730–56.
13. Liberti M V., Locasale JW. The Warburg Effect: How Does it Benefit Cancer Cells? Trends Biochem Sci [Internet]. 2016;41(3):211–8. Available from: http://dx.doi.org/10.1016/j.tibs.2015.12.001
14. Liberti M V, Locasale JW, Biology C, Biology C. The Warburg Effect: How Does it Benefit Cancer Cells? 2017;41(3):211–8.
15. Fodil M, Blanckaert V, Ulmann L, Mimouni V. Contribution of n-3 Long-Chain Polyunsaturated Fatty Acids to the Prevention of Breast Cancer Risk Factors. 2022;
16. Subramani R, Poudel S, Smith KD, Estrada A, Lakshmanaswamy R. Metabolomics of Breast Cancer: A Review. 2022;

ARTÍCULO 1

Assessing Volatile Breath Patterns: Distinguishing Breast Cancer Patients from Healthy Women Using Electronic Nose

(En proceso de publicación)

Assessing Volatile Breath Patterns: Distinguishing Breast Cancer Patients from Healthy Women using Electronic Nose

María José Santoyo Treviño[a], Sergio Zarazúa Guzmán[a], Francisco Javier Pérez Vázquez[a] Rogelio Flores-Ramírez [a], José Zermeño Nava [b]

Affiliations: [a] *Coordinación para la innovación y aplicación para la Ciencia y la Tecnología, Mexico; and* [b] *Hospital Central "Dr Ignacio Morones Prieto*

Abstract

Background: Breast cancer is a malignant disease affecting breast tissue, predominantly in women and men. It typically begins with the uncontrolled growth of abnormal cells. Early detection is crucial for successful treatment, as symptoms may not manifest in the early stages.

Aims of the study: Assess the difference of patterns of volatile prints from exhaled breath from breast cancer (BC) patients and healthy women by electronic nose technique.

Material and methods: In this study, the sensor's response to exhaled breath from two groups, breast cancer patients and healthy controls, was assessed using an electronic nose (Cyranose). The collected data underwent several analyses, including principal component analysis (PCA), Canonical Analysis of Principal Coordinates (CAP), Partial Least Squares - Discriminant Analysis (PLS-DA), and ROC curves. These analyses

aimed to determine the test's diagnostic efficacy and discriminatory power in distinguishing between BC patients and healthy individuals.

Results: A separation was found between the patients with BC and healthy, explaining 95.9% of the variability of the data. Subsequently, a CAP was obtained with a correct classification of 100%, and the PLS-DA got an accuracy of 100%. With the results of axis CAP1, a ROC curve was performed, resulting in a sensitivity of 100% and a specificity of 100%.

Conclusions: Electronic nose technology has shown promise in detecting breast cancer by analyzing exhaled breath. This non-invasive method identifies volatile organic compounds produced by cancer cells, allowing for early disease detection and monitoring.

Keywords: Breast Neoplasms; exhaled breath; multivariate analysis; screening.

Introduction

Breast cancer is an oncologic process in which healthy mammary cells glands degenerate into tumors due to excessive proliferating and multiplying cells, which leads to tumor formation; in this process, these tumors can spread to other organs by hematogenic via as well as lymphatic (1).

According to the tumor characteristics, breast cancer can be classified into different types. This classification depends on the size and extent of the primary tumor, the anatomical area where it forms, and the invasiveness of this cell's excessive proliferation (2).

According to World Health Organization (WHO) (3), at least 1.38 million new breast cancer cases are detected yearly. Data suggests that the incidence of breast cancer around the world is of 2 261 419 new cases. Around 98% of these cases are diagnosed

in women. Global Cancer Observatory (GLOBOCAN) (4) reported that by 2020, breast cancer caused more than 500,000 deaths of women worldwide, and Institute for Health Metrics and Evaluation (IHME) (5) published that 70% of deaths occurred in developing countries. Thus, breast cancer in developing countries is a public health concern since it is usually diagnosed in advanced stages (6). In México, for example, breast cancer has become the second leading cause of death in women aged 30 to 54, with an incidence of 35.4 cases per 100,000 women (7). However, in recent years the incidence of malignant breast tumors also has increased in younger women.

In recent years, there has been increasing concern about male breast cancer. Although incidence and mortality are lower than in women, cases grow yearly, even when considered a sporadic disease (less than 1% of all breast cancers). The risk of a man being diagnosed with breast cancer during life is about 1 in 833 (8).

One of the most significant prevailing global concerns is that about half the worldwide population has no access to essential health services. Therefore, breast cancer in women is already detected in advanced stages. If current trends continue in America, the number of breast cancer cases is predicted to increase by 34% by 2030. Furthermore, in Latin America and the Caribbean, breast cancer is the most common type among women and the second leading cause of mortality.

Diagnosis and screening of BC are primarily based on self and clinical exploration. However, the self-exploring method has many limitations, such as frequent knowledge gaps about breast cancer and screening techniques, fear, and fatalism, among other social problems (9). In this regard, fewer people in Latin America take a self-exploring test frequently compared with many other countries; for example, only 1% of the Colombian population uses this technique to obtain information about breast cancer. In Mexico, 10% use self-examination to prevent BC from appearing. (10) Moreover in

México, the costs of attention for BC vary depending on the stage that disease is diagnosed, for example BC diagnosed at early stages may have an average cost of 5230.78, compared to advanced diagnosis where treatment costs increases to 7789.92 USD per person. (11)

The gold standard for BC diagnosis is mammography which,is only indicated for women aged 40 or more. (12) However, mammography has is limited, requiring high-quality equipment and expert professionals. (9), (37) he recommendation for the initiation and periodicity of mammography as a screening method may be variable. The World Health Organization (WHO) and Panamerican Health Organization (PAHO) established that mammography should be performed once every two years. However, even with this, it is still a problem in countries like Mexico, since only in 2014, 15% of the total women population had access to mammography as a screening test; today, it is predicted that only 20% of Mexican women have access to this test, while in many European countries, at least 75% of them can reach breast cancer health care. (14)

Other relevant techniques for cancer screening are ultrasound and nuclear magnetic resonance imaging (MRI) (15). Ultrasound has become very useful for observing changes in breast tissue, such as mass formation, that are impossible to watch on mammography. (16).

One of the most significant current discussions about the development of this pathology is that breast cancer mortality is highly related to the sensitivity of the detection method used (17). It is becoming increasingly difficult to ignore that the current early diagnosis of breast cancer is limited because the disease develops asymptomatically, and diagnostic techniques have low early sensitivity and specificity (18).

The PAHO and WHO proposed that developing new noninvasive techniques can provide some biomarkers for diagnosing and screening cancer. Therefore, scientists are looking at elaborating new tools for breast cancer identification at different stages. Other screening molecular methods are focused on *"omic"* sciences, such as metabolomics and volatolomics.

In recent years cancer research approaches have involved the study of molecular different molecular characteristics between damaged cells and their healthy counterparts. To reveal representative biomarkers from many disease phenotypes, such as breast cancer, which can be helpful as possible therapeutic targets. From this perspective, new analytical strategies based on metabolomics have emerged as potential tools that can completely compress many biological processes [46, 61].

In broad terms, metabolomics is a science dedicated to the global study of metabolites, their composition, and their response to interventions or changes in the environment, such as cells, tissues, or fluids, which can complement biochemical information obtained from genes and proteins (21). In recent years, metabolomics has had a substantial clinical approach to health since the identification of biomarkers could reveal the diagnosis of a disease from a simple analysis and a relatively low cost. (19). Recently, people's exhaled breath has taken great relevance as a low-cost, fast, safe, and non-invasive biological sample that can assess many pathological conditions. Human exhaled breath is a complex composition of gases where more than 3000 compounds have been identified, including small inorganic compounds such as NO, O2, CO2, and volatile organic compounds (hydrocarbons, alcohols, ketones, aldehydes, and esters. (22)

Aim of the study: Identify the differences between patterns of volatile prints from exhaled breath from breast cancer (BC) patients and healthy women by electronic nose technique.

Material and methods

Subjects and sample collection.

For the present study, 37 controls and 32 breast cancer (BC) patients were recruited from the Department of Gynecology and Obstetrics of the Hospital Dr. Ignacio Morones Prieto in San Luis Potosí, México; subjects were women aged from 30 to 74 years old, in an open, prospective observational cohort study design.

For selection criteria, all women with breast cancer diagnosis detected by physical examination, ultrasonography mammography (mammograms on a BI-RADS scale of 1 or 2), and biopsy were included. Patients with breast or other types of cancer antecedents were not included in this study. [Bioethical committee registration: CONBIOETICA-24-CEI-001-20160427 (96-18)]

Samples of exhaled breath of patients were collected. Before sample collection, the patient rinsed their mouth with 50 mL of water and was asked to take three deep inhalations and exhale deeply in a Breath Collection Bag (BRA). Bags were designed to collect gases and protect light-sensitive compounds. The bags were purged with nitrogen before the analyses. Patients were asked to present themselves with 8 hours of fasting and not smoke or wash their mouths before the sample collection. Samples were transported at 4°C under standardized conditions and stored at -20ºC until analysis.

Electronic nose (LabiNose 43) analysis.

Cyranose 320, a portable electronic nose with 32 chemoreceptors (sensors), will be used for breath analyses. Sensors are composed of carbon polymers incorporated

into a matrix that adsorbs volatile organic compounds from the exhaled breath, causing an increase in electrical resistance, which results in a specific fingerprint (breath print) caused by differences in labinose's electrical resistance.

Each sensor has different properties that influence the adsorption of volatile organic compounds, producing various degrees of response due to its polymer composition (polyvinyl butyral, polyvinyl acetate, polystyrene, and poly-ethylene oxide) and to the conduction nanoparticles such as black carbon and carbon nanotubes.

Statistical analyses.

Multivariate analyses were performed through CDAnalysisTM-Chemometric Data Analysis software to establish differences between the healthy and sick groups. The variables used were the sensory response measured as a differential of the electrical resistance determined by each sensor.

Multivariate analyses were performed based on the 32 Cyranose sensors' resistance that was obtained from the fractional difference: $\partial R/Ro = (Rmax-Ro)/Ro$ where R is the maximum system response of each sensor, and Ro is the reference resistance of each sensor with ultra-pure nitrogen.

Subsequently, with these variables, a Savitzky-Golay filter was carried out to establish differences between groups, as well as a principal component analysis (PCA) to reduce the dimensionality of the initial data set of the 32 sensors to a set of principal components that capture the most variance in the data. A canonical analysis of primary coordinates (CAP) was carried out through the multivariate data that could best discriminate between the study groups.

The significance of class discrimination was assessed through a permutation test. The analysis focused on the CAP1 axis and employed the Receiver Operating Characteristic (ROC) curve, considering it encompassed the entire dataset. The

specificity/sensitivity ratio was maximized while maintaining a 95% confidence interval (CI) to determine the threshold value or cut-off point.

Results

Characteristics of BC patients and controls are shown in Table 1. A total of 69 women participated in the study; thirty-two of them were diagnosed with BC, and 37 were controls. The average ages were 49 years for BC patients and 54 for controls.

Molecular results show that Luminal B cancer was the most predominant type in 32 % of the patients.

Forty-eight percent of the participants in this study did not show other comorbidities besides BC. However, 16 % of them were reported with DM2 and 36 % with hypertension. Table 2.

In Figure 1, PCA analysis is presented, and a natural separation was observed among the chemical fingerprints of patients with breast cancer and controls. The overall PCA of both groups explains 95.9% of the data variation (PC1= 68.8% and PC2=27.1%) (Fig 1.) The existence of a real significant difference in the separation observed by PCA was estimated through the PERMANOVA test, and results show a significant difference between the control and BC groups ($p < 0.01$)

CAP analysis (Table 3) separated the chemical fingerprints of the BC group of the control group with an r^2 value of 0.9498. In the CAP model, 98 % of correct classification was obtained. (Fig 2).

Using the sensor's response values from the CAP model, the CAP1 axis yielded a cut-off point -0.093. This threshold resulted in a sensitivity of 100% (87.9 -100%) and a specificity of 100% (88.5-100%). Furthermore, both the positive and negative

predictive values were 100%. The overall accuracy achieved through the ROC curve was 100%. (Figure 3).

Discussion

As discussed before, disease research has involved the study of different molecular characteristics between damaged cells and their healthy counterparts in revealing representative biomarkers of the disease phenotype and potential therapeutic targets. From this perspective, new analytical strategies based on the “omics” sciences (genomics, proteomics, transcriptomics, and metabolomics) have emerged as potential tools, allowing a comprehensive understanding of biological processes. (23) “omics” markers can improve the prediction of the appearance of breast cancer in its early stages, as well as its diagnosis beyond standard features, and even allow the development of personalized therapy. (24)

Metabolomics has emerged as a powerful tool in clinical research, including in the field of oncology. (19) By analyzing the metabolic profile of biological samples such as blood, urine, or tissue, metabolomics can provide valuable insights into disease diagnosis, progression, and response to treatment. (20)

Breast cancer is one area where metabolomics has shown promise. Studies have identified changes in several metabolic pathways during breast cancer development, including alterations in amino acid metabolism, lipid metabolism, and energy metabolism. These changes can be detected using various analytical techniques, such as mass spectrometry (MS), nuclear magnetic resonance imaging (MRI), and gas chromatography-mass spectrometry (GC-MS). (25)

Identifying biomarkers through metabolomics analysis can have important clinical implications, such as facilitating early detection and diagnosis of breast cancer, monitoring treatment response, and predicting disease recurrence. (26) Furthermore,

metabolomics may also help identify new therapeutic targets and improve our understanding of the underlying mechanisms of breast cancer. (27)

Overall, metabolomics is a promising approach for breast cancer research and has the potential to contribute to improved patient outcomes through personalized medicine.

Jové Mariona et al. (28) compared the metabolomic serum profiles of healthy women with women diagnosed with breast cancer; they found an elevated level of products of branched-chain amino acid metabolism such as 2-hydroxymethyl butyric acid, 2-hydroxy-methyl pentanoic acid, and 3-methyl glutaric acid, also found some glycolysis products like glucose, pyruvate, dihydroxyacetone, and finally, there was an increase of antioxidant metabolites and lipid metabolism products which can be listed as follows: urea, uric acid, 2-methyluric acid, retinoic acid, and many ceramides. Besides, it was observed that alterations in these metabolic pathways increase as the severity of the tumor increases. (28)

Denkert *et al.* (29) also found that certain amino acids such as tyrosine, phenylalanine, and tryptophan were elevated in breast cancer tissue, indicating increased protein turnover and activation of pathways involved in tumor growth and proliferation. They also observed elevated lactate and pyruvate levels, suggesting increased glycolysis and lactate production, a hallmark of cancer metabolism. (30)

Studies have also shown that alterations in the metabolism of nucleotides, choline, and phospholipids are commonly observed in breast cancer. For instance, high levels of choline-containing compounds have been found in breast tumors, suggesting an increased rate of cell membrane turnover. (31) Additionally, high levels of nucleotides, such as uridine, have been associated with more aggressive forms of breast cancer. (32)

These findings demonstrate that breast cancer is associated with significant alterations in multiple metabolic pathways, including amino acid metabolism, lipid metabolism, glycolysis, and nucleotide metabolism. (33) These metabolic changes are likely to play a crucial role in cancer development, growth, and progression and could potentially serve as biomarkers for breast cancer diagnosis and prognosis. (34)

Recently, people's exhaled breath has taken great relevance as a low-cost, fast, safe, and non-invasive biological sample that can assess many pathological conditions. Human exhaled breath is a complex composition of gases where more than 3000 compounds have been identified, which are included small inorganic compounds such as NO, O_2, CO_2, and volatile organic compounds (hydrocarbons, alcohols, ketones, aldehydes, esters); it also can be found many non-volatile inorganic compounds such as isoprostanes, cytokines, leukotrienes, and hydrogen peroxide. (22),(35)

Breast cancer screening based on the analysis of exhaled breath is not confirmatory because it is accompanied by increased oxidative stress, which can cause damage to DNA, proteins, and lipids. Induction of cytochrome P450 enzymes leads to lipid peroxidation of polyunsaturated fatty acids in cell membranes. It results in the overexpression of volatile alkanes and their derivatives in the breath, ultimately affecting the abundance of VOCs in the exhaled breath. (36) Free radical-induced oxidation of amino acids and proteins has also been shown to generate some hydrocarbons and malonaldehyde. (37–39)

The vast array of molecules available results in various characteristics, including polarity, molecular weight, stability, chemical reactivity, and functional groups. (40) Various analytical platforms and configurations are utilized to cover the analyzed metabolome comprehensively. Nuclear magnetic resonance (NMR), mass

spectrometry (MS), and electronic noses are the most common technology platforms for identifying and measuring metabolites. (41)

Various metabolomics investigations have been carried out on breast cancer patients utilizing GC-MS analysis of exhaled breath. In 2014, Phillips conducted a significant study that evaluated the breath of four distinct groups consisting of 35 patients with positive cancer, 79 patients with negative cancer, 93 patients with normal mammographies, and 37 patients with abnormal mammographies were evaluated, resulting in the identification of distinctive chemical fingerprints. This study found that healthy women with normal mammographies had different chemical fingerprints than women who underwent breast biopsy and tested positive for cancer. (42)(43) The study also reported a sensitivity of 81.8%, a specificity of 70%, and an accuracy of 79%, as determined by ROC curves. (44–46)

Peng and colleagues investigated the use of electronic nose technology for a breast cancer diagnosis; the study aimed to evaluate the diagnostic accuracy of a nanosensor array in detecting breast cancer from exhaled breath samples.

The study included 35 healthy individuals, 30 patients with benign breast disease, and 25 patients with BC. Results showed that the nanosensor array could distinguish between healthy individuals and those with breast cancer with a sensitivity of 95% and a specificity of 88%. The nanosensor array also demonstrated high diagnostic accuracy in distinguishing between benign breast disease and breast cancer, with a sensitivity of 90% and a specificity of 83%. (47,48)

In 2020, Töreyin and colleagues published a study that investigated the use of electronic nose technology for breast cancer detection, which aimed to evaluate the diagnostic accuracy of an electronic nose in detecting breast cancer from exhaled breath samples. This study included 18 healthy individuals and 30 patients with BC.

Results showed that the electronic nose could distinguish between healthy individuals and those with breast cancer with an accuracy of 88%. The electronic nose also demonstrated high diagnostic accuracy in determining between different stages of breast cancer, with an accuracy of 83% for early-stage breast cancer and 93% for advanced-stage breast cancer. (49)

Overall, this study provides evidence that electronic nose technology can be used as a non-invasive and accurate tool for diagnosing breast cancer. (50)

Diaz de León and colleagues investigated using a portable electronic nose device for breast cancer detection. The study concluded that the portable electronic nose device could be a valuable tool for non-invasive breast cancer diagnosis. However, but further studies are needed to validate the results and investigate further. (51)

The study included 244 participants and found that the instrument had high diagnostic accuracy for detecting breast cancer from exhaled breath samples, with a sensitivity of 87.7% and a specificity of 80.8%.

Conclusion

Electronic nose technology has shown promising results in the field of medical diagnosis. One area where it has been extensively researched is in detecting breast cancer through exhaled breath analysis.

Exhaled breath analysis is a non-invasive method that detects volatile organic compounds (VOCs) produced by cancer cells. These VOCs can be identified using an electronic nose, a device that mimics the human olfactory system by detecting and analyzing the chemical composition of the exhaled breath.

Several studies have evaluated electronic nose technology's efficacy in the diagnosis of breast cancer.

The advantage of using an electronic nose for breast cancer diagnosis is its non-invasive nature, which is less uncomfortable for the patient than traditional biopsy methods. In addition, the electronic nose can detect cancer at an early stage, leading to earlier treatment and better outcomes.

However, more research is needed to validate the accuracy of the electronic nose for breast cancer diagnosis and identify the specific VOCs associated with breast cancer. Despite this, the potential of electronic nose technology in medical diagnosis is promising and warrants further investigation.

Conflict of Interest Disclosures (includes financial disclosures): All authors have no conflicts of interest to disclose.

Acknowledgments: The authors would like to acknowledge Hospital Central “Dr. Ignacio Morones Prieto”.

Tables

Table 1. Anthropometric characteristics of Controls and BC patients

	Healthy controls n=37	**Breast cancer patients n=35**		
Parameter	Average (±SD)	Average (±SD)	t-Value	P Value
Height (m)	1.55±0.032	1.56±0.047	0.16	0.873
Weight (Kg)	69.5±15.67	66.13±14.63	-0.75	0.454
MBI (Kg/m^2)	28.73±5.5	27.12±4.6	1.48	0.14
Age	54±12.18	49±11.22	-0.83	0.409

Table 2. Clinical characteristics of study population

Parameter	**n**	**Porcentaje (%)**
Molecular Type		
Luminal A	18	6
Luminal B	32	12
Triple Negative	12	4
HER-2	12	4
Undetermined	26	9
Stage		
0	3	8
I	4	12
II	14	40
III	8	24
IV	6	16
Comorbility	n	Porcentaje (%)
DM 2	6	16
Hipertention	13	36
None	18	52

Table 3. Multivariate analysis characteristics.

Multivariate Analysis Type	
% Variation (PCA)	
PC1	68.3
PC2	27
PC3	2.1
CAP	
R^2 CAP	0.9498
% Of Correct classification	98
Mis-classification error (%)	2
P value	0.001

Figures and figure legends

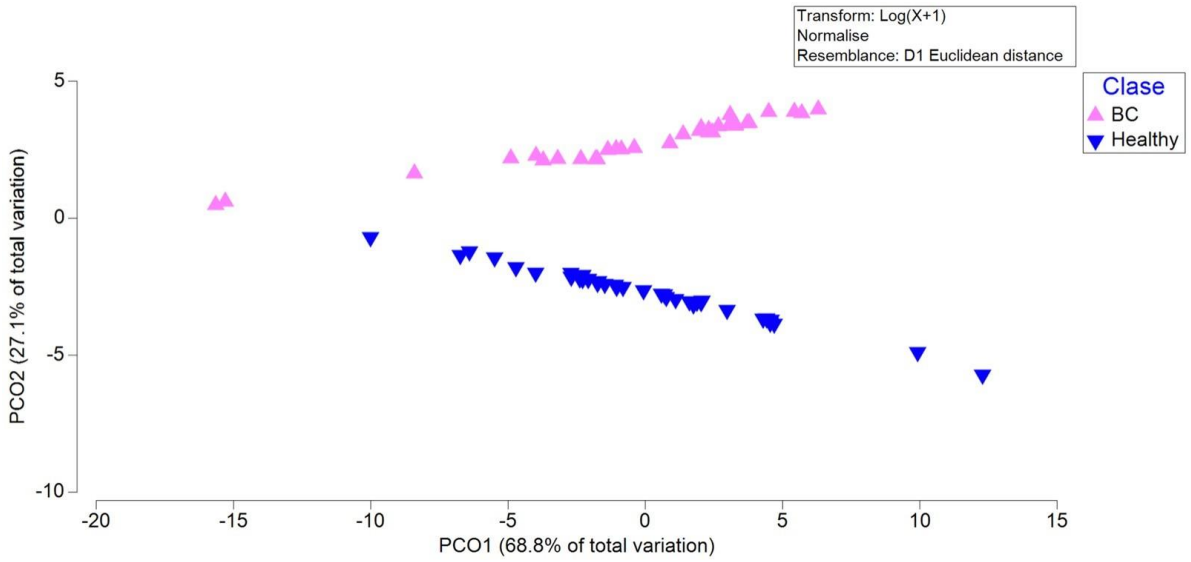


Figure 1. Principal component analysis (PCA) of study populations. Breast Cancer patients (BC) and healthy controls (Healthy).

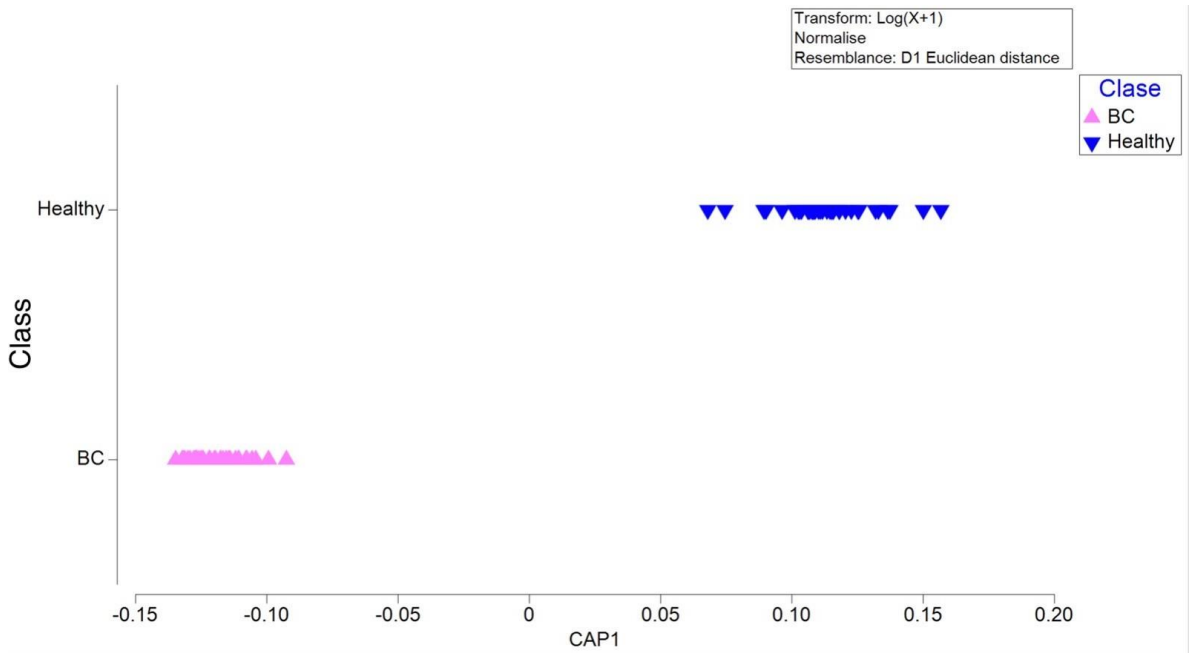


Figure 2. Canonical analysis of principal coordinates (CAP) in different populations. Breast Cancer patients (BC) and healthy controls (Healthy).

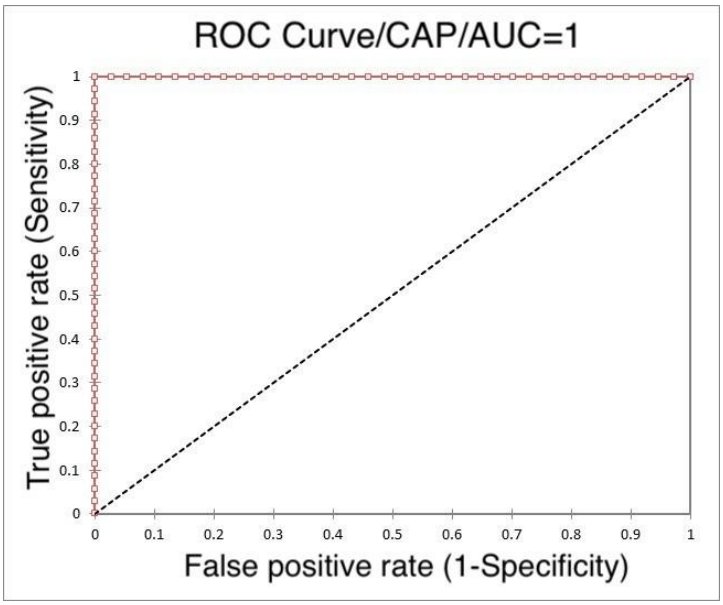


Figure 3 The ROC curve for breast cancer (BC) screening using the CAP1 axis resulted in an AUC of 1, indicating perfect classification performance. The chosen cut-off point of -0.093 and a 95% confidence interval, contributed to this result.

References.

1. Butcher DT, Alliston T, Weaver VM. REVIEWS A tense situation: forcing tumour progression. 2009;9(FEbRuARy).
2. Eliyatkin N, Yalcin E, Zengel B, Aktaş S, Vardar E. Molecular Classification of Breast Carcinoma: From Traditional, Old-Fashioned Way to A New Age, and A New Way. J Breast Heal. 2015;11(2):59–66.
3. WHO. Breast cancer. 2018.
4. GLOBOCAN. Global Cancer Observatory. 2018.
5. Intitute for Health Metrics and Evaluation (IHME). Data Visualizations. 2019.
6. Coleman MP, Quaresma M, Berrino F, Lutz JM, De Angelis R, Capocaccia R, et al. Cancer survival in five continents: a worldwide population-based study (CONCORD). Lancet Oncol. 2008;9(8):730–56.
7. Mohar-Betancourt A, Reynoso-Noverón N, Armas-Texta D, Gutiérrez-Delgado C, Torres-Domínguez JA. Cancer Trends in Mexico: Essential Data for the Creation and Follow-Up of Public Policies. J Glob Oncol. 2017 Dec;3(6):740–8.
8. Yalaza M, Inan A, Bozer M. Male Breast Cancer. J Breast Heal. 2016 Jan;12(1):1–8.
9. Doede AL, Mitchell EM, Wilson D, Panagides R, Oriá MOB. Knowledge, beliefs, and attitudes about breast cancer screening in Latin America and the Caribbean: An in-depth narrative review. J Glob Oncol. 2018;2018(4).
10. Romieu I, Biessy C, Torres-Mejía G, Ángeles-Llerenas A, Sánchez GI, Borrero M, et al. Project profile: a multicenter study on breast cancer in young women in Latin America (PRECAMA study). Salud Publica Mex. 2019 Oct;61(5, sep-oct):601.
11. Santiago-carrillo MA. Análisis del coste directo de la atención médica y

quirúrgica del cáncer de mama . Estudio comparativo entre etapas temprana y tardía en tercer nivel de atención. 2022;91(1):28–33.

12. Knaul FM, Nigenda G, Lozano R, Arreola-Ornelas H, Langer A, Frenk J. Cáncer de mama en México: Una prioridad apremiante. Salud Publica Mex. 2009;51(SUPPL.2):335–44.
13. Secretaria de Salud. NORMA Oficial Mexicana NOM-041-SSA2-2002, Para la prevención, diagnóstico, tratamiento, control y vigilancia epidemiológica del cáncer de mama. 2016. p. 5–6.
14. Uscanga-Sánchez S, Torres-Mejía G, Ángeles-Llerenas A, Domínguez-Malpica R, Lazcano-Ponce E. Indicadores del proceso de tamizaje de cáncer de mama en México: un estudio de caso. Salud Publica Mex. 2014 Sep;56(5):528.
15. Cedolini C, Bertozzi S, Londero AP, Bernardi S, Seriau L, Concina S, et al. Type of Breast Cancer Diagnosis, Screening, and Survival. Clin Breast Cancer. 2014 Aug 1;14(4):235–40.
16. National Institute of Biomedical Imaging and Bioengineering. Ultrasound. 2016.
17. Marmot MG, Altman DG, Cameron DA, Dewar JA, Thompson SG, Wilcox M. The benefits and harms of breast cancer screening: An independent review. Br J Cancer [Internet]. 2013;108(11):2205–40. Available from: http://dx.doi.org/10.1038/bjc.2013.177
18. Li J, Peng Y, Liu Y, Li W, Jin Y, Tang Z, et al. Investigation of potential breath biomarkers for the early diagnosis of breast cancer using gas chromatography–mass spectrometry. Clin Chim Acta. 2014 Sep 25;436:59–67.
19. Zhang A, Sun H, Qiu S, Wang X. Metabolomics in noninvasive breast cancer. Clin Chim Acta. 2013 Sep;424:3–7.

20. Méndez Rodríguez KB, Santoyo Treviño MJ, Saldaña Villanueva K, Maribel RA, Flores Ramírez R, Pérez Vázquez FJ. Metabolómica como nueva herramienta para el diagnóstico oportuno en enfermedades no transmisibles. Rev Salud Ambient. 2019;19(2):109–15.
21. Klassen A, Faccio AT, Canuto GAB, da Cruz PLR, Ribeiro HC, Tavares MFM, et al. Metabolomics: Definitions and significance in systems biology. In: Advances in Experimental Medicine and Biology. Springer New York LLC; 2017. p. 3–17.
22. Phillips M, Cataneo RN, Ditkoff BA, Fisher P, Greenberg J, Gunawardena R, et al. Prediction of breast cancer using volatile biomarkers in the breath. Breast Cancer Res Treat. 2006 Sep;99(1):19–21.
23. Barash O, Zhang W, Halpern JM, Hua Q-L, Pan Y-Y, Kayal H, et al. Differentiation between genetic mutations of breast cancer by breath volatolomics. Oncotarget. 2015 Dec;6(42).
24. Willmann L, Schlimpert M, Halbach S, Erbes T, Stickeler E, Kammerer B. Metabolic profiling of breast cancer: Differences in central metabolism between subtypes of breast cancer cell lines. J Chromatogr B. 2015;1000:95–104.
25. Gu H, Gowda GAN, Raftery D. Metabolic profiling: Are we en route to better diagnostic tests for cancer? Futur Oncol. 2012;8(10):1207–10.
26. Feizi N, Hashemi-Nasab FS, Golpelichi F, Sabouruh N, Parastar H. Recent trends in application of chemometric methods for GC-MS and GC×GC-MS-based metabolomic studies. TrAC - Trends Anal Chem [Internet]. 2021;138:116239. Available from: https://doi.org/10.1016/j.trac.2021.116239
27. Adebo OA, Oyeyinka SA, Adebiyi JA, Feng X, Wilkin JD, Kewuyemi YO, et al.

Application of gas chromatography–mass spectrometry (GC-MS)-based metabolomics for the study of fermented cereal and legume foods: A review. Int J Food Sci Technol. 2021;56(4):1514–34.

28. Jové M, Collado R, Quiles JL, Ramírez-Tortosa M-C, Sol J, Ruiz-Sanjuan M, et al. A plasma metabolomic signature discloses human breast cancer. Oncotarget. 2017 Mar;8(12):19522–33.
29. Denkert C, Bucher E, Hilvo M, Salek R, Orešič M, Griffin J, et al. Metabolomics of human breast cancer: new approaches for tumor typing and biomarker discovery. Genome Med. 2012 Apr;4(4):37.
30. Ash C. Metabolic regulation of microbiota. Science (80-). 2018;362(6418):1016B.
31. Sitter B, Bathen TF, Singstad TE, Fjøsne HE, Lundgren S, Halgunset J, et al. Quantification of metabolites in breast cancer patients with different clinical prognosis using HR MAS MR spectroscopy. NMR Biomed. 2010;23(4):424–31.
32. Kominsky DJ, Klawitter J, Brown JL, Boros LG, Melo J V., Eckhardt SG, et al. Abnormalities in glucose uptake and metabolism in imatinib-resistant human BCR-ABL-positive cells. Clin Cancer Res. 2009;15(10):3442–50.
33. Min HK, Lim S, Chung BC, Moon MH. Shotgun lipidomics for candidate biomarkers of urinary phospholipids in prostate cancer. Anal Bioanal Chem. 2011;399(2):823–30.
34. Lin B. 乳鼠心肌提取 HHS Public Access. Physiol Behav. 2017;176(12):139–48.
35. Min HK, Lim S, Chung BC, Moon MH. Shotgun lipidomics for candidate biomarkers of urinary phospholipids in prostate cancer. Anal Bioanal Chem

[Internet]. 2011 Jan 17;399(2):823–30. Available from: http://link.springer.com/10.1007/s00216-010-4290-7

36. Phillips M, Altorki N, Austin JHM, Cameron RB, Cataneo RN, Kloss R, et al. Detection of lung cancer using weighted digital analysis of breath biomarkers. Clin Chim Acta [Internet]. 2008 Jul;393(2):76–84. Available from: https://linkinghub.elsevier.com/retrieve/pii/S0009898108001083

37. Ratiu IA, Ligor T, Bocos-Bintintan V, Mayhew CA, Buszewski B. Volatile Organic Compounds in Exhaled Breath as Fingerprints of Lung Cancer, Asthma and COPD. J Clin Med [Internet]. 2020 Dec 24;10(1):32. Available from: https://www.mdpi.com/2077-0383/10/1/32

38. Paredi P, Kharitonov SA, Barnes PJ. Analysis of Expired Air for Oxidation Products. Am J Respir Crit Care Med [Internet]. 2002 Dec 15;166(supplement_1):S31–7. Available from: https://www.atsjournals.org/doi/10.1164/rccm.2206012

39. Heunks LMA. Respiratory muscle function and free radicals: from cell to COPD. Thorax [Internet]. 2000 Aug 1;55(8):704–16. Available from: https://thorax.bmj.com/lookup/doi/10.1136/thorax.55.8.704

40. Patti GJ, Yanes O, Siuzdak G. Metabolomics: the apogee of the omics trilogy. Nat Rev Mol Cell Biol [Internet]. 2012 Apr 22;13(4):263–9. Available from: http://www.nature.com/articles/nrm3314

41. Ellis DI, Goodacre R. Metabolic fingerprinting in disease diagnosis: biomedical applications of infrared and Raman spectroscopy. Analyst [Internet]. 2006;131(8):875. Available from: http://xlink.rsc.org/?DOI=b602376m

42. Capuano R, Catini A, Paolesse R, Di Natale C. Sensors for Lung Cancer Diagnosis. J Clin Med [Internet]. 2019 Feb 11;8(2):235. Available from:

http://www.mdpi.com/2077-0383/8/2/235

43. Broza YY, Mochalski P, Ruzsanyi V, Amann A, Haick H. Hybrid Volatolomics and Disease Detection. Angew Chemie Int Ed [Internet]. 2015 Sep 14;54(38):11036–48. Available from: https://onlinelibrary.wiley.com/doi/10.1002/anie.201500153

44. Phillips M, Cataneo RN, Saunders C, Hope P, Schmitt P, Wai J. Volatile biomarkers in the breath of women with breast cancer. J Breath Res [Internet]. 2010 Jun 1;4(2):026003. Available from: https://iopscience.iop.org/article/10.1088/1752-7155/4/2/026003

45. Broza YY, Haick H. Nanomaterial-based sensors for detection of disease by volatile organic compounds. Nanomedicine [Internet]. 2013 May;8(5):785–806. Available from: https://www.futuremedicine.com/doi/10.2217/nnm.13.64

46. Dragonieri S, Annema JT, Schot R, van der Schee MPC, Spanevello A, Carratú P, et al. An electronic nose in the discrimination of patients with non-small cell lung cancer and COPD. Lung Cancer [Internet]. 2009 May;64(2):166–70. Available from: https://linkinghub.elsevier.com/retrieve/pii/S0169500208004194

47. Peng G, Hakim M, Broza YY, Billan S, Abdah-Bortnyak R, Kuten A, et al. Detection of lung, breast, colorectal, and prostate cancers from exhaled breath using a single array of nanosensors. Br J Cancer [Internet]. 2010 Aug 20;103(4):542–51. Available from: http://www.nature.com/articles/6605810

48. Licht J-C, Grasemann H. Potential of the Electronic Nose for the Detection of Respiratory Diseases with and without Infection. Int J Mol Sci [Internet]. 2020 Dec 10;21(24):9416. Available from: https://www.mdpi.com/1422-0067/21/24/9416

49. Töreyin ZN, Ghosh M, Göksel Ö, Göksel T, Godderis L. Exhaled Breath Analysis in Diagnosis of Malignant Pleural Mesothelioma: Systematic Review. Int J Environ Res Public Health [Internet]. 2020 Feb 10;17(3):1110. Available from: https://www.mdpi.com/1660-4601/17/3/1110

50. van der Sar IG, Wijbenga N, Nakshbandi G, Aerts JGJ V., Manintveld OC, Wijsenbeek MS, et al. The smell of lung disease: a review of the current status of electronic nose technology. Respir Res [Internet]. 2021 Dec 17;22(1):246. Available from: https://respiratory-research.biomedcentral.com/articles/10.1186/s12931-021-01835-4

51. Díaz de León-Martínez L, Rodríguez-Aguilar M, Gorocica-Rosete P, Domínguez-Reyes CA, Martínez-Bustos V, Tenorio-Torres JA, et al. Identification of profiles of volatile organic compounds in exhaled breath by means of an electronic nose as a proposal for a screening method for breast cancer: a case-control study. J Breath Res [Internet]. 2020 Oct 1;14(4):046009. Available from: https://iopscience.iop.org/article/10.1088/1752-7163/aba83f

52. Edge, Stephen B; Byrd, David R; Compton, Carolyn C; Fritz, April G; Greene, Frederick L; Trotti A. AJCC CANCER STAGING MANUAL. 2010.

53. Vranic S, Schmitt F, Sapino A, Costa JL, Reddy S, Castro M, et al. Apocrine carcinoma of the breast: A comprehensive review. Histol Histopathol. 2013;28(11):1393–409.

54. Alexander B. Olawaiye, Thomas P. Baker, M. Kay Washington DGM. The New (Version 9) American Joint Committee on Cancer Tumor, Node, Metastasis Staging for Cervical Cancer. CA A Cancer J Clin. 2021;71(4).

55. . Magny SJ, Shikhman R K AL. Breast Imaging Reporting and Data System.

StatPearls Publishing. 2021;

56. Aure MR, Vitelli V, Jernström S, Kumar S, Krohn M, Due EU, et al. Integrative clustering reveals a novel split in the luminal A subtype of breast cancer with impact on outcome. Breast Cancer Res. 2017;19(1):1–18.
57. Mercado CL. BI-RADS Update. Radiol Clin North Am. 2014 May 1;52(3):481–7.
58. Tanaka K, Tsumura H. Eighth edition of the American Joint Committee on Cancer staging system for soft tissue sarcoma of the trunk and extremity: in search of a better staging system. Ann Transl Med. 2019;7(S1):S11–S11.
59. Centro Nacional de Equidad de Género y Salud Reproductiva. Información Estadística Cáncer de Mama. 2016.
60. Maggi N, Gazzarata R, Ruggiero C, Lombardo C, Giacomini M. Cancer precision medicine today: Towards omic information in healthcare systems. Tumori. 2019;105(1):38–46.
61. American Institute for Cancer Research. Worldwide cancer data. 2018.
62. International Agency for Research on Cancer. Cancer Today. 2018.
63. Frigolet ME, Gutiérrez-Aguilar R. Ciencias “ómicas”, ¿cómo ayudan a las ciencias de la salud? Rev Digit Univ. 2017;18(7):0–15.
64. Oeffinger KC, Fontham ETH, Etzioni R, Herzig A, Michaelson JS, Shih YCT, et al. Breast cancer screening for women at average risk: 2015 Guideline update from the American cancer society. JAMA - J Am Med Assoc. 2015;314(15):1599–614.
65. Sahora K, Fernández-del Castillo C. Surgical Management of Mucinous Cystic Neoplastic Lesions of the Pancreas. Pancreat Cancer, Cyst Neoplasms Endocr Tumors Diagnosis Manag. 2015;24(February):243–8.

66. Souza-Silva ÉA, Reyes-Garcés N, Gómez-Ríos GA, Boyaci E, Bojko B, Pawliszyn J. A critical review of the state of the art of solid-phase microextraction of complex matrices III. Bioanalytical and clinical applications. TrAC - Trends Anal Chem [Internet]. 2015;71:249–64. Available from: http://dx.doi.org/10.1016/j.trac.2015.04.017

67. Wengert GJ, Helbich TH, Kapetas P, Baltzer PA, Pinker K. Density and tailored breast cancer screening: practice and prediction – an overview. Acta Radiol Open. 2018;7(9):205846011879121.

68. Merino MJ. Breast. Gattuso's Differ Diagnosis Surg Pathol. 2021;419:721–62.

69. Nardin S, Mora E, Varughese FM, D'Avanzo F, Vachanaram AR, Rossi V, et al. Breast Cancer Survivorship, Quality of Life, and Late Toxicities. Front Oncol. 2020;10(June):1–6.

70. Smolarz B, Zadrożna Nowak A, Romanowicz H. Breast Cancer—Epidemiology, Classification, Pathogenesis and Treatment (Review of Literature). Cancers (Basel). 2022;14(10):1–27.

71. Thomas CA. Cancer Today - IARC, 150. Cancer Today - IARC, 150 69372 Lyon CEDEX 08, France. 2020.

72. Elmore JG, Armstrong K, Lehman CD, Fletcher SW. CLINICIAN ' S CORNER Screening for Breast Cancer. 2005;293(10):1245–56.

73. Atoum MF, Alzoughool F, Al-Hourani H. Linkage Between Obesity Leptin and Breast Cancer. Breast Cancer Basic Clin Res. 2020;14.

74. Russo L, Lumeng CN. Properties and functions of adipose tissue macrophages in obesity. Immunology. 2018;155(4):407–17.

75. Silva C, Perestrelo R, Silva P, Tomás H, Câmara JS. Breast cancer metabolomics: From analytical platforms to multivariate data analysis. A

review. Vol. 9, Metabolites. 2019.

76. Lécuyer L, Bala AV, Deschasaux M, Bouchemal N, Triba MN, Vasson MP, et al. NMR metabolomic signatures reveal predictive plasma metabolites associated with long-term risk of developing breast cancer. Int J Epidemiol. 2018;47(2):484–94.

77. Tang X, Lin CC, Spasojevic I, Iversen ES, Chi JT, Marks JR. A joint analysis of metabolomics and genetics of breast cancer. Breast Cancer Res. 2014;16(4):1–15.

78. Yu L, Jiang C, Huang S, Gong X, Wang S, Shen P. Analysis of urinary metabolites for breast cancer patients receiving chemotherapy by CE-MS coupled with on-line concentration. Clin Biochem [Internet]. 2013;46(12):1065–73. Available from: http://dx.doi.org/10.1016/j.clinbiochem.2013.05.049

79. Ross JS, Hatzis C, Symmans WF, Pusztai L, Hortobágyi GN. Commercialized Multigene Predictors of Clinical Outcome for Breast Cancer. Oncologist. 2008;13(5):477–93.

80. Sreelakshmi M, George JG, Mishra R, Aneesh TP. Targeting apoptosis by inhibiting cMET amplification due to EGFR resistance using benzofuran derivatives. Res J Chem Environ. 2019;23(11):11–8.

81. Abbaszadeh Z, Çeşmeli S, Biray Avcı Ç. Crucial players in glycolysis: Cancer progress. Gene. 2020;726:144158.

82. Ganapathy-Kanniappan S, Geschwind J-F. Tumor glycolysis as a target for cancer therapy: Mol Cancer [Internet]. 2013;12(1):1–11. Available from: https://www.ncbi.nlm.nih.gov/pmc/articles/PMC4223729/pdf/1476-4598-12-152.pdf

83. Liberti M V., Locasale JW. The Warburg Effect: How Does it Benefit Cancer

Cells? Trends Biochem Sci [Internet]. 2016;41(3):211–8. Available from: http://dx.doi.org/10.1016/j.tibs.2015.12.001

84. Weljie AM, Jirik FR. Hypoxia-induced metabolic shifts in cancer cells: Moving beyond the Warburg effect. Int J Biochem Cell Biol. 2011;43(7):981–9.

85. Silva CL, Perestrelo R, Silva P, Tomás H, Câmara JS. Volatile metabolomic signature of human breast cancer cell lines. Sci Rep. 2017;7(November 2016):1–8.

86. Pereira J, Porto-Figueira P, Cavaco C, Taunk K, Rapole S, Dhakne R, et al. Breath analysis as a potential and non-invasive frontier in disease diagnosis: An overview. Metabolites. 2015;5(1):3–55.

87. Phillips M, Cataneo RN, Greenberg J, Gunawardena R, Naidu A, Rahbari-Oskoui F. Effect of age on the breath methylated alkane contour, a display of apparent new markers of oxidative stress. J Lab Clin Med. 2000;136(3):243–9.

88. Martinez NJ, Rai G, Yasgar A, Lea WA, Sun H, Wang Y, et al. A high-throughput screen identifies 2,9-diazaspiro[5.5]undecanes as inducers of the endoplasmic reticulum stress response with cytotoxic activity in 3D glioma cell models. PLoS One. 2016;11(8):1–19.

89. Schallschmidt K, Becker R, Jung C, Bremser W, Walles T, Neudecker J, et al. Comparison of volatile organic compounds from lung cancer patients and healthy controls - Challenges and limitations of an observational study. J Breath Res [Internet]. 2016;10(4):46007. Available from: http://dx.doi.org/10.1088/1752-7155/10/4/046007

90. Phillips M, Cataneo RN, Saunders C, Hope P, Schmitt P, Wai J. Volatile biomarkers in the breath of women with breast cancer. J Breath Res. 2010;4(2).

91. Phillips M, Cataneo RN, Greenberg J, Grodman R, Gunawardena R, Naidu A. Effect of oxygen on breath markers of oxidative stress. Eur Respir J. 2003;21(1):48–51.

92. Yu L, Jiang C, Huang S, Gong X, Wang S, Shen P. Analysis of urinary metabolites for breast cancer patients receiving chemotherapy by CE-MS coupled with on-line concentration. Clin Biochem. 2013 Aug 1;46(12):1065–73.

93. Cailleux A, Cogny M, Allain P. Blood isoprene concentrations in humans and in some animal species. Biochem Med Metab Biol. 1992;47(2):157–60.

94. Janfaza S, Khorsand B, Nikkhah M, Zahiri J. Digging deeper into volatile organic compounds associated with cancer. Biol Methods Protoc. 2019;4(1):1–11.

95. Smith D, Wang T, Spanel P. On-line, simultaneous quantification of ethanol, some metabolites and water vapour in breath following the ingestion of alcohol. Physiol Meas. 2002;23(3):477–89.

96. Hakim M, Broza YY, Barash O, Peled N, Phillips M, Amann A, et al. Volatile organic compounds of lung cancer and possible biochemical pathways. Chem Rev. 2012;112(11):5949–66.

97. Zhang X, Lv Y, Wang H, Tao X, Zhang X. Classification identification of abalone flavoring liquids based on metal sensor array. Carpathian J Food Sci Technol. 2016;8(2):107–12.

98. You L, Tong R, Li M, Liu Y, Xue J, Lu Y, et al. Ac c ep te us cr ip t Ac c us cr t. Nat Biotechnol [Internet]. 2014;3(3):1–16. Available from: https://doi.org/10.1016/j.jbiotec.2020.05.008%0Ahttps://doi.org/10.1016/j.celrep.2019.01.024%0Ahttps://doi.org/10.1007/s13258-019-00830-w%0Ahttp://dx.doi.org/10.1016/j.cell.2014.05.010%0Ahttp://dx.doi.org/10.1038/

nbt.2842%0Ahttp://dx.doi.org/10.1038/mt.20

99. Patel V B. Molecular Aspects of Alcohol and Nutrition. 2015.

100. a. S, W. F, T. M, C. A, J. S, W. M, et al. Release of volatile organic compounds from the lung cancer cell line NCI-H2087 in vitro. Anticancer Res [Internet]. 2009;29(1):419–26. Available from: http://ovidsp.ovid.com/ovidweb.cgi?T=JS&PAGE=reference&D=emed9&NEWS=N&AN=2009107121

101. Mikalauskas S, Mikalauskiene L, Bruns H, Nickkholgh A, Hoffmann K, Longerich T, et al. Dietary glycine protects from chemotherapy-induced hepatotoxicity. Amino Acids. 2011;40(4):1139–50.

102. Moestue SA, Borgan E, Huuse EM, Lindholm EM, Sitter B, Børresen-Dale AL, et al. Distinct choline metabolic profiles are associated with differences in gene expression for basal-like and luminal-like breast cancer xenograft models. BMC Cancer. 2010;10:1–12.

103. Fujimoto. Metabolite Profiling Identifies a Key Role for Glycine in Rapid Cancer Cell Proliferation. Bone. 2008;23(1):1–7.

104. Cao MD, Giskeødegård GF, Bathen TF, Sitter B, Bofin A, Lønning PE, et al. Prognostic value of metabolic response in breast cancer patients receiving neoadjuvant chemotherapy. BMC Cancer [Internet]. 2012;12(1):39. Available from: http://www.biomedcentral.com/1471-2407/12/39

105. Peeling J, Sutherland G. High-Resolution 1H NMR spectroscopy studies of extracts of human cerebral neoplasms. Magn Reson Med. 1992;24(1):123–36.

106. Platten M, Wick W, Van Den Eynde BJ. Tryptophan catabolism in cancer: Beyond IDO and tryptophan depletion. Cancer Res. 2012;72(21):5435–40.

107. Scioscia KA, Snyderman CH, Wagner R. Altered serum amino acid profiles in

head and neck cancer. Nutr Cancer. 1998;30(2):144–7.

108. Farwick M, Siewe RM, Kramer R. Glycine betaine uptake after hyperosmotic shift in Corynebacterium glutamicum. J Bacteriol. 1995;177(16):4690–5.

ARTÍCULO 2

Identification of urinary metabolites of Breast Cancer patients

(En proceso de publicación)

Identification of urinary metabolites of Breast Cancer patients

María José Santoyo Treviño[a], Sergio Zarazúa Guzmán[a], Francisco Javier Pérez Vázquez[a]
Rogelio Flores-Ramírez [a], José Zermeño Nava [b]

Affiliations: [a] *Coordinación para la innovación y aplicación para la Ciencia y la Tecnología, Mexico; and* [b] *Hospital Central "Dr Ignacio Morones Prieto*

Abstract:

Background: Breast cancer is a malignant disease that affects breast tissue, predominantly in women but also in men. It typically begins with the uncontrolled growth of abnormal cells, forming a lump or mass. Early detection is crucial for successful treatment, as symptoms may not manifest in the early stages. Regular screenings, such as mammograms, along with awareness of potential symptoms like breast lumps, changes in size or shape, nipple discharge, or skin abnormalities, can aid in early diagnosis and improved outcomes. Early detection through regular screenings and awareness campaigns is crucial for successful treatment and improved survival rates.

Material and methods: In this study, the sensor's response of urine from two groups of breast cancer (BC) patients, was assessed using CG-MS technique. The collected data underwent several analyses, including principal component analysis (PCA), Canonical Analysis of Principal Coordinates (CAP), Partial Least Squares - Discriminant Analysis (PLS-DA), and a similarity percentage analysis (SIMPER).

Results: A separation was found between the patients with BC 0-2 BI-RADS group and 3-4 group explaining 31.5% of the variability of the data. Subsequently, a CAP was obtained with a correct classification of 61.9%. Finally, a SIMPER analysis was performed showing the compounds that contribute more the variability.

Conclusions: The present study states a precedent for non-invasive BC screening techniques. For future findings, this will be important to allow more people to get an early BC diagnosis, especially for people with limited access to healthcare services.

Introduction

Breast cancer is defined as an oncologic process in which healthy cells of the mammary gland degenerate into tumors, as a result of the excessive proliferating and multiplying cells, which leads to tumor formation, in this process, these tumors can spread to other organs by hematogenic via as well as lymphatically (1).

According to the tumor characteristics, breast cancer can be classified into different types. This classification depends on the size and extent of the main tumor, the anatomical area where it forms, and the invasiveness of this cell's excessive proliferation has (2).

According to the American Joint Committee on Cancer (AJCC) (52), breast cancer is classified by the TNM system, which is based on three main characteristics described later in this paper.

The letter T refers to the size and extent of the main tumor, which is usually called the primary tumor, the N refers to the number of nearby lymph nodes that have cancer, and finally, the letter M is used to refer to whether cancer has metastasized, which means that cancer has spread from the primary tumor to other parts of the body. This TNM system is an internationally accepted system that is based on retrospective survival analysis in many patient samples representing all stages of the disease. This reflects the clinical evaluation methods and treatments that can be used on a particular study population (2,53).

Therefore, depending on the characteristics of the tumor, breast cancer may be classified into 4 stages, from 0 to IV which are characterized by invasiveness, tumor shape and measure and, organ dissemination.(54–57)

Currently, many different factors can influence the decisions about cancer treatment options, however, one of the most important in this decision-making is the type of cancer and stage of the disease (58).

Recently, studies have shown that the high risk in the development of breast cancer may be due to the combination of different factors, for example, age, (women older than 40 years old), menstrual life over 40 years, and factors like obesity sedentary life, and smoking habit and genetic factors. (59,60)In this regard, in recent years, many studies have shown that increased body mass index (BMI) (≥30 kg/m^2) and/or perimenopausal weight gain might be associated with a higher risk of breast cancer development among postmenopausal women.

Cancer is currently one of the leading causes of death worldwide, in 2019 this disease caused around 8.2 million deaths (5). Also, many studies have reported that the types of cancer that cause the highest number of deaths are mainly lung, liver, stomach, colon, and breast (61). In women, breast cancer is the most common type of cancer, in 2020 an incidence of 46.3% was reported higher than the other most common cancers. (62)

One of the most significant current worldwide concerns is that about half of the world's population has no access to basic health services. Consequently, breast cancer in women is already detected in advanced stages. If current trends continue in the Americas, the number of breast cancer cases is predicted to increase by 34% by 2030. Furthermore, in Latin America and the Caribbean, breast cancer is the most

common type of cancer among women, and represents the second leading cause of mortality.(60)

Therefore, scientists are looking at the development of new tools for breast cancer identification at different stages, there are currently different screening molecular methods focused on “omic” sciences, defined as tools that can study a large number of molecules, that are involved in the physiological functioning of an organism. (20). Omic sciences have helped scientists to have a complete idea of the cause of certain diseases, such as breast cancer.

In recent years the approaches of cancer research involves the study of molecular characteristics that are different between damaged cells and their healthy counterparts, to reveal representative biomarkers from many disease phenotypes, such as breast cancer, which may be usefully as well as possible therapeutic targets.(63) From this perspective, new analytical strategies based on metabolomics have emerged as potential tools that can give a complete appreciation of many biological processes(64,65).

In broad terms, metabolomics is definied as a science dedicated to the global study of metabolites, their composition, and response to interventions or changes in the esurounding area, in cells, tissues, or fluids, which can complement biochemical information obtained from genes and proteins (21,66). Metabolomics is currently undergoing an eminent clinical approach to health, since the identification of biomarkers could reveal the diagnosis of a disease from a simple analysis and a relatively low cost.(6)(67) Many studies have shown the presence of alterations in several metabolic pathways during breast cancer development.(68) These studies have found these modifications using different analytical techniques such as mass spectroscopy (MS) and nuclear magnetic resonance imaging (MRI) (19).

Material and methods

2.1 Subjects and sample collection.

For the present study, 46 breast cancer (BC) patients were recruited from the Department of gynecology and Obstetrics of the Hospital Dr.Ignacio Morones Prieto in San Luis Potosí, México; subjects were women aged from 30 to 83 years in an open prospective observational cohort study design: 2 of them with carcinoma ductal in situ diagnosis, 9 with IA stage ductal carcinoma, 23 with stage II carcinoma (14 IIA and 9 IIB), 11 with III stages (4 IIIA, 6 IIIB, and, 1 IIIC) and 1 with IV stage carcinoma.

This study included women with breast cancer diagnosis detected by physical examination, ultrasonography mammography (with mammograms on a BI-RADS scale of 1 or 2), and/or biopsy. Patients with breast or other types of cancer antecedents were not contempled in this study.

First-morning micturition samples were collected in sterile 100 mL plastic containers and stored at -80 ºC until analyses. [Bioethical committee registration: CONBIOETICA-24-CEI-001-20160427 (96-18)]

2.2 Optimization of the sample preparation procedure

The optimization and selection of sample preparation procedures were performed by testing and comparing the efficiency of solid-phase microextraction (SPME), and liquid-liquid extraction (LLE) techniques. For LLE trials, simple hexane and dichloromethane: acetone extractions were tested, however, it was not possible the obtention of volatile metabolites from urine with this technique.

For SPME fiber selection, tests for comparison and analysis of two SPME fibers (Supelco, Belafonte USA) extractions efficiency were performed.

PDMS (100 mm) and DVB/CAR/PDMS (55 mm/30 mm) stationary phases, were tested and compared. In the end, DBV/CAR/PDMS fiber was selected.

For technique optimization, urine samples from healthy subjects were used as an extraction matrix.

2.3 SPME extraction procedure

Duplicates of urine of each study subject were thawed at room temperature, and aliquots of 4 mL were taken for analyses.

After the addition of 500 μL of NaCl (5%) and 500 μL 0.1 N of HCl solutions, samples were transferred to a headspace glass vial capped with a PTFE septum and an aluminum cap (Agilent, California, USA).

Samples were placed in a heating and stirring plate, the temperature was adjusted to 40 ± 1ºC, stirred with a 0.5 mm × 0.1 mm bar, and after SPME fiber was inserted in the headspace vial and exposed for 30 minutes. After sampling, the SPME fiber was withdrawn into the needle and removed from the headspace vial until GC-MS analyses were performed.

2.4 GC-MS Analyses

An Agilent Technologies (7820A GC System, USA) gas chromatograph equipment was used for analyses, with a G4513A injector/autosampler (USA) coupled to a 5979 series MSD Mass Detector (USA). An Agilent (USA) HP-5MS, with 30 m of length, 0.25 μm stationary phase film thickness, and 0.25 mm of internal diameter column was selected.

After exposure, SPME fiber was inserted into the injection port at 250º C for 10 minutes.

The initial temperature of the oven was 60°C for two minutes, increasing 10ºC/min to 180°C, followed by a 2ºC/min increase to reach 260°C, followed by a 40ºC/min increase to achieve the final temperature of 300ºC. Helium was used as a carrier gas at a constant flow rate of 1 mL/min. GC's total run time was 53 minutes. The identity

of metabolites was determined by comparison of NIST05 spectral library results (with a similarity threshold higher than 40%) included in equipment software with available literature.

2.5 Statistical analysis

The demographic and clinical information of the patients was collected in an EXCEL (Microsoft Excel 2016) database, followed by a multivariate statistical analysis using the PRIMERv6 software with the PERMANOVA package. The variables considered for further analyses were the chromatographic response measured as the area under the curve (AUC) of the analytes of interest. The compounds that were present in less than 20% of the samples analyzed were not considered for further analyses. The identified compounds were considered the most significant those with high responses and with a similarity between the chromatographic profiles of the same group. To establish the relationship between the chemical fingerprints and the study groups (mesaured by the AUC of the chemical compounds), an analysis of variance by permutations (PERMANOVA) was performed. In addition, differences between the variables were established with a non-metric dimensional chart (nDMS) analysis. After, a canonical analysis of principal coordinates (CAP) was carried out through the multivariate data that could best discriminate between the study groups. To evaluate percentage of contribution of each compound for the difference between the groups a similarity percentages (SIMPER) analysis was performed.

Results

Characteristics of BC patients are shown in Table 1. A total of 46 women participated in the study. Twenty-seven of them were not using contraceptives at the time, whereas that 9 of them were using hormonal contraceptive methods. Average values of age

(37 – 61 years), weight (68.20±13.53 kg), height (1.53±0.041m), and BMI (27.33±5.70 Kg/m2) were measured and reported by physicians at the moment of diagnosis.

Histological results show that ductal cancer was the most predominant type, which is present in 74% of the patients.

72 % of the participants in this study didn´t show other comorbidities besides BC. However, 22 % of them, were reported with DM2, 9% with hypertension and DM2.

Compounds identification was performed by gas chromatography coupled to mass spectrometry; responses were obtained from treated urine samples.(Table 2).

Compounds were identified by measuring the intensity of peaks, represented by the area under the curve (AUC) and their retention time (TR).

Urine samples were previously treated, and compound identification was performed by measuring the intensity of peaks, represented by the area under the curve (AUC) and their retention time (TR) through gas chromatography coupled to mass spectrometry.

As result of the analysis, 84 different compounds were identified; however, only 20 of them were considered for statistical analysis due to their similarity percentage to NIST library compounds.

Table 3 shows the compounds identified with their respective similarity percentage and chemical group.

As expected, 60 % of the compounds identified, belong to the hydrocarbon chemical group.

Figure 1.

For statistical analyses, samples were divided into two groups according to their molecular similarities. The first group included those patients who presented 0-II cancer stadiums and, in the second group, patients with III-IV stadiums. In addition,

samples were divided by BI-RADS classification as described below: classifications BI-RADS 0-2 were included in the first group, and the rest in the second group.

The natural variation among metabolites in the study groups' was evaluated through a principal component analysis (PCA). In Figure 2, PCA shows that approximately 31% of the variability can be explained by components 1 (PC1) and 2 (PC2) in the stage analysis. The existence of a real significant difference in the separation observed by PCA was estimated through PERMANOVA test, and results show a significant difference between the different stadium groups ($F= 1.9608$, $p < 0.01$;

Figure 2.

A CAP analysis was performed in order to verify if the previous model allowed 76.09 % of total correct classification, 88% for 0-2 BI-RADS group and 61.90 % for the 3-4 BI-RADS group. (Figure 3).

A SIMPER study was conducted to pinpoint the exact amount that the metabolites contributed to the variations seen in earlier analyses. The results are presented in Table 4. The analysis showed that at least 50% of the contribution was mainly for compounds that belong to the hydrocarbon’s chemical groups.

Table 4. here

Discussion

As it was previously discussed, breast malignant tumors are the most common type of neoplasm in women in the world. Only in 2018, around 2089 million women were diagnosed with breast cancer. (69) (70) In developing countries, such as Latin America and Caribbean countries it has been reported an incidence of 210 100 cases (9.3%), and a mortality of 57984 cases (8.5%) by the year 2020. Mortality, however, represents a major concern in these countries, this trend might be due to the limited

access that most Latin American and Caribbean countries have to healthcare programs. (71)

The majority of prevalent chronic diseases, including breast cancer, are properly diagnosed and treated as part of health programs. Currently, mammography is recognized as the gold standard for diagnosing breast cancer. However, mammography has about a 75-95% sensitivity and an 80-95% specificity, according to Elmore et al. (72) In the majority of cases, mammography assists in the earlier detection of cancer and aids patients in beginning an early course of treatment; yet, access to these healthcare services is very limited in underdeveloped nations. As many authors remark, BC can be influenced by comorbidity with other diseases such as DM2 and hypertension because of the hormonal dysregulation that causes obesity. It has been suggested that hormones such as leptin and adiponectin are closely related to the anti- and pro-inflammatory routes of M1 and M2 BC phenotypes. (73,74)

In recent years, OMICs sciences have been taking an important part in biomedical research for the detection and evaluation of the progression of different kinds of diseases, including BC. Metabolomics is a promising technology that identifies patterns in illnesses and gives in-depth knowledge about the interactions among several biomarkers in biological systems. (75) To effectively target early treatment and enhance patient quality of life, metabolomics methods can be employed in the early detection of BC and for the assessment of the disease's progression. (76)

To reduce costs, metabolomic analyses can be implemented in various types of biofluids such as saliva, serum, plasma, or urine. (25)

In this regard, urine is a biofluid which is highly concentrated in volatile organic compounds (VOMs). In addition, serves as an effective and stress-free method of acquiring biological samples from patients.

VOMs are formed endogenously as a consequence of biological processes related to the apparition and or progression of BC. For this reason, the use of VOMs as biomarkers leads us to the possibility of identifying differences between healthy individuals and BC cancer patients. For example, in the study performed by Tang et al. (año, o número de referencia?), the authors analyzed urine samples of 5 healthy individuals and 75 BC patients, analyzed them by GC-MS and they found differences between metabolic subtypes of ER^+ and ER^- tumors.(77) VOCs profile analysis can give us information on disease progression and therapeutic approaches, e.g., in a study performed by Yu et al. (año), proposing the existence of a relation between chemotherapy treatment response and urinary VOMs in BC patients. They showed 30% of different metabolite concentration changes, in comparison to those that were not receiving a chemotherapeutical treatment, which only showed a 9% of change. (78)

As mentioned previously in this paper, cancer is a very complex disease that during its evolution, promotes changes in normal cells becoming tumoral potential cancerous cells.(79) Primally, cells need energy to develop tumoral characteristics, in this regard cellular environment uses mainly glucose and glutamate to produce energy for cancer cells, this allows to synthesize primary biomolecules such as simple and complex carbohydrates, fatty acids, nucleotides, and amino acids used for cellular proliferation.(80) Literature suggests that in general, altered metabolic energy pathways such as glycolysis and Krebs Cycle are closely associated with cancer.(81) Studies have shown that in cancer, glycolysis is increased because of excessive cell proliferation, known as the “Warburg effect”, its main objective is to produce more energy to maintain the cell proliferation rate in malignant cancer cells.(82) Thus, the altered energetic pathways and hypoxic conditions that cells develop during stages of

malignant cell proliferation led to the release of metabolites that are characteristic of certain malignancies. (83,84)

There are specific VOMs that are closely related to BC development that result to be a part of the biological procedures previously mentioned.

Previous literature reports are coincident with some of the results obtained in this study. Alkanes (notably decane, dodecane, and glycine) are the most predominant group of compounds in these urine samples in BC patients.

Undecane as well, according to Silva et al. (año o número de referencia?) was found in significant concentrations in MCF-7 BC cell lines.(85) These compounds result from the lipid peroxidation of many polyunsaturated fatty acids (PUFA) and phospholipids that compound cell membranes normally. (86,87) In a study performed by Martínez et al. (año o número de referencia?) suggests that different types of undecanes may be responsible for Ca depletion that leads to cytotoxic cellular death.(88) Patients with lung and colon cancer have shown increased amounts of other alkanes such as dodecane, pentane, and methylated alkanes, resulting as a consequence of the altered activity of CYP450.(89) Methylated alkanes are thought to be secondary products of oxidative stress as well. (90,91)

Another interesting fact is that some BC patients that are undergoing chemotherapy develop an activation of limonene and pinene degradation pathways, according to Yu et al. investigations.(92)

In this study, a large number of alkenes was found (hexene, naphthalene, carene, etc.) literature states that these compounds, including isoprene might be linked to the mevalonic acid pathway from cholesterol synthesis.(93)

A different type of compound found in chromatographic analyses was aromatic ones, there are several reports were aromatic chemicals such as toluene or *p*-xylene are

found in cancer patients' samples, however its role in disease ongoing still unknown.(94,95) Despite this, some researchers hypnotized that the amount and type of aromatic compounds such as benzene, toluene, xylene, and 2-5 dimethylfuran, is highly influenced by environmental conditions, Hakim et al. suggested that there is a higher amount of these compounds in smokers vs those patients who don't smoke. (96)

Cyclohexanol also seems to influence the metabolomic profile of breast cancer patients, for example, Silva C. et al. found that Cyclohexanol and phenol were released in BC cell line MCF-7 which suggests that this compound might be a product of late PUFA peroxidation metabolism.(85)

Alcohols are another family of compounds that seem to be relevant in a wide variety of cancer types, however, one of the main sources of alcohol in body fluids is the diet.(96,97) Despite that, alcohols can be detected in urine and other biofluids as a consequence of the pathological progression of some cancers. In addition, alcohols can be products from hydrocarbon metabolism that is closely related to the PUFA peroxidation process. Alcohol metabolism is mainly catalyzed by Dehydrogenases (ADHs) in the cytosol of hepatocytes. (98) Methyl alcohol is converted into acetaldehyde by ADHs which is also known to be a carcinogenic compound. (99) Acetaldehyde is oxidated in mitochondria to acetate and released to the bloodstream later on producing CO_2 species and possibly generating ROS.(99) Ethyl hexanol, on the other hand, is a compound that resulted to be decreased in blood and uirne concentrations with papillary thyroid carcinoma, colorectal cancer, and NCI-H2087 lung cancer cell line.(100) Ethyl hexanol decrease is associated with its important role in its consumption by the cell during tumor proliferation. (100)

Considering all biological processes, amino acids play an essential role in breast cancer development, being their metabolism and essential axis for tumor proliferation.(101) Mikalauskas et al. study showed that there were significant differences in urinary profiles of amino acids among patients before and after undergoing chemotherapy.(101) Other studies have proposed that glycine has also a fundamental role during brain cancer progression, due to its positive correlation with the tumor grade. In breast cancer models, for example, higher levels of glycine have been detected in basal-like breast cancer models in comparison with luminal-like breast cancer models, and higher glycine concentrations have been related to bad disease evolution prognosis.(102,103)

Glycine appears to be a common metabolite present in BC survivors that underwent a chemotherapeutic treatment.(104,105) Glycine is synthesized from the Cho-glycine-betaine pathway or glycolysis intermediates, in addition is (106)an essential component from glutathione and has an indirect effect on free radical formation and oxidative stress induction. (105,107,108)

Conclusion

Our study gave a general perspective on BC development. According to the results obtained in CAP analyses, it was possible to observe a difference on the influence of the metabolites during disease progression. In addition, it was possible to determine some metabolites that mainly influence these differences, for example, glycine due to its role in the formation of glycolysis intermediates. Other alkanes and alkenes were also significant in BC development because of its role during PUFA peroxidation which is very important to start the development of malignant tumors.

Despite all the findings presented here, there are still limitations since this study was performed with a nontargeted metabolomics approach.

As a perspective for this study, it is necessary to evaluate the accurate influence of each metabolite presented in this study, including the targeted metabolomics approach counting controls are needed to validate our findings.

However, this states a precedent for non-invasive BC screening techniques. For future findings, this will be important to allow more people to get an early BC diagnosis, especially for people with limited access to healthcare services.

Tables

Table 1. Anthropometric patient characteristics.

	BI-RADS 0-2 n=25	**BI-RADS 3-6 n=21**		
Parameter	Average ($\pm$SD)	Average($\pm$SD)	T-Value	p-Value
Height (m)	1.56$\pm$0.032	1.6$\pm$0.047	-1.97	0.06
Weighr (Kg)	69.8$\pm$16.3	66.22$\pm$9.85	0.87	0.391
BMI (Kg/m^2)	28.82$\pm$6.7	28.13$\pm$5.2	1.39	0.17
Age	52.4$\pm$14.7	46.3$\pm$10.7	1.58	0.122
	Stages I-II n=11	**Stages III-IV n=34**		
Parametr	Media ($\pm DE$)	Media ($\pm DE$)	Valor-t	Valor de p
Height (m)	1.59±0.05	1.58±0.08	0.56	0.582
Weight (Kg)	71.9±15.2	65.9±12.3	1.32	0.201
BMI (Kg/m2)	28.3±7	28.17±7.5	1.39	0.17
Age	51.1±13	41.8±12.7	0.65	0.519

Table 2. Clinical patient characteristics.

Parameter	N	Percentage (%)
Molecular Type		
Luminal A	22	48
Luminal B	18	39
Triple Negative	2	4
HER-2	1	2
Indeterminado	3	7
Stage		
0	2	4
I	9	20
II	23	50
III	11	24
IV	1	2

Table 3: Compounds found in patient's urine samples.

Compound	Molecular weight (g/mol)	CAS-ID	Chemical group
Limonene	136.24	5989-27-5	Hydrocarbon
Dodecane	170.33	112-40-3	Hydrocarbon
Tetradecane	198.39	629-59-4	Hydrocarbon
Glycine	75.07	56-40-6	Hydrocarbon
Toluene	92.14	108-88-3	Hydrocarbon
1-Methoxycyclohexane	114.19	931-56-6	Ether
Hexane	86.18	110-54-3	Hydrocarbon
Propanoic acid 2-metyl Esther			Esther
Phenol-*p*-tert-butyl	150.22	98-54-4	Alcohol
Heptafluorobutyric acid, n-tetradecyl ester	410.41	7365-36-8	Esther
Naphthalene	128.16.	91-20-3	Hydrocarbon
Carene	136.23	498-15-7	Hydrocarbon
Cyclohexene	82.14	110-83-8	Hydrocarbon
Alpha pinene	136.23	80-56-8	Hydrocarbon
Pentanoic acid, 2,2,4-trimethyl-3-carboxyisopropyl, isobutyl ester	286.41	N/A	Esther
5H-Naphtho[2,3-c]carbazole, 5-methyl-	281.3	100025-44-3	Alcohol
9-Methyl carbazole	181.23	1484-12-4	Carbazole
Cyclohexanol	100.158	108-93-0	Alcohol
Octadecane	254.5	593-45-3	Hydrocarbon
Undecane	156.31	1120-21-4	Hydrocarbon

Table 4. Similarity percentages and accumulated contribution of componds to PCA.

BI-RADS		
Compound	Contrubution %	Accumulated contribution (%)
Limonene	8.49	8.49
Heptafluorobutyric acid, n-tetradecyl ester	8.26	16.75
alpha pinene	8.00	24.75
Tetradecane	7.93	32.68
Propanoic acid 2-metyl Esther	6.96	39.64
Octadecane	6.56	46.2
Ciclohexanol	6.20	52.4
Dodecane	5.94	58.34
Carene	5.64	63.98
Glycine	4.95	68.93
Naphthalene	4.24	73.17
Pentanoic acid, 2,2,4-trimethyl-3-carboxyisopropyl, isobutyl ester	3.97	77.14
metyl carbazole	3.85	80.99
Hexane	3.78	84.77
Ciclohexene	3.77	88.54
Phenol p tert butil	3.67	92.21
Toluene	3.61	95.82
Undecane	2.28	98.1
5H-Naphtho[2,3-c]carbazole, 5-methyl-	1.89	99.99
1-Methoxycyclohexane	0.01	100

Figures and figure legends

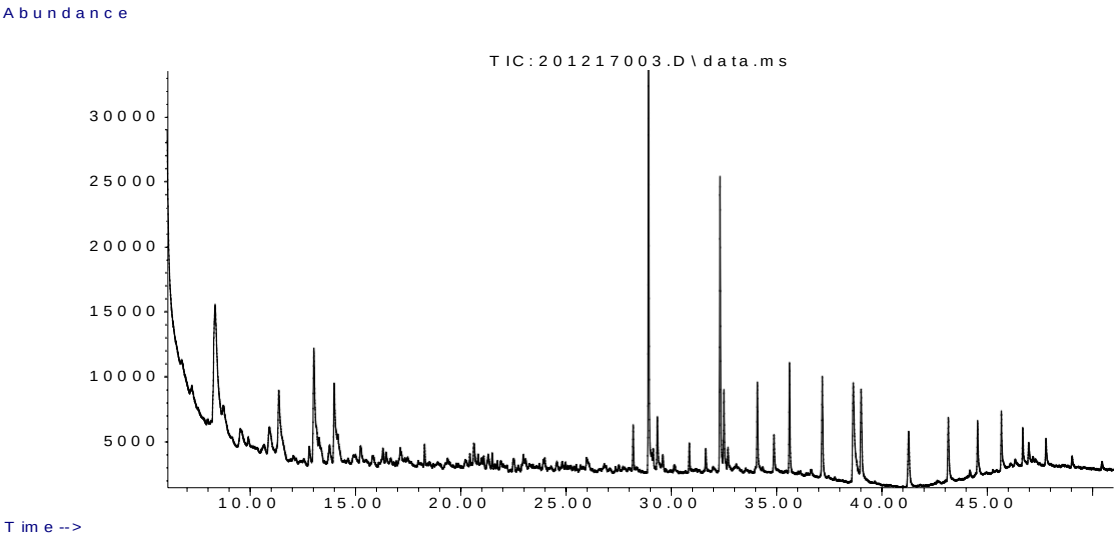


Figure 1. Urine samples chromatogram.

Figure 2.

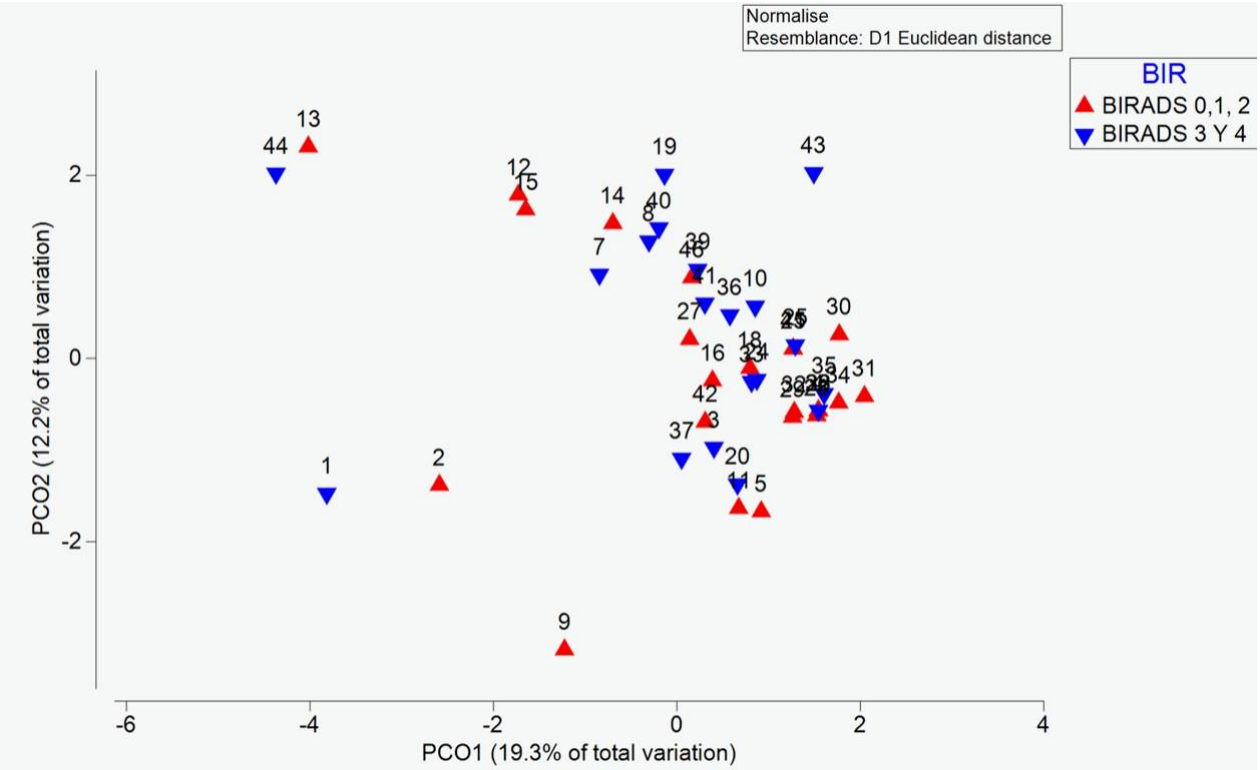


Figure 2. Principal component analysis (PCA) of study populations. Breast Cancer patients with BI-RADS 0,1,2 patients, and BI-RADS 3 and 4 patients. Total of variation 31.5%.

Figure 3.

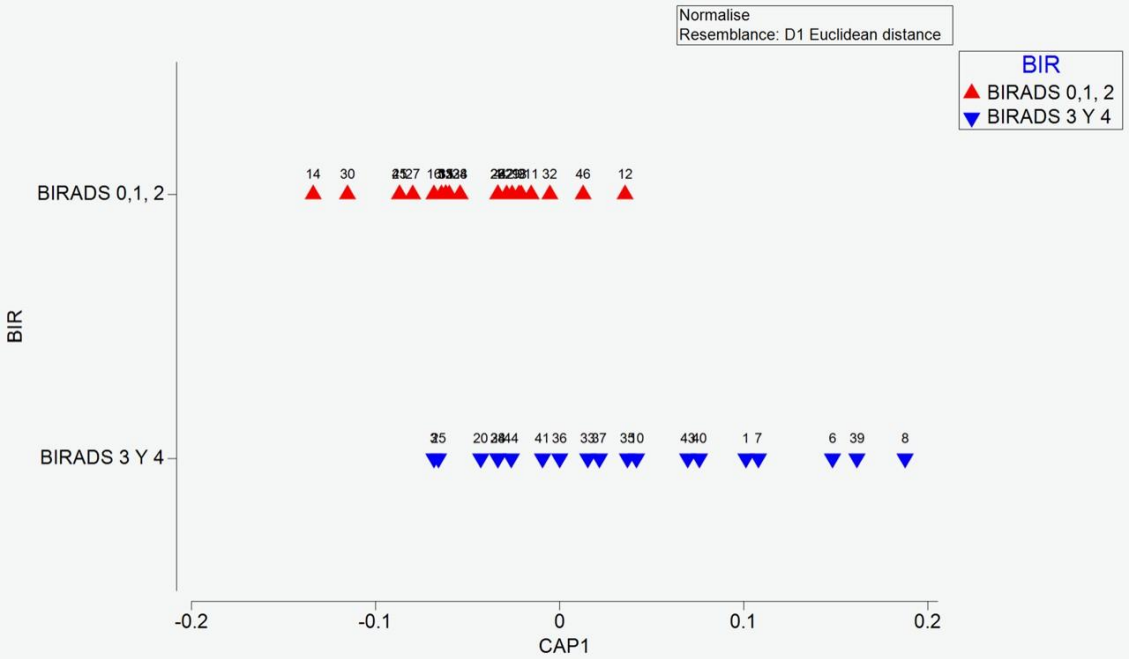


Figure 3. Canonical analysis of principal coordinates (CAP) in different populations. Breast Cancer patients with BI-RADS 0,1,2 patients, and BI-RADS 3 and 4 patients.

References

1. Butcher DT, Alliston T, Weaver VM. REVIEWS A tense situation: forcing tumour progression. 2009;9(FEbRuARy).
2. Eliyatkin N, Yalcin E, Zengel B, Aktaş S, Vardar E. Molecular Classification of Breast Carcinoma: From Traditional, Old-Fashioned Way to A New Age, and A New Way. J Breast Heal. 2015;11(2):59–66.
3. WHO. Breast cancer. 2018.
4. GLOBOCAN. Global Cancer Observatory. 2018.
5. Intitute for Health Metrics and Evaluation (IHME). Data Visualizations. 2019.
6. Coleman MP, Quaresma M, Berrino F, Lutz JM, De Angelis R, Capocaccia R, et al. Cancer survival in five continents: a worldwide population-based study (CONCORD). Lancet Oncol. 2008;9(8):730–56.
7. Mohar-Betancourt A, Reynoso-Noverón N, Armas-Texta D, Gutiérrez-Delgado C, Torres-Domínguez JA. Cancer Trends in Mexico: Essential Data for the Creation and Follow-Up of Public Policies. J Glob Oncol. 2017 Dec;3(6):740–8.
8. Yalaza M, Inan A, Bozer M. Male Breast Cancer. J Breast Heal. 2016 Jan;12(1):1–8.
9. Doede AL, Mitchell EM, Wilson D, Panagides R, Oriá MOB. Knowledge, beliefs, and attitudes about breast cancer screening in Latin America and the Caribbean: An in-depth narrative review. J Glob Oncol. 2018;2018(4).
10. Romieu I, Biessy C, Torres-Mejía G, Ángeles-Llerenas A, Sánchez GI, Borrero M, et al. Project profile: a multicenter study on breast cancer in young women in Latin America (PRECAMA study). Salud Publica Mex. 2019 Oct;61(5, sep-oct):601.

11. Santiago-carrillo MA. Análisis del coste directo de la atención médica y quirúrgica del cáncer de mama . Estudio comparativo entre etapas temprana y tardía en tercer nivel de atención. 2022;91(1):28–33.
12. Knaul FM, Nigenda G, Lozano R, Arreola-Ornelas H, Langer A, Frenk J. Cáncer de mama en México: Una prioridad apremiante. Salud Publica Mex. 2009;51(SUPPL.2):335–44.
13. Secretaria de Salud. NORMA Oficial Mexicana NOM-041-SSA2-2002, Para la prevención, diagnóstico, tratamiento, control y vigilancia epidemiológica del cáncer de mama. 2016. p. 5–6.
14. Uscanga-Sánchez S, Torres-Mejía G, Ángeles-Llerenas A, Domínguez-Malpica R, Lazcano-Ponce E. Indicadores del proceso de tamizaje de cáncer de mama en México: un estudio de caso. Salud Publica Mex. 2014 Sep;56(5):528.
15. Cedolini C, Bertozzi S, Londero AP, Bernardi S, Seriau L, Concina S, et al. Type of Breast Cancer Diagnosis, Screening, and Survival. Clin Breast Cancer. 2014 Aug 1;14(4):235–40.
16. National Institute of Biomedical Imaging and Bioengineering. Ultrasound. 2016.
17. Marmot MG, Altman DG, Cameron DA, Dewar JA, Thompson SG, Wilcox M. The benefits and harms of breast cancer screening: An independent review. Br J Cancer [Internet]. 2013;108(11):2205–40. Available from: http://dx.doi.org/10.1038/bjc.2013.177
18. Li J, Peng Y, Liu Y, Li W, Jin Y, Tang Z, et al. Investigation of potential breath biomarkers for the early diagnosis of breast cancer using gas chromatography–mass spectrometry. Clin Chim Acta. 2014 Sep 25;436:59–67.
19. Zhang A, Sun H, Qiu S, Wang X. Metabolomics in noninvasive breast cancer.

Clin Chim Acta. 2013 Sep;424:3–7.

20. Méndez Rodríguez KB, Santoyo Treviño MJ, Saldaña Villanueva K, Maribel RA, Flores Ramírez R, Pérez Vázquez FJ. Metabolómica como nueva herramienta para el diagnóstico oportuno en enfermedades no transmisibles. Rev Salud Ambient. 2019;19(2):109–15.
21. Klassen A, Faccio AT, Canuto GAB, da Cruz PLR, Ribeiro HC, Tavares MFM, et al. Metabolomics: Definitions and significance in systems biology. In: Advances in Experimental Medicine and Biology. Springer New York LLC; 2017. p. 3–17.
22. Phillips M, Cataneo RN, Ditkoff BA, Fisher P, Greenberg J, Gunawardena R, et al. Prediction of breast cancer using volatile biomarkers in the breath. Breast Cancer Res Treat. 2006 Sep;99(1):19–21.
23. Barash O, Zhang W, Halpern JM, Hua Q-L, Pan Y-Y, Kayal H, et al. Differentiation between genetic mutations of breast cancer by breath volatolomics. Oncotarget. 2015 Dec;6(42).
24. Willmann L, Schlimpert M, Halbach S, Erbes T, Stickeler E, Kammerer B. Metabolic profiling of breast cancer: Differences in central metabolism between subtypes of breast cancer cell lines. J Chromatogr B. 2015;1000:95–104.
25. Gu H, Gowda GAN, Raftery D. Metabolic profiling: Are we en route to better diagnostic tests for cancer? Futur Oncol. 2012;8(10):1207–10.
26. Feizi N, Hashemi-Nasab FS, Golpelichi F, Sabouruh N, Parastar H. Recent trends in application of chemometric methods for GC-MS and GC×GC-MS-based metabolomic studies. TrAC - Trends Anal Chem [Internet]. 2021;138:116239. Available from: https://doi.org/10.1016/j.trac.2021.116239

27. Adebo OA, Oyeyinka SA, Adebiyi JA, Feng X, Wilkin JD, Kewuyemi YO, et al. Application of gas chromatography–mass spectrometry (GC-MS)-based metabolomics for the study of fermented cereal and legume foods: A review. Int J Food Sci Technol. 2021;56(4):1514–34.
28. Jové M, Collado R, Quiles JL, Ramírez-Tortosa M-C, Sol J, Ruiz-Sanjuan M, et al. A plasma metabolomic signature discloses human breast cancer. Oncotarget. 2017 Mar;8(12):19522–33.
29. Denkert C, Bucher E, Hilvo M, Salek R, Orešič M, Griffin J, et al. Metabolomics of human breast cancer: new approaches for tumor typing and biomarker discovery. Genome Med. 2012 Apr;4(4):37.
30. Ash C. Metabolic regulation of microbiota. Science (80-). 2018;362(6418):1016B.
31. Sitter B, Bathen TF, Singstad TE, Fjøsne HE, Lundgren S, Halgunset J, et al. Quantification of metabolites in breast cancer patients with different clinical prognosis using HR MAS MR spectroscopy. NMR Biomed. 2010;23(4):424–31.
32. Kominsky DJ, Klawitter J, Brown JL, Boros LG, Melo J V., Eckhardt SG, et al. Abnormalities in glucose uptake and metabolism in imatinib-resistant human BCR-ABL-positive cells. Clin Cancer Res. 2009;15(10):3442–50.
33. Min HK, Lim S, Chung BC, Moon MH. Shotgun lipidomics for candidate biomarkers of urinary phospholipids in prostate cancer. Anal Bioanal Chem. 2011;399(2):823–30.
34. Lin B. 乳鼠心肌提取 HHS Public Access. Physiol Behav. 2017;176(12):139–48.
35. Min HK, Lim S, Chung BC, Moon MH. Shotgun lipidomics for candidate

biomarkers of urinary phospholipids in prostate cancer. Anal Bioanal Chem [Internet]. 2011 Jan 17;399(2):823–30. Available from: http://link.springer.com/10.1007/s00216-010-4290-7

36. Phillips M, Altorki N, Austin JHM, Cameron RB, Cataneo RN, Kloss R, et al. Detection of lung cancer using weighted digital analysis of breath biomarkers. Clin Chim Acta [Internet]. 2008 Jul;393(2):76–84. Available from: https://linkinghub.elsevier.com/retrieve/pii/S0009898108001083

37. Ratiu IA, Ligor T, Bocos-Bintintan V, Mayhew CA, Buszewski B. Volatile Organic Compounds in Exhaled Breath as Fingerprints of Lung Cancer, Asthma and COPD. J Clin Med [Internet]. 2020 Dec 24;10(1):32. Available from: https://www.mdpi.com/2077-0383/10/1/32

38. Paredi P, Kharitonov SA, Barnes PJ. Analysis of Expired Air for Oxidation Products. Am J Respir Crit Care Med [Internet]. 2002 Dec 15;166(supplement_1):S31–7. Available from: https://www.atsjournals.org/doi/10.1164/rccm.2206012

39. Heunks LMA. Respiratory muscle function and free radicals: from cell to COPD. Thorax [Internet]. 2000 Aug 1;55(8):704–16. Available from: https://thorax.bmj.com/lookup/doi/10.1136/thorax.55.8.704

40. Patti GJ, Yanes O, Siuzdak G. Metabolomics: the apogee of the omics trilogy. Nat Rev Mol Cell Biol [Internet]. 2012 Apr 22;13(4):263–9. Available from: http://www.nature.com/articles/nrm3314

41. Ellis DI, Goodacre R. Metabolic fingerprinting in disease diagnosis: biomedical applications of infrared and Raman spectroscopy. Analyst [Internet]. 2006;131(8):875. Available from: http://xlink.rsc.org/?DOI=b602376m

42. Capuano R, Catini A, Paolesse R, Di Natale C. Sensors for Lung Cancer

Diagnosis. J Clin Med [Internet]. 2019 Feb 11;8(2):235. Available from: http://www.mdpi.com/2077-0383/8/2/235

43. Broza YY, Mochalski P, Ruzsanyi V, Amann A, Haick H. Hybrid Volatolomics and Disease Detection. Angew Chemie Int Ed [Internet]. 2015 Sep 14;54(38):11036–48. Available from: https://onlinelibrary.wiley.com/doi/10.1002/anie.201500153

44. Phillips M, Cataneo RN, Saunders C, Hope P, Schmitt P, Wai J. Volatile biomarkers in the breath of women with breast cancer. J Breath Res [Internet]. 2010 Jun 1;4(2):026003. Available from: https://iopscience.iop.org/article/10.1088/1752-7155/4/2/026003

45. Broza YY, Haick H. Nanomaterial-based sensors for detection of disease by volatile organic compounds. Nanomedicine [Internet]. 2013 May;8(5):785–806. Available from: https://www.futuremedicine.com/doi/10.2217/nnm.13.64

46. Dragonieri S, Annema JT, Schot R, van der Schee MPC, Spanevello A, Carratú P, et al. An electronic nose in the discrimination of patients with non-small cell lung cancer and COPD. Lung Cancer [Internet]. 2009 May;64(2):166–70. Available from: https://linkinghub.elsevier.com/retrieve/pii/S0169500208004194

47. Peng G, Hakim M, Broza YY, Billan S, Abdah-Bortnyak R, Kuten A, et al. Detection of lung, breast, colorectal, and prostate cancers from exhaled breath using a single array of nanosensors. Br J Cancer [Internet]. 2010 Aug 20;103(4):542–51. Available from: http://www.nature.com/articles/6605810

48. Licht J-C, Grasemann H. Potential of the Electronic Nose for the Detection of Respiratory Diseases with and without Infection. Int J Mol Sci [Internet]. 2020 Dec 10;21(24):9416. Available from: https://www.mdpi.com/1422-

0067/21/24/9416

49. Töreyin ZN, Ghosh M, Göksel Ö, Göksel T, Godderis L. Exhaled Breath Analysis in Diagnosis of Malignant Pleural Mesothelioma: Systematic Review. Int J Environ Res Public Health [Internet]. 2020 Feb 10;17(3):1110. Available from: https://www.mdpi.com/1660-4601/17/3/1110

50. van der Sar IG, Wijbenga N, Nakshbandi G, Aerts JGJ V., Manintveld OC, Wijsenbeek MS, et al. The smell of lung disease: a review of the current status of electronic nose technology. Respir Res [Internet]. 2021 Dec 17;22(1):246. Available from: https://respiratory-research.biomedcentral.com/articles/10.1186/s12931-021-01835-4

51. Díaz de León-Martínez L, Rodríguez-Aguilar M, Gorocica-Rosete P, Domínguez-Reyes CA, Martínez-Bustos V, Tenorio-Torres JA, et al. Identification of profiles of volatile organic compounds in exhaled breath by means of an electronic nose as a proposal for a screening method for breast cancer: a case-control study. J Breath Res [Internet]. 2020 Oct 1;14(4):046009. Available from: https://iopscience.iop.org/article/10.1088/1752-7163/aba83f

52. Edge, Stephen B; Byrd, David R; Compton, Carolyn C; Fritz, April G; Greene, Frederick L; Trotti A. AJCC CANCER STAGING MANUAL. 2010.

53. Vranic S, Schmitt F, Sapino A, Costa JL, Reddy S, Castro M, et al. Apocrine carcinoma of the breast: A comprehensive review. Histol Histopathol. 2013;28(11):1393–409.

54. Alexander B. Olawaiye, Thomas P. Baker, M. Kay Washington DGM. The New (Version 9) American Joint Committee on Cancer Tumor, Node, Metastasis Staging for Cervical Cancer. CA A Cancer J Clin. 2021;71(4).

55. . Magny SJ, Shikhman R K AL. Breast Imaging Reporting and Data System. StatPearls Publishing. 2021;

56. Aure MR, Vitelli V, Jernström S, Kumar S, Krohn M, Due EU, et al. Integrative clustering reveals a novel split in the luminal A subtype of breast cancer with impact on outcome. Breast Cancer Res. 2017;19(1):1–18.

57. Mercado CL. BI-RADS Update. Radiol Clin North Am. 2014 May 1;52(3):481–7.

58. Tanaka K, Tsumura H. Eighth edition of the American Joint Committee on Cancer staging system for soft tissue sarcoma of the trunk and extremity: in search of a better staging system. Ann Transl Med. 2019;7(S1):S11–S11.

59. Centro Nacional de Equidad de Género y Salud Reproductiva. Información Estadística Cáncer de Mama. 2016.

60. Maggi N, Gazzarata R, Ruggiero C, Lombardo C, Giacomini M. Cancer precision medicine today: Towards omic information in healthcare systems. Tumori. 2019;105(1):38–46.

61. American Institute for Cancer Research. Worldwide cancer data. 2018.

62. International Agency for Research on Cancer. Cancer Today. 2018.

63. Frigolet ME, Gutiérrez-Aguilar R. Ciencias “ómicas”, ¿cómo ayudan a las ciencias de la salud? Rev Digit Univ. 2017;18(7):0–15.

64. Oeffinger KC, Fontham ETH, Etzioni R, Herzig A, Michaelson JS, Shih YCT, et al. Breast cancer screening for women at average risk: 2015 Guideline update from the American cancer society. JAMA - J Am Med Assoc. 2015;314(15):1599–614.

65. Sahora K, Fernández-del Castillo C. Surgical Management of Mucinous Cystic Neoplastic Lesions of the Pancreas. Pancreat Cancer, Cyst Neoplasms

Endocr Tumors Diagnosis Manag. 2015;24(February):243–8.

66. Souza-Silva ÉA, Reyes-Garcés N, Gómez-Ríos GA, Boyaci E, Bojko B, Pawliszyn J. A critical review of the state of the art of solid-phase microextraction of complex matrices III. Bioanalytical and clinical applications. TrAC - Trends Anal Chem [Internet]. 2015;71:249–64. Available from: http://dx.doi.org/10.1016/j.trac.2015.04.017

67. Wengert GJ, Helbich TH, Kapetas P, Baltzer PA, Pinker K. Density and tailored breast cancer screening: practice and prediction – an overview. Acta Radiol Open. 2018;7(9):205846011879121.

68. Merino MJ. Breast. Gattuso's Differ Diagnosis Surg Pathol. 2021;419:721–62.

69. Nardin S, Mora E, Varughese FM, D'Avanzo F, Vachanaram AR, Rossi V, et al. Breast Cancer Survivorship, Quality of Life, and Late Toxicities. Front Oncol. 2020;10(June):1–6.

70. Smolarz B, Zadrożna Nowak A, Romanowicz H. Breast Cancer—Epidemiology, Classification, Pathogenesis and Treatment (Review of Literature). Cancers (Basel). 2022;14(10):1–27.

71. Thomas CA. Cancer Today - IARC, 150. Cancer Today - IARC, 150 69372 Lyon CEDEX 08, France. 2020.

72. Elmore JG, Armstrong K, Lehman CD, Fletcher SW. CLINICIAN ' S CORNER Screening for Breast Cancer. 2005;293(10):1245–56.

73. Atoum MF, Alzoughool F, Al-Hourani H. Linkage Between Obesity Leptin and Breast Cancer. Breast Cancer Basic Clin Res. 2020;14.

74. Russo L, Lumeng CN. Properties and functions of adipose tissue macrophages in obesity. Immunology. 2018;155(4):407–17.

75. Silva C, Perestrelo R, Silva P, Tomás H, Câmara JS. Breast cancer

metabolomics: From analytical platforms to multivariate data analysis. A review. Vol. 9, Metabolites. 2019.

76. Lécuyer L, Bala AV, Deschasaux M, Bouchemal N, Triba MN, Vasson MP, et al. NMR metabolomic signatures reveal predictive plasma metabolites associated with long-term risk of developing breast cancer. Int J Epidemiol. 2018;47(2):484–94.

77. Tang X, Lin CC, Spasojevic I, Iversen ES, Chi JT, Marks JR. A joint analysis of metabolomics and genetics of breast cancer. Breast Cancer Res. 2014;16(4):1–15.

78. Yu L, Jiang C, Huang S, Gong X, Wang S, Shen P. Analysis of urinary metabolites for breast cancer patients receiving chemotherapy by CE-MS coupled with on-line concentration. Clin Biochem [Internet]. 2013;46(12):1065–73. Available from: http://dx.doi.org/10.1016/j.clinbiochem.2013.05.049

79. Ross JS, Hatzis C, Symmans WF, Pusztai L, Hortobágyi GN. Commercialized Multigene Predictors of Clinical Outcome for Breast Cancer. Oncologist. 2008;13(5):477–93.

80. Sreelakshmi M, George JG, Mishra R, Aneesh TP. Targeting apoptosis by inhibiting cMET amplification due to EGFR resistance using benzofuran derivatives. Res J Chem Environ. 2019;23(11):11–8.

81. Abbaszadeh Z, Çeşmeli S, Biray Avcı Ç. Crucial players in glycolysis: Cancer progress. Gene. 2020;726:144158.

82. Ganapathy-Kanniappan S, Geschwind J-F. Tumor glycolysis as a target for cancer therapy: Mol Cancer [Internet]. 2013;12(1):1–11. Available from: https://www.ncbi.nlm.nih.gov/pmc/articles/PMC4223729/pdf/1476-4598-12-152.pdf

83. Liberti M V., Locasale JW. The Warburg Effect: How Does it Benefit Cancer Cells? Trends Biochem Sci [Internet]. 2016;41(3):211–8. Available from: http://dx.doi.org/10.1016/j.tibs.2015.12.001

84. Weljie AM, Jirik FR. Hypoxia-induced metabolic shifts in cancer cells: Moving beyond the Warburg effect. Int J Biochem Cell Biol. 2011;43(7):981–9.

85. Silva CL, Perestrelo R, Silva P, Tomás H, Câmara JS. Volatile metabolomic signature of human breast cancer cell lines. Sci Rep. 2017;7(November 2016):1–8.

86. Pereira J, Porto-Figueira P, Cavaco C, Taunk K, Rapole S, Dhakne R, et al. Breath analysis as a potential and non-invasive frontier in disease diagnosis: An overview. Metabolites. 2015;5(1):3–55.

87. Phillips M, Cataneo RN, Greenberg J, Gunawardena R, Naidu A, Rahbari-Oskoui F. Effect of age on the breath methylated alkane contour, a display of apparent new markers of oxidative stress. J Lab Clin Med. 2000;136(3):243–9.

88. Martinez NJ, Rai G, Yasgar A, Lea WA, Sun H, Wang Y, et al. A high-throughput screen identifies 2,9-diazaspiro[5.5]undecanes as inducers of the endoplasmic reticulum stress response with cytotoxic activity in 3D glioma cell models. PLoS One. 2016;11(8):1–19.

89. Schallschmidt K, Becker R, Jung C, Bremser W, Walles T, Neudecker J, et al. Comparison of volatile organic compounds from lung cancer patients and healthy controls - Challenges and limitations of an observational study. J Breath Res [Internet]. 2016;10(4):46007. Available from: http://dx.doi.org/10.1088/1752-7155/10/4/046007

90. Phillips M, Cataneo RN, Saunders C, Hope P, Schmitt P, Wai J. Volatile biomarkers in the breath of women with breast cancer. J Breath Res.

2010;4(2).

91. Phillips M, Cataneo RN, Greenberg J, Grodman R, Gunawardena R, Naidu A. Effect of oxygen on breath markers of oxidative stress. Eur Respir J. 2003;21(1):48–51.

92. Yu L, Jiang C, Huang S, Gong X, Wang S, Shen P. Analysis of urinary metabolites for breast cancer patients receiving chemotherapy by CE-MS coupled with on-line concentration. Clin Biochem. 2013 Aug 1;46(12):1065–73.

93. Cailleux A, Cogny M, Allain P. Blood isoprene concentrations in humans and in some animal species. Biochem Med Metab Biol. 1992;47(2):157–60.

94. Janfaza S, Khorsand B, Nikkhah M, Zahiri J. Digging deeper into volatile organic compounds associated with cancer. Biol Methods Protoc. 2019;4(1):1–11.

95. Smith D, Wang T, Spanel P. On-line, simultaneous quantification of ethanol, some metabolites and water vapour in breath following the ingestion of alcohol. Physiol Meas. 2002;23(3):477–89.

96. Hakim M, Broza YY, Barash O, Peled N, Phillips M, Amann A, et al. Volatile organic compounds of lung cancer and possible biochemical pathways. Chem Rev. 2012;112(11):5949–66.

97. Zhang X, Lv Y, Wang H, Tao X, Zhang X. Classification identification of abalone flavoring liquids based on metal sensor array. Carpathian J Food Sci Technol. 2016;8(2):107–12.

98. You L, Tong R, Li M, Liu Y, Xue J, Lu Y, et al. Ac c ep te us cr ip t Ac c us cr t. Nat Biotechnol [Internet]. 2014;3(3):1–16. Available from: https://doi.org/10.1016/j.jbiotec.2020.05.008%0Ahttps://doi.org/10.1016/j.celrep.2019.01.024%0Ahttps://doi.org/10.1007/s13258-019-00830-

w%0Ahttp://dx.doi.org/10.1016/j.cell.2014.05.010%0Ahttp://dx.doi.org/10.1038/nbt.2842%0Ahttp://dx.doi.org/10.1038/mt.20

99. Patel V B. Molecular Aspects of Alcohol and Nutrition. 2015.

100. a. S, W. F, T. M, C. A, J. S, W. M, et al. Release of volatile organic compounds from the lung cancer cell line NCI-H2087 in vitro. Anticancer Res [Internet]. 2009;29(1):419–26. Available from: http://ovidsp.ovid.com/ovidweb.cgi?T=JS&PAGE=reference&D=emed9&NEWS=N&AN=2009107121

101. Mikalauskas S, Mikalauskiene L, Bruns H, Nickkholgh A, Hoffmann K, Longerich T, et al. Dietary glycine protects from chemotherapy-induced hepatotoxicity. Amino Acids. 2011;40(4):1139–50.

102. Moestue SA, Borgan E, Huuse EM, Lindholm EM, Sitter B, Børresen-Dale AL, et al. Distinct choline metabolic profiles are associated with differences in gene expression for basal-like and luminal-like breast cancer xenograft models. BMC Cancer. 2010;10:1–12.

103. Fujimoto. Metabolite Profiling Identifies a Key Role for Glycine in Rapid Cancer Cell Proliferation. Bone. 2008;23(1):1–7.

104. Cao MD, Giskeødegård GF, Bathen TF, Sitter B, Bofin A, Lønning PE, et al. Prognostic value of metabolic response in breast cancer patients receiving neoadjuvant chemotherapy. BMC Cancer [Internet]. 2012;12(1):39. Available from: http://www.biomedcentral.com/1471-2407/12/39

105. Peeling J, Sutherland G. High-Resolution 1H NMR spectroscopy studies of extracts of human cerebral neoplasms. Magn Reson Med. 1992;24(1):123–36.

106. Platten M, Wick W, Van Den Eynde BJ. Tryptophan catabolism in cancer: Beyond IDO and tryptophan depletion. Cancer Res. 2012;72(21):5435–40.

107. Scioscia KA, Snyderman CH, Wagner R. Altered serum amino acid profiles in head and neck cancer. Nutr Cancer. 1998;30(2):144–7.
108. Farwick M, Siewe RM, Kramer R. Glycine betaine uptake after hyperosmotic shift in Corynebacterium glutamicum. J Bacteriol. 1995;177(16):4690–5.

COMPROBANTE DE ENVÍO DE PUBLICACIÓN

Fecha: 27 de junio de 2023, 12:02:57 GMT-6
Para: FRANCISCO JAVIER PEREZ VAZQUEZ <javier.perez@uaslp.mx>
Asunto: Submission to Archives of Medical Research - manuscript number
Responder-Para: Archives of Medical Research <support@elsevier.com>

This is an automated message.

Manuscript Number: ARCMED-D-23-00529
Assessing Volatile Breath Patterns: Distinguishing Breast Cancer Patients from Healthy Women using Electronic Nose

Dear Francisco Perez,

Your above referenced submission has been assigned a manuscript number: ARCMED-D-23-00529.

To track the status of your manuscript, please log in as an author at https://www.editorialmanager.com/arcmed/, and navigate to the "Submissions Being Processed" folder.

Thank you for submitting your work to this journal.

Kind regards,
Archives of Medical Research

More information and support

You will find information relevant for you as an author on Elsevier's Author Hub: https://www.elsevier.com/authors

OTROS PRODUCTOS

NARODOWA AGENCJA WYMIANY AKADEMICKIEJ

Warsaw, on 30 August 2021

DECISION
OF THE DIRECTOR OF THE POLISH NATIONAL AGENCY FOR ACADEMIC EXCHANGE (NAWA)
No. BPN/FRC/2021/1/00011/DEC/1

Based on art. 104 of the act of June 14th 1960 the Code of Administrative Procedure (i.e. Journal of Laws of 2018, item 2096, as amended, hereinafter referred to as the CAP) and art. 25 paragraph 1 of the Act of July 7th, 2017 on the Polish National Agency for Academic Exchange (Journal of Laws, item 1530, as amended, hereinafter referred to as the PNAAE), art. 323 paragraph 1 point 4 of the act of July 20th 2018 the Higher Education and Science Law (Journal of Laws of 2018, item 1668, as amended, hereinafter referred to as the HESL) and based on art. 324 paragraph 1 sentence 2 point 2 of the HESL, after considering the application submitted by: Maria José Santoyo under Exchange Program for students and scientists as part of bilateral cooperation – offer for incoming students and scientists:

I hereby grant

To Ms.
Maria José Santoyo
born on 24.11.1991
citizen of Mexico

1) the scholarship of the Polish National Agency for Academic Exchange in the maximum amount of 13200 PLN (in words: thirteen thousand two hundred and 0/100 zlotys), for scientific stay in the academic year 2021/22;
2) the right to undertake and perform the above mentioned studies with exemption from fees referred to in art. 324 par. 1 of the HESL

subject to the completion of the following action - accepting the Agreement within 30 days from the date the Agreement is made available in the NAWA ICT system.

Failure to meet the above mentioned deadline shall be tantamount to resignation from the conclusion of the Agreement

Substantiation

The Agency refrained from substantiating the decision due to the fact that it fully approves the applicant's request.
This decision was issued under the authority of the Director of the Polish National Agency for Academic Exchange.

FINAL REPORT RESEARCH STAY:

It is known that people are exposed to several kinds of environmental pollutants through different exposure routes in complex scenarios, that is why understanding the nature and magnitude of exposure and the negative consequences on human health have become a critical issue in public health matters. Additionally, because of the increasing knowledge of exposure routes and their effects on human health, that is the reason why there are needed new tools to analyze the increasingly complex exposure scenarios and help policymakers in the decision-making.

Due to these reasons, this investigation aimed to develop a method for the determination of selected biomarkers and their metabolites from popular xenobiotics in urine samples absorbed in the gel of disposable diapers from infants and children without micturition control. It is an innovative project which results can bring many advantages for future diagnosis of babies and small children in a noninvasive and nonstress full way.

The aim for this internship is divided into three principal tasks, which will be described in the following report.

1.- Develop a method for unaltered urine extraction from the absorbent material of baby diapers.

This part of the research aimed to find a suitable salt that can extract unaltered liquid urine from the polymer of the diaper.

The salts that were tested were: Sodium chlorine, sodium carbonate, potassium acetate, sodium nitrate, ammonium oxalate, potassium bromate, and ammonium bromate.

To extract a considerable amount of urine from the diaper material, trials with different types of salts to prevent the polymer and the urine from a gel structure formation and extract the liquid urine were performed.

The procedure of salting the urine consisted of adding to the urine 0.5 g of the selected salt to the sorbent of the diaper, followed by the filtration of the sample with a nylon filter in a syringe to obtain the maximum amount possible of liquid urine in an Eppendorf tube.

After the extraction, the properties of the urine were evaluated (to determine if it was unaltered or not).

Conventional urine strips were used to determine if the composition of urine was modified during the salting process.

It was added approx. 100 µL of the extracted urine to the test strip and color changing of the parameters were evaluated with changes in RGB values of each parameter. The evaluated parameters were as follows: leucocytes, nitrites, specific gravity, urobilinogen, proteins, pH, blood, ketones, bilirubin, and glucose.

For the determination of the RGB values, a smartphone application was used.

At the end of the performance of these experiments, our investigation group evaluated which salt had the minor modifications in the urine test strip parameters.

It was sodium chloride the salt the appears to produce less changes in the color of the parameters in the strips.

2.-Develop an analytical methodology for dialkyl phosphates (DAPs) determination and quantification in urine samples.

There is a wide variety of xenobiotics that can affect newborns' and children’s health. These products are mainly pesticides.

For this part of the project, it was decided to focus on organophosphate pesticides metabolites (dialkyl phosphates) identification and quantification in urine samples.

Organophosphate pesticides are well known for their endocrine disruptor properties and are associated with neurological development alterations in children and newborns.

These pesticides are mainly metabolized by Cytochrome P 450 in the human liver and converted into highly hydrophilic products that can be excreted in the urine.

The main products that can be found in urine are different types of dialkyl phosphates such as Dimethylphosphate (DMP), Diethylphosphate (DEP), Dimethylthiophpsphate (DMTP) Diethylthiophosphate (DETP), Dimethyldithiophosphate (DMDTP), and Diethyldithiophosphate (DEDTP).

For the identification of these metabolites, two different analytical techniques:g chromatography coupled to mass spectrometry (GC-MS) and liquid chromatography and tandem mass spectrometry (LC-MS/MS) were used, which will be described in the next section of this report.

2.1 GC-MS procedure development.

For DAPs extraction from urine, it was reproduced a previous methodology using hexane and dichloromethane (DCM) as extraction solvents. As DAPs are highly hydrophilic compounds, they are not suitable for GC determination, however, it was performed a derivatization procedure with pentafluorobenzyl bromide (PFBB) to convert these metabolites into less hydrophilic compounds, suitable for GC-MS analysis.

2.2 LC MS/MS procedure development.

In parallel to GC analysis, an LC-MS/MS -based procedure for the compound’s identification without the derivatization process was developed.

In the first place, the optimization of the mass spectrometry conditions was performed, followed by optimization for LC conditions such as column, buffer, solvent, and gradient selection.

Once chromatographic and mass spectrometer conditions were set, the selection for the extraction procedure of DAPs from human urine was performed.

Conclusions

During this internship, it was possible to develop a methodology for urine extraction from baby diapers. In addition, it was possible to establish a mythology useful for DAPs extraction from human urine and the identification of them by LC-MS/MS.

This investigation will allow researchers in the future to have a reference procedure for the identification and analysis of organophosphates metabolites in newborns and infants urine.

In the future the analysis of these compounds in real newborn samples is expected.

In parallel, Gdańsk University of Technology researchers and students are developing techniques for the identification and quantification of other different groups of xenobiotics, such as phthalates and plastics.

In further studies is vitally important to monitor the exposure to environmental xenobiotics which can represent a hazardous component for the human health especially during fetal and embryonic stages and during the first years of life o children. Understanding the mechanisms of exposure of toxic substances, their metabolism, and their effects on the body, may lead in the future to the development of a policy for sustainable and responsible use of substances that can be considered harmful to children and for general population.

However, there are still many challenges to overcome, such as the efficient, and non-invasive collection of biological samples for the determination of long-term health

effects of exposure to environmental hazardous xenobiotics as well as the investigation of possible synergistic effects of combinations of them. Future efforts may include the analysis and search for new exposure, and effect biomarkers, especially in non-invasive samples such as urine, sweat or breath.

Finally, as a personal accomplishment, this internship allowed me to build professional relationships and collaborations with experimented researchers on the field of analytical chemistry and toxicology that will allow forming future academic cooperation between our countries and academic institutions, thus creating networks that will allow us to strengthen and expand knowledge, for future applications in these areas.

GDAŃSK UNIVERSITY OF TECHNOLOGY

RESEARCH UNIVERSITY

L.dz. 36/WCH-BR/2022

Wydział Chemiczny PG/ *Faculty of Chemistry, GUT*

Gdańsk, 28.02.2022

ZAŚWIADCZENIE O ODBYTYM POBYCIE BADAWCZYM /
RESEARCH STAY CONFIRMATION CERTIFCATE

realizowanego w ramach programu wymiany osobowej studentów i naukowców w ramach współpracy bilateralnej – oferta przyjazdowa Narodowej Agencji Wymiany Akademickiej /
within the exchange program for students and scientists as part of bilateral cooperation – offer for incoming students and scientists of Polish National Agency for Academic Exchange

Instytucja przyjmująca Stażystę/kę na pobyt badawczy / *Institution accepting the research student*
Katedra Chemii Analitycznej/ *Department of Analytical Chemistrii*
Wydział Chemiczny / *Faculty of Chemistry*
Politechnika Gdańska / *Gdańsk University of Technology*
ul. G. Narutowicza 11/12
80-233 Gdańsk
nazwa i adres Instytucji przyjmującej na staż / *name and address of the accepting institution*

potwierdza odbycie stażu przez / *research stay confirmation by*
María José Santoyo Treviño
imię i nazwisko Stażysty/ki / *name and surname of the research student*

w terminie / *duration* : **od *4.09.2021* do *26.02.2022* / *from 4.09.2021 to 26.02.2022***
5 miesięcy/22 dni --- *5months/22days*

temat badań/zakres stażu / *subject matter/scope of research*:
Determination of selected biomarkers of exposure to popular xenobiotics in samples of children's urine absorbed in disposable children's diapers".

Podczas stażu opiekę nad Stażystą/ką sprawował /
Academic supervisor of the student during the research stay
dr hab. inż. Justyna Płotka-Wasylka, prof. PG
Imię i nazwisko Opiekuna stażu / *name and surname of the academic supervisor*

2022 -02- 28

.....
data, podpis i pieczęć Jednostki/Instytucji przyjmującej na staż /
date, signature and stamp of the Unit/Institute accepting for the research stay

.....
data, podpis i pieczęć Działu Międzynarodowej Współpracy Akademickiej PG
date, signature and stamp of the International Relations Office at Gdańsk Tech

POLITECHNIKA GDAŃSKA
ul. G. Narutowicza 11/12
80-233 Gdańsk

tel. (+48 58) 347-23-94
e-mail: agata.kot-wasik@pg.edu.pl
www.pg.edu.pl

Mexican Journal of Oncology
Gaceta Mexicana de Oncología

ORIGINAL ARTICLE

Evaluation of sialic acid concentrations and its association with the use of hormonal contraceptives

*Andrea G. Rodríguez-López[1], Mónica L. Córdova-Serrano[2], Ana L. González-Bolaños[2], Karen B. Téllez-Blanco[2], Laura S. Marín-Jaureguí[3], Ma. José Santoyo-Treviño[4], and Aida C. Hernández-Arteaga[4]**

[1]Physical-Mathematical Department; [2]Faculty of Chemical Sciences; [3]Division of Molecular and Translational Medicine, Research Center of Health Science and Biomedicine; [4]Coordination for Innovation and Application of Science and Technology. Universidad Autónoma de San Luis Potosí, S.L.P., Mexico*

Abstract

Objective: *To evaluate the relationship between the use of HC and the concentration of Sialic acid (CSA) detected in the saliva of 120 women from San Luís Potosí, Mexico.* **Methods:** *Three groups were evaluated: control group, HC intake shorter than 4 years, and HC intake longer than 4 years. A questionnaire was applied, anthropometric measures were taken, and collection of saliva samples was carried out. The samples were analyzed with Raman-SERS, for which silver nanoparticles were synthesized, and the measurement of SA was held.* **Results:** *The concentrations were divided into three SA groups: normal (≤ 4 mg/dL), risk (> 4-7 mg/dL), and altered (> 7 mg/dL). The maximum CSA was detected, which was 9.2 mg/dL. All the volunteers who used the intrauterine system (IUS) and most of the ones who used the pills and subdermal implant (SI) presented risk and altered concentrations.* **Conclusions:** *That there is an association between the HC and the CSA, presenting a higher concentration, those volunteers who used IUS, contraceptive pills, and SI.*

Keywords: *Breast cancer. Hormonal contraceptive. Sialic acid. SERS. Raman spectroscopy.*

Evaluación de las concentraciones de ácido siálico y su asociación con el uso de anticonceptivos

Resumen

Objetivo: *Evaluar la relación entre el uso de anticonceptivos hormonales (AH) y las concentraciones de ácido siálico (AS) detectado en saliva de mujeres de San Luis Potosí, México.* **Métodos:** *Se formaron tres grupos: grupo control, ingesta de AH menor a 4 años e ingesta de AH mayor a 4 años. Se aplicó un cuestionario, se tomaron medidas antropométricas y se llevó a cabo la recolección de muestras de saliva. Las muestras se analizaron con Raman-SERS (surface-enhanced Raman spectroscopy); para lo cual se sintetizaron nanopartículas de plata y se llevó a cabo la medición de concentración de AS, comparando los resultados obtenidos con una curva de calibración.* **Resultados:** *Se dividieron en tres grupos las concentraciones de AS: normal (≤ 4 mg/dl), riesgo (> 4-7 mg/dl) y alterado (> 7 mg/dl). La concentración máxima de AS detectado fue de 9.2 mg/dl. Todas las voluntarias que utilizan sistema intrauterino (SIU) y la mayoría de las que utilizan píldoras e implante subdérmico (IS), presentaron concentraciones de riesgo y valores de AS alterados.* **Conclusiones:** *Existe una asociación entre el uso de AH y las concentraciones de AS, presentando una mayor concentración de AS aquellas voluntarias que utilizan SIU, píldoras anticonceptivas e IS.*

Palabras clave: *Cáncer de mama. Ácido siálico. Anticonceptivo hormonal. SERS. Espectroscopia Raman.*

Correspondence:
*Aida C. Hernández-Arteaga
E-mail: aida.arteaga@uaslp.mx

Date of reception: 04-06-2022
Date of acceptance: 23-06-2022
DOI: 10.24875/j.gamo.22000081

Available online: 02-09-2022
Gac Mex Oncol. 2022;21(3):89-98
www.gamo-smeo.com

Science of the Total Environment 836 (2022) 155339

Contents lists available at ScienceDirect

Science of the Total Environment

journal homepage: www.elsevier.com/locate/scitotenv

Review

End-of-life management of single-use baby diapers: Analysis of technical, health and environment aspects

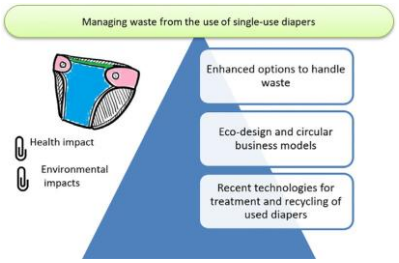
Justyna Płotka-Wasylka [a,*], Patrycja Makoś-Chełstowska [b], Aleksandra Kurowska-Susdorf [c], María José Santoyo Treviño [d], Sergio Zarazúa Guzmán [d], Heba Mostafa [e], Mauro Cordella [f]

[a] Department of Analytical Chemistry, Faculty of Chemistry, Gdańsk University of Technology, 11/12 G. Narutowicza St., 80-233 Gdańsk, Poland
[b] Department of Process Engineering and Chemical Technology, Faculty of Chemistry, Gdańsk University of Technology, 11/12 G. Narutowicza Street, 80-233 Gdańsk, Poland
[c] The Naval Academy, Faculty of Humanities and Social Sciences, 69 Śmidowicza Street, 81-127 Gdynia, Poland
[d] Laboratory of Toxicology, Faculty of Chemistry, Autonomous University of San Luis Potosí, Av. Manuel Nava 6, Zona Universitaria, CP 78210 San Luis Potosí, Mexico
[e] Analytical Chemistry Department, Faculty of Pharmacy, Cairo University, Cairo 11562, Egypt
[f] TECNALIA, Basque Research and Technology Alliance (BRTA), Astondo Bidea, Edificio 700, 48160 Derio, Spain

HIGHLIGHTS

- Environmental and health concerns can be associated with baby diapers.
- Single use and disposable diapers are analysed, focusing on materials and waste.
- Enhanced design, use and waste management options are provided.
- Systemic challenges and future perspectives are described.
- Holistically safe(r) and sustainable diapering processes are addressed.

GRAPHICAL ABSTRACT



ARTICLE INFO

Editor: Dimitra A Lambropoulou

Keywords:
Chemical safety
Ecodesign
Environmental impacts
Reusable baby diapers
Single-use baby diapers
Waste management

ABSTRACT

Single-use baby diapers belongs to an important group of products used in the parenting journey because of their high performance and convenience. Single-use baby diapers are normally thrown away after one-time use, resulting in a waste management problem. The goal of this paper was to better understand main environmental concerns of different types of diapers and address how to reduce them, with a special consideration of waste management strategies and user behaviour practices. Furthermore, health and environmental hazards potentially associated with materials included in diapers, or substances formed from diapers during the waste treatment stage, are also analysed (e.g., phthalates, pesticides, dioXins, pesticides). Three main types of baby diapers have been analysed: single-use baby diapers, reusable baby diapers, and biodegradable single-use diapers. Each type of diaper comes with technical characteristics and environmental concerns and challenges, which are discussed in this paper to support the development of measures for the safe(r) and sustainable design, use and end of life management of baby diapers.

Contents

* Corresponding author.
E-mail address: juswasyl@pg.edu.pl (J. Płotka-Wasylka).

http://dx.doi.org/10.1016/j.scitotenv.2022.155339
Received 2 March 2022; Received in revised form 13 April 2022; Accepted 13 April 2022
Available online 20 April 2022

Science of the Total Environment 856 (2023) 158859

Contents lists available at ScienceDirect

Science of the Total Environment

journal homepage: www.elsevier.com/locate/scitotenv

How pesticides affect neonates? - Exposure, health implications and determination of metabolites

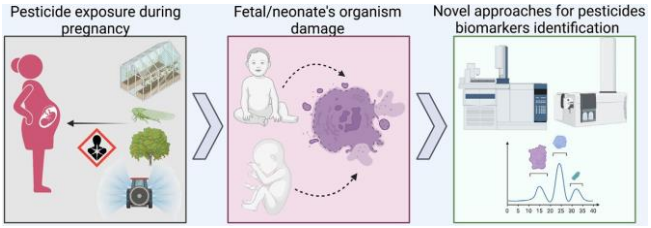
María José Santoyo Treviño [a,e], Marina Pereira-Coelho [b], Andrea Guadalupe Rodríguez López [c], Sergio Zarazúa [e], Luiz Augusto dos Santos Madureira [b], Tomasz Majchrzak [d,*], Justyna Płotka-Wasylka [d,*]

[a] Coordinación para la innovación y aplicación para la Ciencia y la Tecnología, Mexico
[b] Department of Chemistry, Federal University of Santa Catarina, 88040-900 Florianópolis, SC, Brazil
[c] Departamento Físico-Matemático, Facultad de Ingeniería, Universidad Autónoma de San Luis Potosí, Mexico
[d] Department of Analytical Chemistry, Faculty of Chemistry and BioTechMed Center, Gdańsk University of Technology, 11/12 G. Narutowicza Str., 80-233 Gdańsk, Poland
[e] Laboratorio de Neurotoxicología, Facultad de Ciencias Químicas, Universidad Autónoma de San Luis Potosí, Mexico

HIGHLIGHTS

- Exposure to pesticides and their metabolites starts in fetal period through placenta.
- Neonates exposure to pesticides can lead to life-long abnormalities and diseases.
- Pesticide determination in neonates biofluids should differ from those from adults.
- Novel analytical approaches focus on low volume analysis and high throughput.
- Untargeted analysis gives information for toXicity assessment in biological systems.

GRAPHICAL ABSTRACT



ARTICLE INFO

Editor: Dimitra A Lambropoulou

Keywords:
Xenobiotics
Pesticides
Heavy metals
Persistent organic pollutants
Children
Fetus
Health
Analytical techniques

ABSTRACT

This review covers key information related to the effects of pesticides on fetal and child health. All humans are exposed to environmental toXicants, however child's health, due to their high vulnerability, should be of special concern. They are continuously exposed to environmental Xenobiotics including a wide variety of pesticides, and other pollutants. These compounds can enter the child's body through various routes, both during fetal life, in the first days of life with breast milk, as well as during environmental exposure in later years of life. Consequently, in the body, some of them are metabolized and excreted with urine or faces, while others accumulate in tissues causing toXic effects. This review will provide information on the types of pesticides, their pathways of uptake and metabolism in children's bodies. Determination of the impact of them on children's organism performance is possible through effective identification of these compounds and their metabolites in children's tissues and biofluids. Therefore, the main procedures for the determination of pesticides are reviewed and future trends in this field are indicated. We believe that this comprehensive review can be a good starting place for the future readers interested in the impact of environmental Xenobiotics on the health of children as well as the aspects relates with the analytical methods that can be used for analysis and monitoring of these pollutants in children's tissues and biofluids.

* Corresponding authors.
E-mail addresses: tomasz.majchrzak@pg.edu.pl (T. Majchrzak), juswasyl@pg.edu.pl (J. Płotka-Wasylka).

http://dx.doi.org/10.1016/j.scitotenv.2022.158859
Received 29 July 2022; Received in revised form 14 September 2022; Accepted 15 September 2022
Available online 17 September 2022

Metabolómica como nueva herramienta para el diagnóstico oportuno en enfermedades no transmisibles

Metabolómica como uma nova ferramenta para o diagnóstico oportuno em doenças não transmissíveis

Metabolomics as a new tool for timely diagnosis in noncommunicable diseases

Karen Beatriz Méndez Rodríguez, María José Santoyo Treviño, Kelvin Saldaña Villanueva, Maribel Rodríguez Aguilar, Rogelio Flores Ramírez, Francisco Javier Pérez Vázquez

CONACYT Research Fellow, Coordinación para la Innovación y Aplicación de la Ciencia y la Tecnología (CIACYT). Universidad Autónoma de San Luis Potosí.

Cita: Méndez Rodríguez KB, Santoyo Treviño MJ, Saldaña Villanueva K, Rodríguez Aguilar M, Flores Ramírez R, Pérez Vázquez FJ. Metabolómica como nueva herramienta para el diagnóstico oportuno en enfermedades no transmisibles. Rev. salud ambient. 2019; 19(2):109-115.

Recibido: 5 de febrero de 2019. **Aceptado:** 12 de agosto de 2019. **Publicado:** 15 de diciembre de 2019.

Autor para correspondencia: Francisco Javier Pérez Vázquez.
Correo e: francisco.perezvaz@gmail.com
CONACYT Research Fellow, Coordinación para la Innovación y Aplicación de la Ciencia y la Tecnología (CIACYT), Universidad Autónoma de San Luis Potosí, Avenida Sierra Leona No. 550, Colonia Lomas Segunda Sección, CP 78210, San Luis Potosí, SLP, México.

Financiación: Consejo Nacional de Ciencia y Tecnología a través del proyecto "Indicadores metabolómicos para la detección temprana de enfermedades ocupacionales en trabajadores precarios ESTRATEGIA STOP (Salud en el trabajo para Ocupaciones Precarias) /FOSEC SS/IMSS/ISSSTE (A3-S-38681).

Declaración de conflicto de intereses: Los autores declaran que no existen conflictos de intereses que hayan influido en la realización y la preparación de este trabajo.

Declaraciones de autoría: Todos los autores contribuyeron al diseño del estudio y la redacción del artículo. Asimismo, todos los autores aprobaron la versión final.

Resumen

En los últimos años, el empleo de las ciencias "ómicas" en la optimización del diagnóstico temprano y no invasivo de diferentes tipos de enfermedades, ha cobrado gran importancia, principalmente en enfermedades crónico-degenerativas; por otro lado, también han sido empleadas para la evaluación de la exposición a determinados contaminantes ambientales, infecciones bacterianas y virales, entre otras aplicaciones. Entre las ciencias ómicas destacan principalmente la genómica, transcriptómica, proteómica, y actualmente ha cobrado gran relevancia la metabolómica. Gracias a los múltiples avances tanto en la genómica como en la proteómica, se han logrado establecer algunos elementos para el posible diagnóstico de enfermedades crónico-degenerativas. Sin embargo, aún no se han dilucidado por completo los cambios metabólicos que se llevan a cabo durante los procesos patológicos de distintas enfermedades. Por esta razón, la metabolómica ha surgido como una disciplina con una aplicación muy importante para la identificación de componentes oportunos en el desarrollo de algunas enfermedades.

Palabras clave: biomarcador; metabolómica; ómica; salud.

Resumo

Nos últimos anos, o emprego das ciências "ómicas", na otimização do diagnóstico precoce e não invasivo de diferentes tipos de doenças tem ganhado grande relevância, principalmente em doenças crónico-degenerativas. Por outro lado, também têm sido utilizadas para a avaliação da exposição a determinados contaminantes ambientais, infecções bacterianas e virais, entre outras aplicações. Entre as ciências ómicas, destacam-se principalmente a genómica, transcriptómica, proteómica e atualmente, tem adquirido grande relevância a metabolómica. Graças aos múltiplos avanços tanto na genómica quanto na proteómica, tem-se conseguido estabelecer alguns elementos para o possível diagnóstico de doenças crónico-degenerativas. Entretanto, ainda não se esclareceu completamente as mudanças metabólicas que ocorrem durante os processos patológicos de diferentes doenças.

molecules

MDPI

Review

Nanosorbents as Materials for Extraction Processes of Environmental Contaminants and Others

María José Santoyo Treviño [1], Sergio Zarazúa [1] and Justyna Płotka-Wasylka [2,*]

[1] Laboratory of Neurotoxicology, Faculty of Chemistry, Autonomous University of San Luis Potosí, Av. Manuel Nava 6, Zona Universitaria, San Luis Potosí 78210, Mexico; mjst1191@gmail.com (M.J.S.T.); sergio.zarazua@uaslp.mx (S.Z.)

[2] Department of Analytical Chemistry, Faculty of Chemistry and BioTechMed Center, Gdańsk University of Technology, 11/12 G. Narutowicza St., 80-233 Gdańsk, Poland

* Correspondence: juswasyl@pg.edu.pl or plotkajustyna@gmail.com

Abstract: The aim of this work focuses on the application of nanomaterials (NMs) in different sorptive extraction techniques for the analysis of organic contaminants from environmental samples of distinct matrix compositions. Without any doubt, the integration of specific NMs such as carbonaceous nanomaterials, magnetic nanoparticles (MNPs), metal–organic frameworks (MOFs), silica nanoparticles, and ion-imprinted NPs with solid-phase extraction techniques counting *d*-SPE, solid-phase microextraction (SPME), and stir bar sorptive extraction (SBSE) impact on the improvements in analytical performance. The application of NMs as sorbents in the extraction of organic pollutants in environmental samples allows for providing better sensitivity, repeatability, reproducibility, and reusability.

Keywords: nanomaterials; nanoparticles; extraction techniques; sorbent; sample preparation

Citation: Santoyo Treviño, M.J.; Zarazúa, S.; Płotka-Wasylka, J. Nanosorbents as Materials for Extraction Processes of Environmental Contaminants and Others. *Molecules* **2022**, *27*, 1067. https://doi.org/10.3390/molecules27031067

Academic Editor: Simone Morais

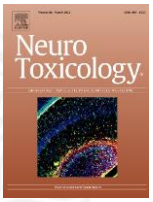
Received: 21 December 2021
Accepted: 2 February 2022
Published: 5 February 2022

Publisher's Note: MDPI stays neutral with regard to jurisdictional claims in published maps and institutional affiliations.

1. Introduction

Much research on the application of nanomaterials in different scientific and industrial fields is being performed in areas such as oil processing, sensors, water treatment, building materials, catalysis, food, and construction [1]. NMs have also become an object of interest in the field of analytical chemistry activities [2]. Over the last two decades, NMs have been widely applied as sorbents for the extraction of environmental contaminants [2]. This is due to their unique properties such as large surface area and fast adsorption capability, and they also present high selectivity and efficiency for environmental pollutants. The most popular NMs used as sorbents for these purposes are magnetic nanoparticles (MNPs), nano-based metal–organic frameworks (N-MOFs), silica nanoparticles (SiNPs), carbon nanomaterials (CNMs), and nano-imprinted polymers (NIPs). These NMs are utilized for the isolation and pre-concentration of environmental pollutants in such techniques as solid-phase extraction (SPE), solid-phase microextraction (SPME), magnetic solid-phase extraction (MSPE), and dispersive solid-phase extraction (DSPE). It is worth mentioning that the application of these nanomaterial sorbents impacts the extraction efficiency of these techniques.

This review summarizes the basic features of analytical options that can be used for the extraction and preconcentration of pollutants in environmental samples based on the integration amongst different kinds of NMs and several types of microextraction techniques. In addition, environmental samples characterized by different matrix compositions are considered. The issue of the article focusing on the application of nanosorbents as materials for extraction processes was highly treated in the literature [3–6]. However, this review contains essential and the most significant information for researchers who deal with the complex issues connected with environmental samples and can be an easy start for future researchers in this area. In the present article, future authors will find not only information on the types of NMs applied in sample preparation processes, but also general knowledge



NeuroToxicology

Certificate of Reviewing

Awarded for 1 review in March 2023
presented to

MARÍA SANTOYO

in recognition of the review contributed to the journal

The Editors of NeuroToxicology

Science of the Total Environment

Certificate of Reviewing

Awarded for 2 reviews between March 2023 and June 2023
presented to

MARÍA SANTOYO

in recognition of the review contributed to the journal

The Editors of Science of the Total Environment

ELSEVIER

Trends in Analytical Chemistry

Certificate of Reviewing

Awarded since April 2022 (1 review)
presented to

MARÍA SANTOYO

in recognition of the review contributed to the journal

The Editors of Trends in Analytical Chemistry

Elsevier Reviewer RECOGNITION

BRITISH COUNCIL

Certificate

This is to certify that

María José Santoyo Treviño

participated as a Mentee in the Mentoring in Action - Women and Girls in STEM Online programme. The mentoring programme took place between August and November 2022 and consisted of a minimum of 12 hours' mentoring.

Awarded by

British Council, Inova Consultancy, WiTEC

Signed

Brian Douglas Young
Country Director Mexico, British Council

Signed

Marina Larios
Director, Inova Consultancy

December 2022

WiTEC EUROPEAN ASSOCIATION FOR WOMEN IN SCIENCE ENGINEERING & TECHNOLOGY

inova consultancy

PRESENTACIÓN DE POWER POINT

Identificación de biomarcadores de riesgo en el desarrollo de cáncer de mama mediante el uso de herramientas metabolómicas.

M. C. María José Santoyo Treviño

1

1

Comité Tutelar

Co-directores:
Dr. Sergio Zarazúa Guzmán
Dr. Francisco Javier Pérez Vázquez

Asesores internos:
Dr. Rogelio Flores Ramírez
Dr. Fernando Díaz-Barriga Martínez

Asesor externo:
Dr. Jorge Alegría Torres

Asesor clínico:
Dr. José de Jesús Zermeño Nava

2

2

Jurado

Presidente de sinodales
Dra. Leticia Yáñez Estrada

Sinodales:
Dra. Patricia Cossio Torres
Dra. Saray Aranda Romo

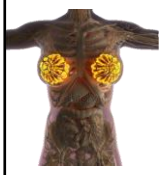
Sinodales externos:
Dr. Jorge Alegría Torres
Dr. José de Jesús Zermeño Nava

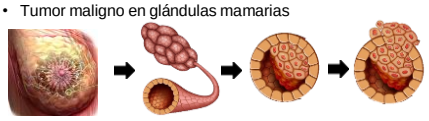
3

3

Cáncer de mama

- Tumor maligno en glándulas mamarias





- Puede diseminarse fuera de la mama a través de los

Vasos sanguíneos

Nódulos linfáticos

Darci T. Butcher et al 2009.

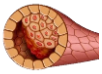
4

4

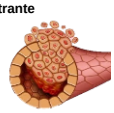
Clasificación

Grado de infiltración

- *IN SITU*



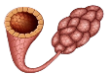
- Infiltrante



Anatomía

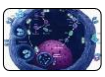
- Ductal

- Lobulillar



Proteínas expresadas

- Luminal A
- Luminal B
- HER-2 enriched
- Triple negativo
- Normal Like



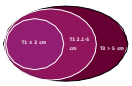
Shuster, Gallimidi et al. 2011.

6

6

Clasificación TNM

T: Extensión y tamaño del tumor

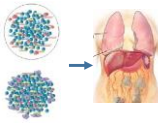


T4: El tumor se ha extendido a órganos vecinos

N: Grado de propagación en nódulos linfáticos adyacentes

N0: No existe en invasión de nódulos linfáticos.
N1: Nódulos axilares ( )
N2: Nódulos axilares o nódulos mamarios (ó)
N3: Nódulos axila res y nódulos mamarios (ó)
Nódulos infraclaviculares ( ) o supraclaviculares ( )

M: Metástasis



M0: Sin signos de cáncer en partes del cuerpo lejanas al tejido mamario.
M1: Cáncer invasivo en partes lejanas al tejido mamario

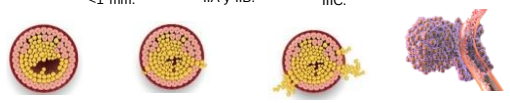
Catherine F. Cowella et al. 2013, breastcancer.org, American Joint Committee on Cancer (AJCC)

8

8

Estadios del cáncer de mama

0	I	II	III	IV
• Cáncer no invasivo • CDIS • Enfermedad de Paget.	• Carcinoma infiltrante • Categorías IA y IB • Células invasoras <1 mm.	• Tumores 2 a 5 cm • De 1 a 3 nódulos linfáticos • Categorías IIA y IIB.	• Tumor <5 cm • 4-9 nódulos linfáticos. • Categorías IIIA, IIIB y IIIC.	• Cáncer extendido a órganos lejanos.



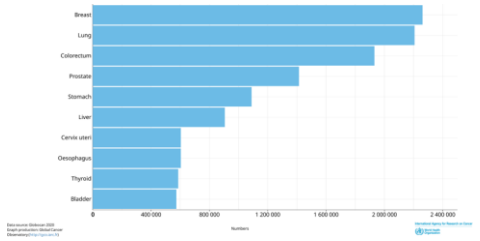
Peter A Banks,, Thomas L Bollen, et al 2012, Herreros Villanueva et al. 2013

9

9

Epidemiología

Nuevos casos hasta el año 2020



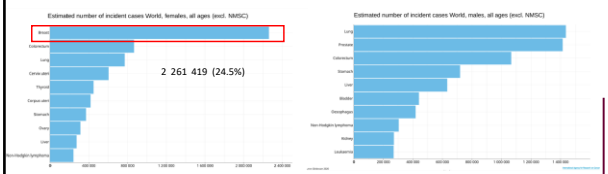
GLOBOCAN 2020.

10

10

Epidemiología

Nuevos casos en el año 2020



El 70% ocurre en países vías de desarrollo

Las cifras aumentan por el bajo acceso a la detección oportuna.

GLOBOCAN 2020, IHME, 2020.

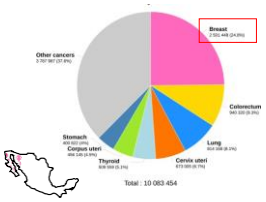
11

11

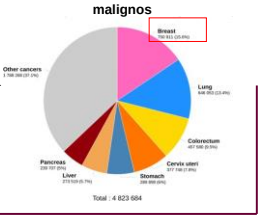
Epidemiología

- **Primera causa de muerte en mujeres de 30 a 54 años de edad.**

Nuevos casos en el año 2020



Muertes asociadas a tumores malignos



1990 a 2015, INEGI-SSA/DGIS, SSA., GLOBOCAN 2018

13

13

Impacto económico

- Estadio avanzado mayor costo en estudios de laboratorio-gabinete, cirugías, hospitalizaciones e interconsultas.
- Costo promedio de atención por paciente es de $99280.38 MXN en estadio inicial.
- Costo promedio de atención por paciente es de $148 023.60.38 MXN en estadio avanzado.

Montiel-Jarquín, et. al (2023). Análisis del coste directo de la atención médica y quirúrgica del cáncer de mama. Estudio comparativo entre etapas temprana y tardía en tercer nivel de atención. *Cirugía y cirujanos*, *91*(1), 28-33.

14

14

Diagnóstico

Autoexploración.
- **Mujeres mayores de 20 años.**

Examen clínico.
- **Exploración clínica de la mama.**
- **Historia clínica.**
- **Mujeres mayores de 25 años.**

Pruebas histopatológcas.

Pruebas de imagen.
- **Ultrasonido.**
- **Ecografía.**
- **Mastografía (mayores de 40 años).**

Torres Mejía Gabriela, Detección temprana y manejo integral del cáncer de mama, 2011.

15

15

Diagnóstico

- **Categorías de evaluación Breast Imaging Reporting and Data System (BI-RADS).**

BI-RADS 0	Evidencia insuficiente
BI-RADS 1	Hallazgo negativo
BI-RADS 2	Hallazgo benigno
BI-RADS 3	Hallazgo posiblemente benigno
BI-RADS 4	Anormalidad sospechosa
BI-RADS 5	Hallazgo maligno
BI-RADS 6	Malignidad demostrado

Li, Peng et al. 2014 16

16

Desventajas

- **Sensibilidad (75-85%) y especificidad (55-98%).**

- **Pruebas de imagen son costosas.**

- **119 especialistas/ 100,000 habitantes**
- **50% en las grandes ciudades.**

- **Cobertura 20% de la población.**

Torres Mejía Gabriela, Detección temprana y manejo integral del cáncer de mama, 2011, INCAN 2014,, ENT, SSA 2018. 17

17

Diagnóstico

Falta de acceso a servicios de salud e información

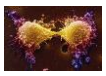
Pocas realizan autoexploración o pruebas de gabinete

Exposición a sustancias químicas

SALUD
SECRETARÍA DE SALUD

C-MIC Colegio Mexicano para la Investigación del Cáncer

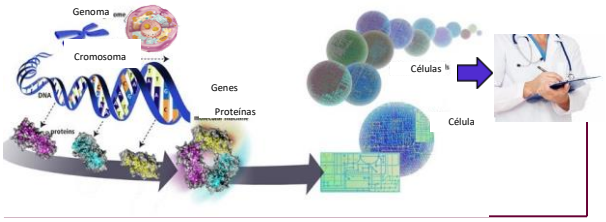
- Cobertura Universal en Salud



ENSANUT, 2012 18

18

Diagnóstico



Scharft 2015, Frente Genet 19

19

Metabolómica

Célula sana

Características moleculares

Célula dañada

Huella metabolómica representativa del fenotipo de la enfermedad

Scharft 2015, Frente Genet 20

20

Metabolómica

Disciplina dedicada al estudio de los metabolitos

Respuesta a las intervenciones o cambios en el ambiente

Células, tejidos o fluidos

Característicos de una patología

Patti GJ, et al. 2012, Davis, B (April 2005). "Growing pains for metabolomics". *The Scientist*. **19** (8): 25–28. 21

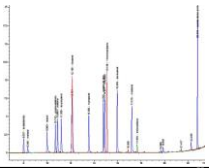
31

21

Metabolómica

METABOLÓMICA NO DIRIGIDA	METABOLÓMICA DIRIGIDA
Estudio del perfil metabolómico y búsqueda de metabolitos diferenciales	Búsqueda y cuantificación de metabolitos específicos

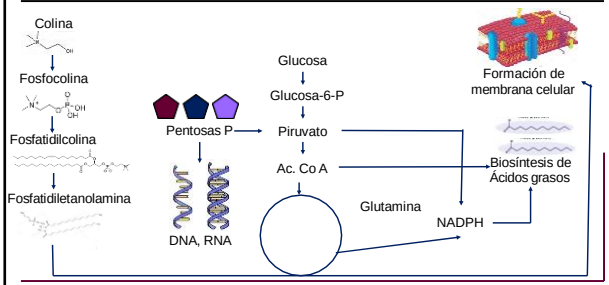
La metabolómica podría proporcionar biomarcadores de una enfermedad



Patti GJ, et al. 2012

22

Rutas metabólicas alteradas



Carsten Denkert, et al. 2012, Catherine Oakman et.al, 2011

23

Justificación

Aumento de cáncer de mama en México
Métodos de diagnóstico:
- Costosos
- Baja cobertura o alcance

OPS, México no tiene la capacidad para invertir en tecnologías de diagnóstico.

Desarrollo de métodos más: → • Sensibles, • Específicos

Para el tamizaje de riesgo de desarrollo de la enfermedad.

Metabolómica:
Marcadores de riesgo para desarrollo de cáncer de mama.

24

Objetivo general

Identificar patrones de metabolitos para el riesgo en el desarrollo de cáncer de mama mediante el uso de herramientas metabolómicas

25

Hipótesis

Los patrones metabolómicos de mujeres con diagnóstico de cáncer de mama presentan diferencias con respecto al de las mujeres sanas y con diferentes estadios de la enfermedad

26

Objetivos específicos

1. Estandarizar las técnicas de nariz electrónica y cromatografía de gases-masas para identificar patrones de metabolitos en muestras de aliento exhalado y orina.
2. Calcular la sensibilidad y especificidad del método estadístico empleado en el análisis de los patrones obtenidos de la nariz electrónica, a través de la elaboración de curvas ROC.

27

Objetivos específicos

3. Identificar patrones de metabolitos en orina y aliento exhalado mediante metabolómica no dirigida, empleando cromatografía de gases-masas y nariz electrónica.

4. Comparar los patrones metabolómicos de compuestos orgánicos volátiles globales entre mujeres sanas, pacientes con cáncer de mama y pacientes con cáncer de mama que cursan los estadios I-II y III-IV.

28

28

Metodología

29

Lugar de realización

Departamento de ginecología y obtetricia del hospital central Dr. Ignacio Morones Prieto

Coordinación para la Innovación y aplicación de la Ciencia y la Tecnología (CIACYT)

30

30

Diseño del estudio

Aprobado por parte del Comité de Bioética de

HOSPITAL CENTRAL
DR. IGNACIO MORONES PRIETO

31

31

Diseño del estudio

- Estudio piloto
- Transversal
- N= 30 Individuos por grupo

Babbie E.Fundamentos de la investigación social (3ª), Thomson editores, México (2000), pp. 232-256, José Antonio García-García, et. al, Cálculo del tamaño de la muestra en investigación en educación médicaInv Ed Med 2013;2(8):217-224

32

32

Grupos de estudio

Mujeres sanas	Mujeres con CaM
Mayores de 18 años	Mayores de 18 años
Clínicamente sanas	Diagnóstico de cáncer de mama.

33

33

Grupos de estudio

Criterios de inclusión

CaM

1.- Mujeres.
2.- Mayores de 18 años.
3.- Diagnóstico de cáncer de mamá en estadios (0-IV) con estudios confirmatorios (mastografía/ultrasonido/diagnóstico histopatológico).

Sanas

1.- Mujeres
2.- Mayores de 18 años de edad.

34

Grupos de estudio

Criterios de no inclusión

1.- Mujeres que se encuentren bajo tratamiento hormonal o quimioterapia.
2.- Mujeres que hayan padecido otro tipo de cáncer.
3.- Mujeres fumadoras.

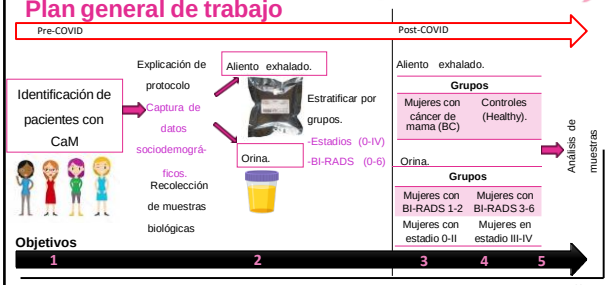
Criterios de no eliminación

1.- Que decidan ya no participar en el estudio.
2.- Imposibilidad para la toma de muestra.

35

Metodología

Plan general de trabajo



36

Metodología

Identificación de pacientes con cáncer de mama

HC hospital de referencia. Pacientes con alta sospecha de CaM.

- Exploración clínica

- Mastografía

- BI-RADS

- Biopsia

Médico realiza el diagnóstico CaM

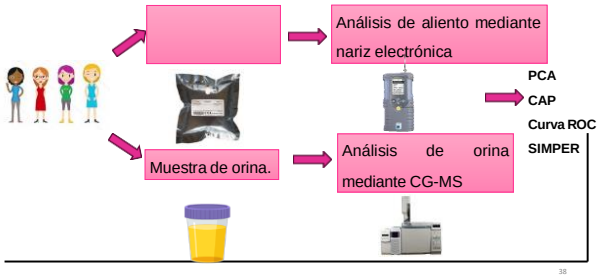
Controles

Invitación para participar en el estudio.

Tanto las pacientes como las mujeres sanas fueron invitadas a participar por parte del personal de salud de HC (médicos residentes, internos, estudiantes de posgrado).

37

Metodología



38

Metodología

Recolección de muestra de aliento exhalado.

Procesamiento y análisis de muestra

39

Metodología

Estandarizar la técnica para identificación de patrones de metabolitos en muestras de aliento exhalado y orina en mujeres de cada uno de los grupos.

Temperatura ambiente durante 2 minutos.

Incubación durante 20 minutos a 37°C.

Muestra incubada se inyecta directamente en la nariz electrónica.

Nariz electrónica *Cyranose®* 320

Lectura

Análisis de datos

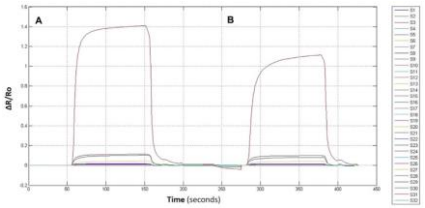
CONDICIONES DEL EQUIPO
Línea base: 40 segundos (120 ml/min)
Lectura de muestra: 90 segundos
Limpieza de sensores e inyectores: 80 segundos (180 ml/min)

40

40

Metodología

Análisis de COVs por nariz electrónica cyranose® 320



Sensograma característico de pacientes con CaM y mujeres sanas.
A) Sensograma de paciente con cáncer de mama B) Sensograma de mujer sana.

41

41

Metodología

Análisis estádistico de muestras de aliento exhalado.

Grupos

Mujeres con cáncer de mama (BC). Controles (Healthy).

→ Variables: Cambios en las respuestas de los sensores.

→ Análisis de componentes principales PCA.

→ Análisis PERMANOVA

→ Análisis predictivo CAP

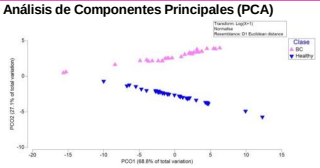
→ Curva ROC

42

42

Metodología

Análisis de Componentes Principales (PCA)



Sensor	PC1 Factor de Carga	PC1 % contribución	PC2 Factor de Carga	PC2 % contribución
S1	0.994	3.347	-0.047	0.131
S2	0.994	3.344	-0.032	0.061
S3	0.990	3.315	-0.007	0.003
S4	0.988	3.305	-0.092	0.509
S5	0.902	2.756	0.427	11.053
S6	0.834	2.353	-0.151	1.376
S7	0.867	2.544	-0.400	9.696
S8	0.920	2.866	-0.337	6.877
S9	0.993	3.338	0.094	0.536
S10	0.978	3.237	-0.178	1.917
S11	0.933	2.946	0.346	7.238
S12	0.994	3.345	-0.032	0.063
S13	0.977	3.230	-0.142	1.213
S14	0.966	3.158	-0.227	3.116
S15	0.996	3.357	0.057	0.197
S16	0.996	3.356	-0.024	0.036
S17	0.997	3.362	0.050	0.153
S18	0.995	3.351	0.025	0.039
S19	0.995	3.351	0.038	0.088
S20	0.947	3.038	-0.299	5.392
S21	0.982	3.260	0.142	1.214
S22	0.990	3.316	-0.125	0.942
S23	0.855	2.474	0.478	13.701
S24	0.877	2.600	-0.470	13.338
S25	0.996	3.356	-0.044	0.117
S26	0.975	3.220	0.211	2.704
S27	0.996	3.356	-0.050	0.153
S28	0.988	3.305	-0.013	0.010
S29	0.995	3.349	0.063	0.240
S30	0.982	3.262	0.119	0.855
S31	0.829	2.328	0.510	15.727
S32	0.984	3.274	0.147	1.305
Total		100.000		100.000

Total de explicación de variabilidad: 95.9%:
- *PC1 (68.8%)*
- *PC2 (27.1%)*

P<0.0001 (Análisis de varianza basado en permutaciones (PERMANOVA))

43

43

Metodología

Recolección de muestra de orina

- Chorro medio
- Frascos estériles de 50 mL
- Ayuno de 12 horas

Procesamiento de muestra y análisis por CG-MS

44

44

Metodología

Estandarización del procedimento para el análisis de las muestras de orina

Condiciones modificadas			
Tipo de fibra.	Polidimetil-siloxano (PDMS)	Divinilbencen/Carboxen/Polidimetil-siloxano (DVB/CAR/PDMS)	
Tiempo de incubación (min).	20	40	60
Tiempo de exposición (min).	60	45	30
Temperatura de exposición (°C).	60	40	30

45

45

Metodología

Procesamiento y análisis de muestra de orina.

400 µl

Condiciones cromatográficas	
Temperatura de exposición	250º C/10 min
Rampa de temperatura	60°C hold 2 min 10°C/min a 180 1°C/min a 200 2°C/min a 265 30°C/min a 300
Modo	Scan

Exposición 30 min/ 60ºC Fibra PDMS

Análisis cromatográfico

46

46

Metodología

Análisis estádistico de muestras de orina.

Grupos

Mujeres con BI-RADS 1-2 vs Mujeres con BI-RADS 3-6

Mujeres con estadio 0-II vs Mujeres en estadio III-IV

→ Comparación de compuestos con base de datos. NIST → Variables: Areas bajo la curva (AUC).

→ Análisis de componentes principales PCA. → Análisis PERMANOVA → Análisis predictivo CAP → Análisis SIMPER

47

47

Resultados y discusión

48

48

Aliento exhalado

49

49

Resultados

Caracterísiticas antropométricas de las pacientes n= 72

	Mujeres sanas n=37	Mujeres con cáncer de mama n=35		
Parametro	Media $\pm DE$	Media $\pm DE$	Valor-t	Valor de p
Altura (m)	1.55±0.032	1.56±0.05	0.16	0.87
Peso (Kg)	69.5±15.67	66.13±14.63	-0.75	0.45
IMC (Kg/m²)	28.73±5.5	27.12±4.60	1.48	0.14
Edad	54±12.18	49±11.22	-0.83	0.41

50

50

Resultados

Caracterísiticas clínicas las participantes

Parámetro	n	Porcentaje (%)
Tipo molecular		
Luminal A	18	6
Luminal B	32	12
Triple Negativo	12	4
HER-2	12	4
Indeterminado	26	9
Estadio		
0	3	8
I	4	12
II	14	40
III	8	24
IV	6	16

51

51

Resultados

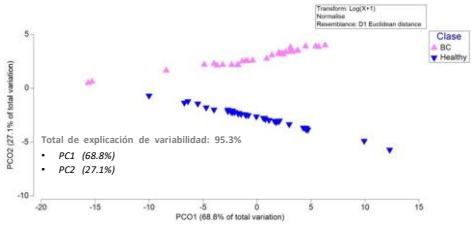
Comorbilidades de las pacientes con cáncer de mama

Comorbilidad	n	Porcentaje (%)
DM 2	6	16
Hipertensión	13	36
Ninguna	18	52

52

Resultados

Análisis de componentes principales (PCA).

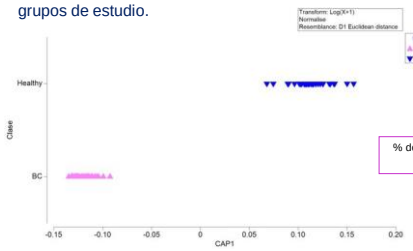


P < 0.001

53

Resultados

Análisis canónico de coordenadas principales. Distribución de los diferentes grupos de estudio.



R= 0.95

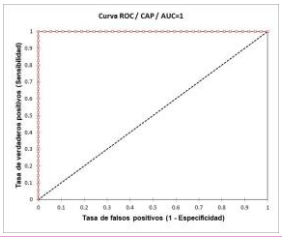
P < 0.01

% de correcta clasificación= 95%

54

Resultados

Recieving operating characteristics (ROC) curves



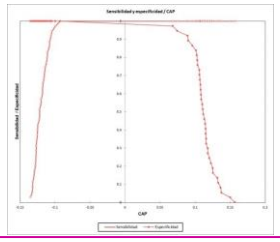
Valor de cohorte

-0.093

55

Resultados

Calcular la sensibilidad y especificidad de los métodos utilizados a través de la elaboración de curvas ROC.



R= 0.95

Sensibilidad: 100%

Especificidad: 100%

56

Discusión

222 pacientes con BC en estadios iniciales

Análisis de aliento exhalado con E-Nose 320

Análisis Machine learning

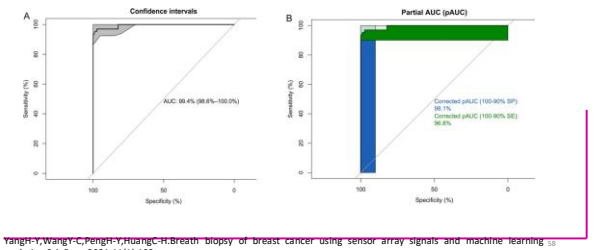
Model	Luminal A		Luminal B		HER2/neu		Triple-negative	
	Accuracy	Kappa	Accuracy	Kappa	Accuracy	Kappa	Accuracy	Kappa
k-nearest neighbors	0.61	0.22	0.60	0.20	0.67	0.34	0.84	0.69
Naive Bayes	0.59	0.18	0.53	0.06	0.64	0.28	0.68	0.36
Decision tree	0.62	0.27	0.71	0.41	0.97	0.94	0.99	0.98
Neural network	0.53	0.11	0.56	0.12	0.65	0.29	0.77	0.54
Support vector machines (linear kernel)	0.51	0.02	0.54	0.08	0.60	0.20	0.77	0.54
Support vector machines (radial kernel)	0.57	0.15	0.59	0.18	0.59	0.19	0.84	0.67
Support vector machines (polynomial kernel)	0.63	0.26	0.63	0.25	0.66	0.32	0.85	0.69
Random forest	0.74	0.49	0.83	0.66	0.98	0.95	0.99	0.97

Table 3. Leave-one-out cross-validated discrimination accuracy and reliability of molecular phenotyping of breast cancer using machine learning algorithms.

Yang H-Yet.al.Breath biopsy of breast cancer using sensor array signals and machine learning analysis. *Sci Rep*. 2021;11(1):103.

57

Discusión



YangH-Y,WangY-C,PengH-Y,HuangC-H.Breath biopsy of breast cancer using sensor array signals and machine learning analysis. *Sci Rep.* 2021;11(1):103.

58

58

Discusión

36 pacientes con BC y 45 mujeres sanas.

Obtención de muestras de aliento

Análisis y comparación con diferentes tipos de E-nose y ANN

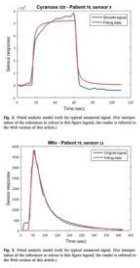


Table 1
Validation results of the ANN models obtained for the two sets of data "Group 1" and "Group 2" using both the raw data and the one following feature extraction (FE).

Type of IN	"Group"	Data type	Accuracy [%]	Sensitivity [%]	Specificity [%]
M64	1	raw	67.5 ± 21.6	67.5 ± 18.5	67.5 ± 33.4
M64	1	FE	70.4 ± 19.1	73.5 ± 22.6	67.5 ± 23.7
M64	2	raw	73.8 ± 24.6	68.3 ± 28.8	80 ± 28.1
M64	2	FE	76.2 ± 18.5	74.17 ± 25	78.3 ± 15.3
Cyranose 320	1	raw	60.9 ± 22.8	56 ± 29.1	66.7 ± 23.6
Cyranose 320	1	FE	57.4 ± 22	47 ± 29.7	46.7 ± 26.7
Cyranose 320	2	raw	51.7 ± 22.1	51.7 ± 21.4	52.5 ± 36.0
Cyranose 320	2	FE	55 ± 22.2	48.3 ± 18.8	61.7 ± 31.2

Herman-SaffarO, Early non-invasive detection of breast cancer using exhaled breath and urine analysis. *Comput Biol Med*. 2018;96:227-232.

59

59

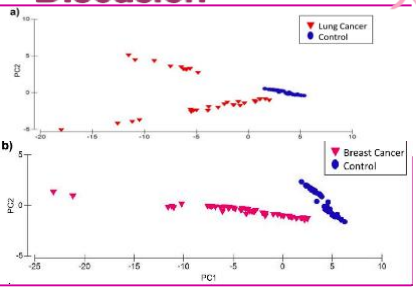
Discusión

203 participantes

30 cáncer de pulmón, 50 COPD, 50 BC, 50 Controles.

Análisis mediante Cyranose 320.

Análisis estadístico PCA, CAP.



Rodríguez-Aguilar, Clinica Chimica Acta 518 (2021)

60

60

Discusión

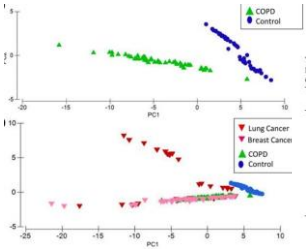


Table 2
Multivariate analysis results of patients with Lung Cancer, Breast Cancer and COPD.

Multivariate analysis	Lung Cancer vs Breast Cancer	Lung Cancer vs COPD	Breast Cancer vs COPD
PCA (% Variation)			
PC1	80.1	72.2	96.3
PC2	17.2	17.5	2
PC3	1.3	7.7	0.9
CAP			
r^2 CAP1	0.91	0.84	0.91
Correct classification (%)	93.05	96.47	100
Mis-classification error (%)	6.94	3.53	0
p value	0.001	0.001	0.001

Rodríguez-Aguilar, Clinica Chimica Acta 518 (2021)

61

61

Orina

62

62

Resultados

Caracterísiticas antropométricas de las pacientes

	Mujeres con cáncer de mama n=46
Parámetro	Media $\pm DE$
Altura (m)	1.53±0.00
Peso (Kg)	68±13.53
IMC (Kg/m²)	28±5.70
Edad	49±12

63

63

Resultados

Caracterísiticas antropométricas de las pacientes n= 46

Parametro	BI-RADS 0-2 n=25 Media $\pm DE$	BI-RADS 3-6 n=21 Media $\pm DE$	Valor-t	Valor de p
Altura (m)	1.56±0.03	1.6±0.05	-1.97	0.06
Peso (Kg)	69.8±16.30	66.22±9.85	0.87	0.39
IMC (Kg/m²)	28.82±6.70	28.13±5.20	1.39	0.17
Edad	52.40±14.70	46.3±10.70	1.58	0.12

64

64

Resultados

Caracterísiticas antropométricas de las pacientes n= 46

Parametro	Estadio I-II n=11 Media $\pm DE$	Estadio III-IV n=34 Media $\pm DE$	Valor-t	Valor de p
Altura (m)	1.59±0.05	1.58±0.08	0.56	0.58
Peso (Kg)	71.9±15.20	65.9±12.30	1.32	0.20
IMC (Kg/m²)	28.3±7.00	28.17±7.50	1.39	0.17
Edad	51.1±13.00	41.8±12.70	0.65	0.52

65

65

Resultados

Caracterísiticas antropométricas de las pacientes

Parámetro	n	Porcentaje (%)
Tipo molecular		
Luminal A	22	48
Luminal B	18	39
Triple Negativo	2	4
HER-2	1	2
Indeterminado	3	7
Estadio		
0	2	4
I	9	20
II	23	50
III	11	24
IV	1	2

66

66

Resultados

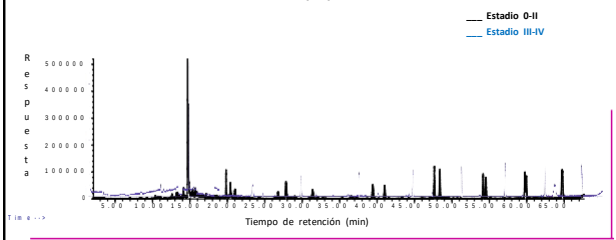
Parámetro	n	Porcentaje (%)
Otras caracteristicas: **Partos**		
Ninguno	11	26.8
1	3	7.3
2	9	22.0
3	8	19.5
4	4	9.8
5 ó mas	6	14.6
Método anticonceptivo utilizado		
Anticonceptivos hormonales	9	22.0
Oculsión tubárica bilateral	7	17.1
Ninguno	21	51.2
Otro	9	22.0

67

67

Resultados

Ejemplos de cromatogramas obtenidos en muestras de orina de pacientes con cáncer de mama



68

68

Resultados

Compuestos identificados por CG-MS

Compuesto	Peso molecular (g/mol)	CAS-ID	Grupo químico
Limoneno	136.24	5989-27-5	Hidrocarburo
Duodecano	170.33	112-40-3	Hidrocarburo
Tetradecano	198.39	629-59-4	Hidrocarburo
Glicina	75.07	56-40-6	Hidrocarburo
Tolueno	92.14	108-88-3	Hidrocarburo
1-Methoxicicloheхano	114.19	931-56-6	Eter
Hexano	86.18	110-54-3	Hidrocarburo
Acido propanóico 2-metil ester			Ester
Fenol-p-tert-butil	150.22	98-54-4	Alcohol
Acido heptafluorobutirico, n-tetradecil ester	410.41	7365-36-8	Ester
Naftaleno	128.16	91-20-3	Hidrocarburo
Careno	136.23	498-15-7	Hidrocarburo
Ciclohexeno	82.14	110-83-8	Hidrocarburo
Alfa pineno	136.23	80-56-8	Hidrocarburo
Acido pentanoico 2,2,4-trimethil-3-carboxisopropyl, isobutil ester	286.41	N/A	Ester
5H-Nafto[2,3-c]carbazol,	281.3	10025-44-3	Alcohol
9-Metilcarbazol	181.23	1484-12-4	Carbazol
Ciclohexanol	100.159	108-93-0	Alcohol
Octadecano	254.5	593-45-3	Hidrocarburo
Undecano	156.31	1120-21-4	Hidrocarburo

69

69

Resultados

Grupos de estudio

Grupo	n	Grupo	n
Mujeres con BI-RADS 1-2	25	Mujeres con BI-RADS 3-6	21
Mujeres con estadio 0-II	11	Mujeres en estadio III-IV	34

70

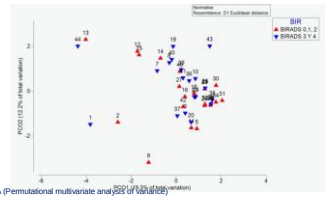
70

Resultados

Análisis de componentes principales.

Total de explicación de variabilidad: 31.5%

- *PC1 (19.3%)*
- *PC2 (12.2%)*



P < 0.001

PERMANOVA (Permutational multivariate analysis of variance)

71

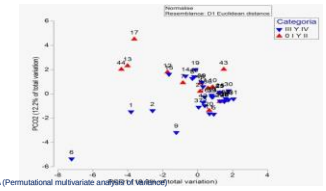
71

Resultados

Análisis de componentes principales.

Total de explicación de variabilidad: 31.5%

- *PC1 (19.3%)*
- *PC2 (12.2%)*



No significativo

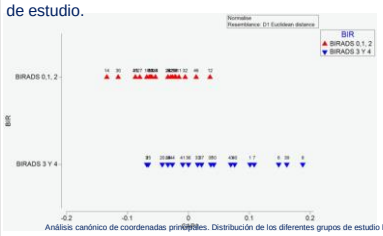
PERMANOVA (Permutational multivariate analysis of variance)

72

72

Resultados

Análisis canónico de coordenadas principales. Distribución de los diferentes grupos de estudio.



R= 0.6198

P > 0.001

% de correcta clasificación= 61.9%

Análisis canónico de coordenadas principales. Distribución de los diferentes grupos de estudio basado en el patrón metabolómico presente en cada muestra.

73

73

Resultados

Análisis de porcentajes de similitud

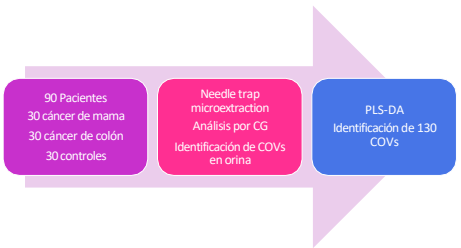
SIMPER

Compuesto	Contribución %	Contribución acumulada (%)
Limoneno	8.49	8.49
Ácido heptafluorobutírico, n-tetradecil ester	8.26	16.75
Alfa pineno	8.00	24.75
Ácido propanoico 2-metil Ester	6.95	31.7
Hexadecano	7.03	38.64
Ciclohexanol	6.20	52.4
Careno	5.64	63.98
Naftaleno	4.24	73.17
Ácido pentanoico 2,2,4-trimethil-3-carboxyisopropil, isobutil ester	3.97	77.14
Metil carbazol	3.85	80.99
Ciclohexeno	3.77	88.54
Tolueno	3.61	95.82
5H-Nafto[2,3-c]carbazol, 5-methl-	1.89	99.99
1-Methoxiciclohexano	0.01	100

74

74

Discusión



P. Porto-Figueira et al. / Analytica Chimica Acta 1023 (2018)

75

75

Discusión

Table 1
Volatile organic metabolites (VOM) identified in all studied groups, frequency of occurrence in each group, relative area and possible metabolic origin reported in the literature for each VOM.

#	Volatile metabolites	RT[a]	RI $_{Exp}$[b]	RI $_{Lit}$[c]	CTL[d]	BC[e]		CC[f]		Origin
						FO[g]	Rel. area (×10[6])	FO	Rel. area (×10[6])	FO	Rel. area (×10[6])	
1	Pentane	4.376	660	650	100	0.06		100	0.20	100	0.18	End[i]/Exo[j]
2	Methyl chloride	4.489	671	---	100	0.15		100	0.47	100	0.55	End/Exo
3	Hexane	4.541	676	600	97	0.05		100	0.05	100	0.07	End/Exo
4	Ethyl ether	4.603	682	640	87	0.01		100	0.05	100	0.09	Unknown
5	Isoprene	4.670	689	633	100	0.80		100	1.43	100	1.71	End
6	Methanethiol	4.820	703	702	100	1.32		100	1.98	100	2.41	End
7	Acetaldehyde	4.855	706	703	97	0.49		100	0.58	100	0.87	End/Exo (D/M[h])
8	Carbon disulphide	5.078	726	723	100	0.20		100	0.49	100	1.20	Exo
9	2,4,4-Trimethylpent-1-ene	5.128	730	750	83	0.03		100	0.07	87	0.08	Unknown
10	Dimethyl sulphide	5.207	737	737	100	0.08		100	0.15	97	0.18	End/Exo (D/M)
11	trans-2-Methyl-1,3-pentadiene	5.452	757	760	83	0.01		100	0.03	97	0.04	Unknown
12	Furan	5.586	768	760	100	20.2		100	37.3	100	38.7	End
13	Acetone	5.842	788	785	100	37.1		100	29.8	100	36.1	End, M
14	Sulphur dioxide	6.605	843	856	37	0.34		---	0.00	97	0.48	Exo Food
15	Tetrahydro-2,2,5,5-tetramethylfuran	6.420	830	---	93	4.61		100	0.78	100	1.41	Unknown
16	2-Methylfuran	6.594	842	843	100	3.04		100	5.31	100	6.52	End
17	Butanal	6.640	845	854	0	0.00		100	0.06	97	0.04	End
18	Ethyl Acetate	7.008	869	870	87	4.15		97	3.95	70	3.42	End/Exo (Food)
19	2-Butanone	7.197	881	881	100	5.07		100	6.70	100	6.65	End
20	3-Methylfuran	7.124	876	877	97	2.04		100	2.54	100	2.73	Exo Food
21	2-Methylbutanal	7.458	897	896	83	0.21		97	0.65	90	0.45	End/Exo (Food)
22	Dichloromethane	7.952	917	914	60	0.46		100	0.97	83	0.54	End/Exo (Food)
23	3-Methyl-2-butanone	7.901	915	918	90	1.42		100	1.69	100	1.76	End
24	Benzene	8.218	927	924	80	0.28		97	0.29	100	0.25	Exo (Pol[k])
25	1,6-Dimethylhepta-1,3,5-triene	8.707	944	---	93	0.23		100	0.85	93	0.48	Unknown
26	Methyl allyl sulphide	8.654	942	943	77	0.42		100	0.35	60	0.16	End/Exo (Food)
27	1,2,5,5-Tetramethyl-1,3-cyclopentadiene	8.991	953	---	100	0.15		100	0.48	100	0.28	Unknown
28	5-tert-Butyl-1,3-cyclopentadiene	8.932	951	---	63	0.31		97	0.29	87	0.43	End/Exo (Food)
29	2-Pentanone	9.371	966	969	100	17.3		100	22.7	100	18.8	Exo (Food)
30	2-Methyl-3-pentanone	10.053	987	989	87	0.55		100	0.28	67	0.21	End
31	2,4-Dimethyl-3-pentanone	10.148	989	995	100	1.32		100	1.19	100	0.71	End/Exo (Food)
32	1,3-Dimethyl-6-methylenecyclohexene	10.782	1006	---	93	0.10		100	0.36	100	0.21	Unknown
33	3-Methyl-2-pentanone	10.908	1008	1005	100	1.06		97	1.63	100	1.40	End/Exo (Food)
34	2-Hexanone	11.105	1013	1082	100	0.49		100	0.97	100	0.75	End
35	Thiophene	11.246	1016	1017	100	0.24		100	0.45	100	0.47	End/Exo (Food)
36	1,3-Dimethyl-1-cyclohexene	11.862	1024	---	100	0.88		100	1.93	93	1.87	Unknown
37	Toluene	12.053	1032	1033	100	2.09		100	2.14	97	3.95	Exo (Pol/Food)
38	2-Hexanone	12.712	1045	1047	100	1.70		100	0.93	100	0.91	End
39	2-Ethyl-4-methylimidazole	12.970	1049	---	100	0.24		100	0.34	100	0.35	Unknown
40	2-Methyl-3-hexanone	12.928	1048	---	80	0.59		100	0.54	100	0.55	Unknown
41	Dimethyl disulphide	13.901	1065	1065	100	100.8		100	107.0	100	88.1	End
42	Hexanal	14.523	1075	1075	93	0.34		100	0.47	100	0.56	End
43	3-Ethyl-2-pentanone	14.947	1082	1033	53	0.12		93	0.76	93	0.22	End (Food)
44	2-Methylthiophene	14.995	1083	1085	97	0.19		100	0.27	90	0.25	Exo (Food)
45	2-methyl-(E)-2-butenal	15.454	1089	1088	83	0.10		100	0.19	100	0.18	End/Exo (Food)
46	6,6-Dimethylhepta-2,4-diene	15.529	1091	---	93	0.07		97	0.11	100	0.12	End/Exo (Food)
47	2-Ethyl-5-methylfuran	15.037	1052	1034	73	0.09		100	0.10	87	0.07	End/Exo (Food)
48	2,2,6-Trimethyl-6-vinyltetrahydropyran	15.833	1095	1093	100	0.25		100	0.20	93	0.33	End/Exo (Food)
49	7,7-Dimethyl-9-oxatricyclo[6.2.2.0(1,6)]undecan-10-one	17.206	1114	---	93	0.12		100	0.21	100	0.23	Unknown
50	4-Heptanone	17.421	1117	1118	100	30.4		100	68.2	100	53.3	End
51	p-Xylene	18.048	1124	1123	100	0.24		100	0.65	100	0.21	Unknown
52	1,5,5-Trimethyl-6-methylene-cyclohexene	18.212	1126	---	100	0.16		100	0.26	100	0.28	Unknown
53	Ethyl methyl disulphide	18.511	1130	1141	97	0.05		100	0.06	70	0.03	Exo (Food)
54	Diallyl sulphide	18.636	1131	1143	70	0.10		93	0.18	57	0.07	Exo (Food)
55	o-Phellandrene	19.211	1138	1143	90	0.10		100	0.07	73	0.11	End/Exo (Food)
56	3-Heptanone	18.685	1145	1148	87	0.08		100	0.10	90	0.10	Unknown
57	α-Terpinene	20.273	1150	1159	100	2.41		100	1.50	100	3.05	End/Exo (Food)
58	1,4-Cineole	20.802	1156	1164	100	1.22		100	0.45	100	0.87	End/Exo (Food)
59	m-Xylene	21.263	1161	1165	77	0.16		87	0.36	33	0.10	Unknown
60	Limonene	21.878	1165	1180	63	0.07		97	0.06	70	0.07	End/Exo (Food)
61	2-Heptanone	22.371	1172	1173	100	0.48		100	1.09	100	0.85	End

P. Porto-Figueira et al. / Analytica Chimica Acta 1023 (2018) 76

76

Discusión

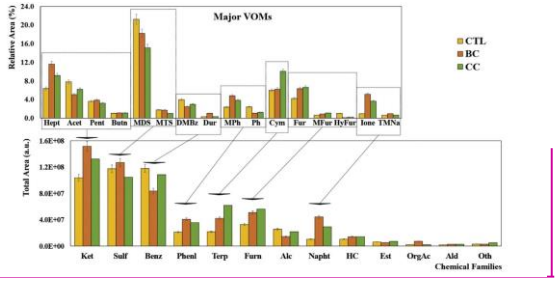
Table 1
Volatile organic metabolites (VOM) identified in all studied groups, frequency of occurrence in each group, relative area and possible metabolic origin reported in the literature for each VOM.

#	Volatile metabolites	RT[a]	RI $_{Exp}$[b]	RI $_{Lit}$[c]	CTL[d]	BC[e]		CC[f]		Origin
						FO[g]	Rel. area (×10[6])	FO	Rel. area (×10[6])	FO	Rel. area (×10[6])	
30	2-Methyl-3-pentanone	10.053	987	989	87	0.55		100	0.28	67	0.21	End
31	2,4-Dimethyl-3-pentanone	10.148	989	995	100	1.32		100	1.19	100	0.71	End/Exo (Food)
32	1,3-Dimethyl-6-methylenecyclohexene	10.782	1006	---	93	0.10		100	0.36	100	0.21	Unknown
33	3-Methyl-2-pentanone	10.908	1008	1005	100	1.06		97	1.63	100	1.40	End/Exo (Food)
34	2-Hexanone	11.105	1013	1082	100	0.49		100	0.97	100	0.75	End
35	Thiophene	11.246	1016	1017	100	0.24		100	0.45	100	0.47	End/Exo (Food)
36	1,3-Dimethyl-1-cyclohexene	11.862	1024	---	100	0.88		100	1.93	93	1.87	Unknown
37	Toluene	12.053	1032	1033	100	2.09		100	2.14	97	3.95	Exo (Pol/Food)
38	2-Hexanone	12.712	1045	1047	100	1.70		100	0.93	100	0.91	End
39	2-Ethyl-4-methylimidazole	12.970	1049	---	100	0.24		100	0.34	100	0.35	Unknown
40	2-Methyl-3-hexanone	12.928	1048	---	80	0.59		100	0.54	100	0.55	Unknown
41	Dimethyl disulphide	13.901	1065	1065	100	100.8		100	107.0	100	88.1	End
42	Hexanal	14.523	1075	1075	93	0.34		100	0.47	100	0.56	End
43	3-Ethyl-2-pentanone	14.947	1082	1033	53	0.12		93	0.76	93	0.22	End (Food)
44	2-Methylthiophene	14.995	1083	1085	97	0.19		100	0.27	90	0.25	Exo (Food)
45	2-methyl-(E)-2-butenal	15.454	1089	1088	83	0.10		100	0.19	100	0.18	End/Exo (Food)
46	6,6-Dimethylhepta-2,4-diene	15.529	1091	---	93	0.07		97	0.11	100	0.12	End/Exo (Food)
47	2-Ethyl-5-methylfuran	15.037	1052	1034	73	0.09		100	0.10	87	0.07	End/Exo (Food)
48	2,2,6-Trimethyl-6-vinyltetrahydropyran	15.833	1095	1093	100	0.25		100	0.20	93	0.33	End/Exo (Food)
49	7,7-Dimethyl-9-oxatricyclo[6.2.2.0(1,6)]undecan-10-one	17.206	1114	---	93	0.12		100	0.21	100	0.23	Unknown
50	4-Heptanone	17.421	1117	1118	100	30.4		100	68.2	100	53.3	End
51	p-Xylene	18.048	1124	1123	100	0.24		100	0.65	100	0.21	Unknown
52	1,5,5-Trimethyl-6-methylene-cyclohexene	18.212	1126	---	100	0.16		100	0.26	100	0.28	Unknown
53	Ethyl methyl disulphide	18.511	1130	1141	97	0.05		100	0.06	70	0.03	Exo (Food)
54	Diallyl sulphide	18.636	1131	1143	70	0.10		93	0.18	57	0.07	Exo (Food)
55	o-Phellandrene	19.211	1138	1143	90	0.10		100	0.07	73	0.11	End/Exo (Food)
56	3-Heptanone	18.685	1145	1148	87	0.08		100	0.10	90	0.10	Unknown
57	α-Terpinene	20.273	1150	1159	100	2.41		100	1.50	100	3.05	End/Exo (Food)
58	1,4-Cineole	20.802	1156	1164	100	1.22		100	0.45	100	0.87	End/Exo (Food)
59	m-Xylene	21.263	1161	1165	77	0.16		87	0.36	33	0.10	Unknown
60	Limonene	21.878	1165	1180	63	0.07		97	0.06	70	0.07	End/Exo (Food)
61	2-Heptanone	22.371	1172	1173	100	0.48		100	1.09	100	0.85	End

P. Porto-Figueira et al. / Analytica Chimica Acta 1023 (2018) 77

77

Discusión



P. Porto-Figueira et al. / Analytica Chimica Acta 1023 (2018) 78

78

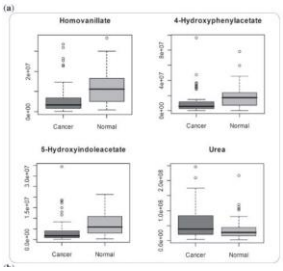
Discusión

Orina de 50 pacientes con cáncer de mama y 50 controles

Análisis por GC-MS

Cuantificación de compuestos y comparación con biblioteca NIST

Análisis estadístico LDA Y SVM, t-Student en comparación de grupos



H.Nam et al., bioinformatics, (2009) 79

79

Discusión

Importancia de la matriz biológica

Aliento exhalado	Orina
No invasiva: La recolección de muestras de orina y aliento es un procedimiento no invasivo y relativamente sencillo en comparación con otros métodos de obtención de muestras biológicas.	
Facilidad de recolección: La toma de muestra de aliento es un procedimiento simple y rápido en comparación con otros métodos de obtención de muestras biológicas. La orina se puede recolectar fácilmente en grandes cantidades y está disponible de forma regular y continua.	
Representatividad del metaboloma sistémico: Tanto la orina como el aliento reflejan el metaboloma sistémico y puede proporcionar información sobre diversos procesos metabólicos y la salud general del individuo.	
Detección en tiempo real: Algunos metabolitos pueden ser detectados en el aliento de forma casi inmediata, lo que permite mediciones en tiempo real.	**Estabilidad de las muestras:** Las muestras de orina suelen ser estables durante el almacenamiento a largo plazo, lo que facilita su procesamiento y análisis en estudios de metabolómica.

Ute Roessner, A. Ian Smith, 2016, Wiley 80

80

Conclusiones

- Se observó un comportamiento diferente en el patrón de metabolitos de personas sanas y mujeres con cáncer de mama en las muestras analizadas por nariz electrónica.
- La obtención de aliento exhalado en las personas de ambos grupos nos brinda la facilidad de obtener una muestra no invasiva para la identificación de metabolitos.
- La elaboración de curvas ROC con los datos obtenidos de aliento exhalado nos permite considerar la nariz electrónica como una herramienta útil para la distinción de 2 grupos.

81

Conclusiones

- El limoneno y el alpha-pineno se han descrito como elementos relevantes en algunos tipos de cáncer.
- Los HC poseen un papel importante en la biosintesís de ácidos grasos la cual se ve exacervada en procesos oncológicos.
- El uso adecuado de las herramientas anteriormente descritas puede proveer un auxiliar útil en la detección oportuna de CM

82

82

Conclusiones

- Aplicaciones clínicas .

BI-RADS	Riesgo Dx
0	13%
I	<1%
II	<1%
III	<2%
IV	4-95%
a	10%
b	50%
c	70%
V	95%
VI	Seguro

83

83

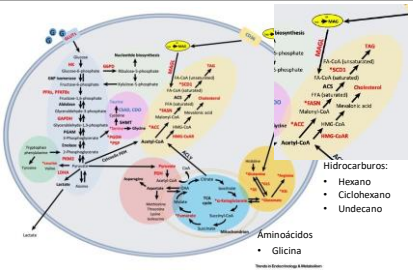
Limitantes

- **La pandemia de COVID-19 limitó muchas de las estrategias que se definieron al inicio para elaborar el presente proyecto.**
- **No fue posible darle seguimiento a las pacientes una vez que comenzaran su tratamiento.**
- **Los grupos empleados para el análisis de orina no proveen suficiente evidencia para determinar si existe alguna diferencia.**
- **No se contó con el suficiente número de pacientes de cada grupo para la consolidación del estudio piloto**
- **No fue posible emparejar el análisis de aliento exalado tanto en CG-MS con nariz electrónica para la obtención de datos confirmatorios.**
- **En el presente proyecto no fue posible hacer una cuantificación dirigida de los metabolitos encontrados en orina.**

84

84

Limitantes



Hidrocarburos:
- Hexano
- Ciclohexano
- Undecano

Aminoácidos
- Glicina

Eylem Kulkoyluoglu-Cotul, 2019

85

85

Perspectivas

- **Es posible realizar un estudio mediante una técnica analítica distinta para respaldar los resultados obtenidos.**
- **Elaborar grupos mas grandes en donde se incluyan una mayor cantidad de pacientes con diversos grupos moleculares para obtener un análisis más específico sobre las diferencias en cada tipo de cáncer.**
- **Realizar un análisis dirigido con metabolitos representativos de cada grupo.**
- **Determinar rutas metabólicas alteradas en los grupos de estudio.**
- **Utilizar la nariz electrónica como técnica confirmatoria y auxiliar en el tamizaje de cáncer de mama**

86

86

Productos

87

87

Estancia académica

NAWA NARODOWA AGENCJA WYMIANY AKADEMICKIEJ

GDAŃSK UNIVERSITY OF TECHNOLOGY

Departamento de Química Analítica

Justyna Plotka-Wasylka, PhD, DSc.eng

"Determination of selected biomarkers of exposure to popular xenobiotics in samples of children's urine absorbed in disposable children's diapers".

Desarrollar un método para la determinación de xenobióticos y/o sus metabolitos a partir de muestras de orina lactantes y niños.

88

88

Productos doctorado

89

89

Productos doctorado

Artículo Aprobado

Review

Nanosorbents as Materials for Extraction Processes of Environmental Contaminants and Others

90

90

Productos doctorado

How pesticides affect neonates? - Exposure, health implications and determination of metabolites

Evaluation of sialic acid concentrations and its association with the use of hormonal contraceptives

91

91

Productos doctorado

Revisiones

92

92

Gracias

93

93