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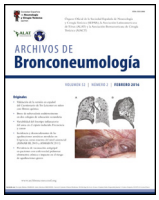

Original Article

# Study of 5 Volatile Organic Compounds in Exhaled Breath in Chronic Obstructive Pulmonary Disease☆

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ABSTRACT

*Introduction:* A major risk factor for chronic obstructive pulmonary disease (COPD) is tobacco smoke, which generates oxidative stress in airways, resulting in the production of volatile organic compounds (VOCs). The purpose of this study was to identify VOCs in exhaled breath and to determine their possible use as disease biomarkers.
*Method:* Exhaled breath from 100 healthy volunteers, divided into 3 groups (never smokers, former smokers and active smokers) and exhaled breath from 57 COPD patients were analyzed. Samples were collected using BioVOC® devices and transferred to universal desorption tubes. Compounds were analyzed by thermal desorption, gas chromatography and mass spectrometry. VOCs analyzed were linear aldehydes and carboxylic acids.
*Results:* The COPD group and healthy controls (never smokers and former smokers) showed statistically significant differences in hexanal concentrations, and never smokers and the COPD group showed statistically significant differences in nonanal concentrations.
*Conclusions:* Hexanal discriminates between COPD patients and healthy non-smoking controls. Nonanal discriminates between smokers and former smokers (with and without COPD) and never smokers.


## Estudio de 5 compuestos orgánicos volátiles en aire exhalado en la enfermedad pulmonar obstructiva crónica

*Palabras clave:*
Enfermedad pulmonar obstructiva crónica
Compuestos orgánicos volátiles
Aire exhalado
Estrés oxidativo

RESUMEN

*Introducción:* Un factor de riesgo importante para el desarrollo de la enfermedad pulmonar obstructiva crónica (EPOC) es el humo del tabaco, que genera estrés oxidativo en las vías respiratorias, dando lugar a la producción de compuestos orgánicos volátiles (VOC). El objetivo del trabajo es su identificación en el aire exhalado y su posible utilidad como biomarcadores de la enfermedad.
*Método:* Se analizó el aire exhalado de 100 voluntarios sanos, clasificados en 3 grupos (no fumadores, exfumadores y fumadores activos) y un grupo de 57 pacientes con EPOC. La muestra de aire exhalado se recogió mediante BioVOC® y se traspasó a tubos de desorción para su posterior análisis por cromatografía de gases y espectrometría de masas. Los VOC analizados fueron aldehídos lineales y ácidos carboxílicos.

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*Resultados:* Hexanal mostró diferencias estadísticamente significativas entre el grupo EPOC y los controles sanos (no fumadores y exfumadores), y nonanal entre el grupo control no fumador y el grupo EPOC.
*Conclusiones:* Hexanal discrimina entre pacientes con EPOC y controles sanos no fumadores y exfumadores. Nonanal diferencia entre fumadores y exfumadores (con o sin EPOC) frente a controles no fumadores.



## Introduction

Chronic obstructive pulmonary disease (COPD) is defined as a respiratory disease characterized by chronic, progressive airflow limitation that is not fully reversible. The main symptoms are dyspnea and cough, sometimes accompanied by expectoration. COPD patients have exacerbations that vary in severity depending on comorbidities.[1]

Smoking is the most important risk factor in the development of COPD, and evidence shows that the risk is proportional to the accumulated consumption of cigarettes.[2] In Spain, 7.6% of male and 5.5% of female non-smokers have COPD,[3] but this figure rises to 39.9% and 15.4% among men and women, respectively, who have smoked for more than 10 years. Other factors, such as alpha-1 antitrypsin deficiency or pneumonia in childhood, also play a role.[1]

According to the World Health Organization (WHO), 2.9 million individuals a year die from COPD, and it is estimated that by 2030, it will be the third cause of death worldwide.[4]

In the EPI-SCAN epidemiological study of COPD in Spain,[5] 73% of confirmed cases did not have a previous diagnosis of COPD, revealing a high degree of underdiagnosis. The standard technique is spirometry, but this method carries a risk of underdiagnosis in the early stages and overdiagnosis in the advanced stages. Other useful methods are available, such as bronchoalveolar lavage or open lung biopsy, but these are too invasive for routine use.[6]

Clinicians involved in diagnosing COPD are continually on the look-out for techniques and parameters that will help them in their decision-making. Biomarkers are biological parameters that provide information on the normal or disease status of an individual or a population.[7] The search for biomarkers to characterize COPD is ongoing, and possible candidates have recently been investigated in sputum,[8] bronchoalveolar lavage,[9] and exhaled air.[10–12]

Exhaled air contains a multitude of volatile organic compounds (VOCs), some of which can be identified as biomarkers that may be of use in the characterization of COPD. The analytical procedure is non-invasive and rapid, and could complement spirometry in both diagnosis and follow-up of this entity. However, this technique is susceptible to contamination of samples by multiple environmental compounds, and this needs to be taken into account when interpreting results.

Tobacco smoke contains over 2000 compounds and a great quantity of free radicals and reactive oxygen and nitrogen species, which increase oxidative stress and pulmonary inflammation.[13] Increased oxidative stress causes lipid peroxidation. The damage generated by this chain reaction produces a large amount of VOCs, including alkanes, aldehydes, and carboxylic acids, which may be excreted by the airways. The presence of these compounds in exhaled air may be a sign of oxidative stress in the airways and the lungs. These VOCs meet the definition of inflammatory biomarkers mentioned above.

A series of benchmark studies on VOCs as biomarkers for COPD in COPD patients compared to clinically healthy controls have been published.[14–18] The studies are similar, but no clear conclusions can be reached as the results vary widely.

The aim of our study was to determine if significant differences really exist between certain VOCs found in the exhaled air of COPD patients compared to healthy controls, and if these substances could be considered as disease biomarkers.

## Patients and Methods

This was a case–control study with consecutive non-probability sampling. A total of 157 volunteers were selected among the employees and patients of the Hospital Central de la Defensa "Gomez Ulla" and the General Air Force Headquarters between October 2014 and December 2015. Two study groups were established, one consisting of 57 clinically stable COPD patients, and the other of 100 healthy controls (never smokers, former smokers, and active smokers).

The inclusion criteria for the 2 groups included consent to participate in the study, age over 40 years, and a smoking status of never, former or active (according to WHO criteria). All individuals completed a questionnaire and an additional clinical examination, including a flow-volume loop. Patients in the COPD group underwent standard tests for the diagnosis and follow-up of their disease, including chest radiograph, flow-volume loop, bronchodilator testing, etc. COPD severity was classified according to the GOLD scale.

Exclusion criteria consisted of any other current or previous lung or tumor disease of any organ or system, or refusal to participate in the study. No gender-based restrictions were applied.

None of the participants were exposed to any special occupational environmental conditions.

Subjects were informed about the aims, risks and benefits, and planned tests and techniques used for conducting the study. They were given written information before signing the informed consent form. All study data were handled in accordance with the provisions of Organic Law 15/1999 on the Protection of Personal Data, 13 December 1999, and Act 41/2002, 14 November 2002, regulating the autonomy of patients and their rights and obligations in relation to clinical information and documentation. The study protocol was approved by the Ethics and Clinical Research Committee of Hospital Central de la Defensa "Gómez Ulla".

This was a targeted chromatographic study with previous selection of the compounds to be studied. VOCs were selected as follows: (1) contaminants derived from the environment or from tobacco smoke were excluded; (2) their metabolic origin had to be known for them to meet criteria for use as biomarkers.

Thus, of more than 250 VOCs in exhaled air described in the consulted literature, only 50 were preselected, on the basis of frequency. Some were endogenous compounds derived from lipid peroxidation. Others were environmental contaminants, and, lastly, another group were compounds of undetermined origin. These latter 2 groups were ruled out, as the origin of the VOCs had to be known in order to select compounds that were of real use as possible biomarkers.

The final selection produced 5 VOCs that met the required criteria: 3 linear, hexanal, hepatanal and nonanal aldehydes (known metabolites of omega 3, omega 6 and omega 9 fatty acid lipid peroxidation); and 2 carboxylic acids, propanoic acid and nonanoic acid (also metabolites of fatty acid lipid peroxidation).[11,12]

After individuals had rested for 1 h, with no oral intake or smoking, the exhaled air sample was collected in BioVOC® breath samples. These easy-to-manage devices do not generate resistance