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# NCT

## Neumología y Cirugía de Tórax

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## THE PROTECTION AND CARE OF HEALTH PERSONNEL, A PRIORITY AND VALUABLE STRATEGY DURING A HEALTH EMERGENCY



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**REVISTA OFICIAL DE:**
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INSTITUTO NACIONAL DE ENFERMEDADES RESPIRATORIAS ISMAEL COSÍO VILLEGAS


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Por el derecho de respirar (for the right to breathe) by Antonio Esparza Castillo, cultural heritage, INER; the second portrays the swabbing process for the detection of COVID-19 in INER personnel during the pandemic.
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*Editorial*
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# The protection and care of health personnel, a priority and valuable strategy during a health emergency

## La protección y atención del personal de salud, una estrategia prioritaria y valiosa durante una emergencia sanitaria

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The year 2020 will be remembered in the history of humanity as the moment of the biggest health emergency never experienced before in the modern era. The reported cases of a severe acute respiratory infection from Wuhan, China[1] looked so far away, but in few weeks and months the world was immerse in the uncertainty, in the threat of the disease, the overload of healthcare services, the scarcity of supplies, death and the impact on other fields like the educational, social economic, work and more. During the months of January and February of that year, health institutions of the country started to prepare themselves to treat this cases, that moment arrived to Mexico on the 27th of February, when the first case in the country was diagnosed, in the National Institute for Respiratory Illness Ismael Cosío Villegas (INER). In the previous week, the INER carried out a wide training program in the proper use of the personal protective equipment (PPE) aimed first and foremost to the frontline staff and afterwards extended to all levels of the workforce, 100% of staff.

The Preventive and Occupational Health Care Coordination, a multidisciplinary group of doctors and nurses, initially it was implemented as a measurement to offer medical attention to all the INER staff, since the primary health services of the ISSSTE were closed. The article of Salazar LMA and collaborators, «COVID-19-Occupational in the National Institute for Respiratory Illness, Mexico City, during the pandemic»,[2] add to the already reported[3] the detail of the work model design for the care of the healthcare staff of the INER during all the pandemic period by SARS-CoV-2 and interesting results as a result of the group work. The INER made available the biggest healthcare workforce to assist in the pandemic, with 4,772 members, from whom almost 60% (2,823) were considered frontline staff. In this way, it was expected that the group of Occupational Health Care took care of a large number of sick people and contacts. It was interesting the complete assistance protocol given to the sick employees, which included the medical consultation to have clinical criteria, the obtaining of samples to detect SARS-CoV-2, the computerized chest tomography to rule out pneumonia, to provide administrative facilities including the internal incapacity for work, the management and follow up in their houses or in the hospital until their recovery and reintegration to their work activities.

The total of assistance during three long years, 12.3% turned out to be positive (4,160), being the largest number due to the Omicron variant.[4] This data agrees with other many different hospitals. Unfortunately, there were three diseased non-active workers during the pandemic period. From the total of 4,772 people, any active worker died during

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the pandemic, it is a very relevant health fact considering the almost 5,000 deaths of the healthcare staff reported in all the country during pandemic. The low prevalence of the nosocomial acquisition of the infection, only 3% of the cases, as well as the nil mortality in the active staff taking care of COVID-19 patients is a success of the Occupational and Preventive Health Care Program and the institutional work, explained by the extensive and continuous campaign on the correct use of the PPE, the availability of this resources and the early detection of sick people, avoiding the contact with the rest of their peers at work.

In a pandemic as the one we lived, it is very important to have in the healthcare centers the enough amount of staff, in good conditions, trained and with the service vocation. Undoubtedly, the doctor Salazar and his group of work did a great job, permanently, 24 hours the seven days of the week, in benefit of the workers as the text shows.

There are many lessons learned during the SARS-CoV-2 pandemic and they must be part of the protocol of action for new possible events. Without doubt, some of these lessons are resource management; offering training to the healthcare staff and provide healthcare through an organized and permanent program that facilitates the work conditions, safety and mental and physical health.

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# Occupational COVID-19 at the National Institute of Respiratory Diseases, Mexico City during the pandemic

## COVID-19 ocupacional en el Instituto Nacional de Enfermedades Respiratorias, Ciudad de México, durante la pandemia

Miguel Ángel Salazar-Lezama,* Hilda Patiño-Gallegos,* Misael Osmar García-Martín,* Fernando Manuel Sosa-Gómez,* Eduardo Becerril-Vargas,* Montserrat Sánchez-Rivera,* Luis Enrique Morales-Bartolo,* Manuel Estrada-Colín,* Nadia León-González,* Edgar Alejandro Monroy-Olascoaga,* Mariano Miguel-Guerra,* María de los Ángeles Martínez-Torres,* Aldo Cantero-Morales,* Nadia Otilia Díaz-Vázquez,* Ana María Vega-Martínez,* Víctor Hugo Ahumada-Topete*

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**ABSTRACT. Introduction:** the pandemic caused by the SARS-CoV-2 virus faced Health Care Workers (HCW's) with achallenge like never before. The Ismael Cosío Villegas National Institute of Respiratory Diseases, (INER), of Mexico, became a care center for patients with COVID-19. **Objective:** to publicize the results of a control program, in INER workers, based on frequent tests in oro/nasopharyngeal sampling to determine the presence of the virus, and thus isolate positive cases and detect asymptomatic ones. **Material and methods:** an oro/nasopharyngeal swab was performed for SARS-CoV-2 test by RT-PCR in all de HCWs who attended to Occupational Medicine Service. In case of being positive, he/she isolated him/herself at home for fourteen days. An epidemiological questionnaire was obteined if the acquisition of the disease had been community or nosocomial. A new sample was taken every 14 days until negative. **Results:** 33,780 tests were performed on 4,772 of the HCW's during the period April 2020-June 2023, of these, 4,160 were found to be positive. The months of January and July 2022 were the months with the most cases, (789 and 636, respectively). The nursing staff was the most affected with 1,106 positive cases. **Conclusions:** the application of a care protocol to the HCWs proved to be efficient in protecting with a low infection rate due to the use of PPE, continuous training and frequent control tests to avoid intrahospital transmission with zero mortality.

**Keywords:** health care workers, SARS-CoV-2 infection, COVID-19.

**RESUMEN. Introducción:** la pandemia ocasionada por el virus SARS-CoV-2 enfrentó al personal de salud a un reto como nunca antes. El Instituto Nacional de Enfermedades Respiratorias Ismael Cosío Villegas, (INER), de México, se convirtió en un centro de atención para pacientes con COVID-19. **Objetivo:** dar a conocer los resultados de un programa de control, en el personal de salud, con base en pruebas frecuentes en toma de muestras nasofaríngeas para determinar la presencia del virus; y así, aislar a los casos positivos y detectar a los asintomáticos. **Material y métodos:** mediante un protocolo de atención definido se tomaron muestras a todo trabajador que acudía a la coordinación. En caso de ser positivo se aislaba en su domicilio por 14 días; en un cuestionario epidemiológico se definió si la adquisición de la enfermedad había sido comunitaria o nosocomial. Posteriormente, cada 14 días se tomaba nueva muestra hasta negativa. El protocolo se modificó en las diferentes olas que se presentaron. **Resultados:** durante el período abril 2020-junio 2023, se efectuaron 33,780 pruebas a 4,772 trabajadores del personal de salud; de éstas 4,160 resultaron ser positivas. Los meses de enero y julio de 2022 fueron los meses con más casos (789 y 636, respectivamente). El personal de enfermería fue el mayormente afectado con 1,106 casos positivos. **Conclusiones:** la pandemia afectó de manera importante al personal de salud del instituto. Sin embargo, el aislamiento oportuno y las pruebas frecuentes evitaron muertes en los trabajadores. La gran mayoría fue de origen comunitario, tal como se reporta en la literatura.

**Palabras clave:** personal de salud, COVID-19, infección por SARS-CoV-2.

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## INTRODUCTION

17 years after 2003 and the epidemic by severe acute respiratory syndrome (SARS), a new coronavirus, the severe acute respiratory syndrome 2 (SARS-CoV-2) was isolated in the bronchoalveolar lavage in various patients with pneumonia of unknown origin in Wuhan, China,[1] which provoke a big amount of infections and a significant number of deaths, which led the World Health Organization (WHO) to declare the coronavirus disease (COVID-19) as an emergency of concern. By the 20th of February 2020 a total of 81,109 confirmed cases by laboratory had been reported.[2] In addition to the previous, cases of nosocomial spread among healthcare staff were reported, some severe and with high mortality.[3,4] Obviously, the healthcare staff was the first line of response to COVID-19, leaving them in a high risk of acquiring the disease, exposing the same patients and the community.

In this context, the National Institute for Respiratory Illness Ismael Cosío Villegas (INER), Mexico City, on the imminent arrival of the virus, it started the preparations of hospital conversion for the care of affected patients by COVID-19 with the training on the correct use of the personal protective equipment to the staff, particularly because there was an important hiring of staff to face the contingency.

Due to the concern to maintain the healthcare staff safe and protected from the disease, the Preventive and Occupational Health Care Coordination was created; so, as far as it is possible, being able to control the spreading among the healthcare staff, detect early complications and do not wear down the staff due to the lack of personnel because of isolation.

This report is the result of the prospective patient cohort of which was already published previously by our team and complete the years 2020-2023.[5]

## MATERIAL AND METHODS

In April 2020, the control care program to the healthcare staff in the external consultation began. The original staff was a total of 4,772: 2,823 in the frontline (nurses, doctors, stretcher-bearers, custodial staff, laboratory), 1,336 in the second line (administrative staff that is not in contact with patients) and 613 that were other part of the staff or of third line. This were registered in a data base on Microsoft Excel 16.16.27l, the electronic file was also checked. Descriptive data was use in the statistical package SPSS statistics version 25 to calculate median and interquartile age of the evaluated groups.

### Consultation care protocol

During the evolution of the pandemic, three processes were implemented: the first was carried out based on the protocol published by Bielicki and collaborators[6] in the «first wave» of cases. This consisted of granting consultation to both symptomatic and asymptomatic patients, in the event that they were contacts of the sick partner. Epidemiological questionnaire (SISVER) and nasopharyngeal sampling for SARS-CoV-2 were performed. Symptomatic patients were clinically assessed based on symptoms, vital signs, oxygen saturation, and chest CT scan. In case of alarm, the probable hospitalization was decided; if not, they were sent to isolation at home until receiving the result of the sample, in case of being positive they were informed by telephone and a questionnaire was carried out to differentiate between community or nosocomial infection. The isolation lasted 14 days, repeating the process until they tested negative to return to work the next day, depending on their symptoms. Contacts were sent to their place of work to wait for results with strict use of personal protective equipment.

The second process, according to the pandemic, was evolving, and due to the massive vaccination of personnel against SARS-CoV-2, as well as the changes in the variants of the virus, it was modified to seven days, based on the modified guidelines of the United States Center for Disease Control (CDC) with a grace of three more, incase of symptoms, in addition to this time a rapid test was carried out for control.[7,8] At the end of June 2023, the five-day policy was adopted in the third process, without a control test.

### Laboratory diagnostic tests for SARS-CoV-2

#### *1. Luminex viral panel*

**RNA extraction.** RNA was extracted from 200 μL of oropharyngeal/nasopharyngeal exudate samples contained in universal transport medium, the extraction was done automatically in the BIONEER ExiPrep 96 equipment, using the BIONEER brand ExiPrep 96 Viral DNA/RNA extraction kit (Ref. K-4614), following the manufacturer's specifications.

#### *2. Luminex*

Detection of HCoV subtypes was performed by xTAG RVP fast v2 assay. The Luminex assay includes reagents to detect 19 viral types and subtypes, including four HCoV species (HKU1, 229E, OC43, and NL63).

#### *3. RT-PCR*

For the viral RNA amplification assay, GeneFinder™ COVID-19 Plus RealAmp Real-Time PCR Kit, Gene Finder brand (Ref. IFMR-45), which amplifies the RNA of the RdRP, N and E genes. For this process, the manufacturer's specifications were followed, the reaction mixture was

made by mixing 10 μL of the master mix and 5 μL of the probe mixture, finally 5 μL of the nucleic acid extract will be added for each sample, to have a final volume of 20 μL. RT-qPCR shall be run in a Quant Studio 5 thermocycler (Applied Biosystems) under the following amplification conditions: 50 °C/20 minutes, 95 °C/5 minutes, followed by 45 cycles of 95 °C/15 seconds and 58 °C/60 seconds.

## RESULTS

From April 2020 to June 3, 2023, 33,780 tests were performed on 4,772 workers; of these, 2,977 were women and 1,795 men, median age 36 years (interquartile range [IQR] 28.00-45.00). In total, of the 33,780 tests performed there were 4,160 positive cases during these years. In the four years, the number of infected cases was: 2020, 737; 2021, 464; 2022, 2,421 (in this year, due to the Omicron variant of the virus, the months of January and July were the ones with the highest number of cases); and 2023, until June 30, 538 cases *(Table 1)*.

*Table 2* separates by lines of care the total staff at that time, and the cases of infection among them, where the first line was the one with the highest number of cases; the staff most affected was nursing (out of a total of 1,420, there were 1,106 positive [77.88%]), followed by doctors (out of a total of 814, there were 574 [70.51%]). The above probably because this staff was the one with the largest number.

The results of the epidemiological questionnaire evaluated whether the acquisition of the infection had been in the community or in the hospital. The result was 4,023 community-acquired and 137 hospital-acquired, for a prevalence of 3% of hospital-acquired cases. *Figure 1* shows the positivity index in the different waves of the pandemic, in the fourth, a higher index is noted because, due to the characteristics of the pandemic, the tests were only carried out on symptomatic personnel, since when different viruses appeared they caused respiratory disease.

In a work published by us,[5] we reported in a period of six months a prevalence of 3.8% in nosocomial acquisition. Of the hospitalized health staff there were 30 cases, of these there were two deaths, contingency personnel, with multiple comorbidities who unfortunately died within the institute and who acquired the infection in the community.

## DISCUSSION

Healthcare staff has experienced a significant burden in the fight against SARS-CoV-2 infection. The first reports indicated a high morbidity and mortality among health personnel;[3,9] but there were no conclusive results that could separate community infection from nosocomial infection. Hunter et al. concluded that the positivity rates in the clinical team of a hospital in England were not consistent with nosocomial infection[10] and that it had previously been reported in China.[11] In our healthcare staff we reported a prevalence of nosocomial infection at the beginning of the pandemic of 3.8%,[5] and as of June 30, 2023 this decreased to 0.7%. The use of personal protective equipment, the use of appropriate high-efficiency face masks and infection control training has been of great help, greatly reducing the risk of nosocomial transmission.[12,13]

**Table 1:** Breakdown by month and year of positive cases.

| Month | 2020 | 2021 | 2022 | 2023 | Total |
|---|---|---|---|---|---|
| January | | 139 | 789 | 194 | 1,122 |
| February | | 14 | 176 | 122 | 312 |
| March | | 20 | 31 | 135 | 186 |
| April | 44 | 5 | 9 | 42 | 100 |
| May | 90 | 6 | 41 | 36 | 173 |
| June | 70 | 8 | 351 | 9 | 438 |
| July | 64 | 94 | 636 | | 794 |
| August | 48 | 84 | 101 | | 233 |
| September | 95 | 37 | 23 | | 155 |
| October | 65 | 11 | 16 | | 92 |
| November | 102 | 7 | 30 | | 139 |
| December | 159 | 39 | 218 | | 416 |
| Total | 737 | 464 | 2,421 | 538 | 4,160 |

**Table 2:** General table of workers divided into the three lines and breakdown of positive cases by sex and episodes of infection.

| Cases April 2020-June 2023 | | | | | | | |
|---|---|---|---|---|---|---|---|
| **Variable** | **Women** | | | **Men** | | | |
| **Line** | **First** | **Second** | **Third** | **First** | **Second** | **Third** | **Overall total of staff** |
| Number | 1,801 | 792 | 384 | 1,022 | 544 | 229 | 4,772 |
| Years of age<br>Median<br>Interquartile range | 32<br>28.00-40.00 | 43<br>32.00-52.00 | 26<br>24.00-29.00 | 32<br>28.00-39.50 | 41<br>32.00-53.00 | 27<br>24.00-37.00 | 36<br>28.00-45.00 |
| Total attention given 33,780 | | | | | | | |
| Positive cases | 2,659 | | | 1,501 | | | 4,160 |
| Cases by number of infection episodes | | | | | | | |
| 1 time positive | 1,690 | | | 941 | | | 2,631 |
| First line | 1,150 | | | 613 | | | 1,763 |
| Second line | 382 | | | 237 | | | 619 |
| Third line | 158 | | | 91 | | | 249 |
| 2 time positive | 881 | | | 501 | | | 1,382 |
| First line | 655 | | | 358 | | | 1,013 |
| Second line | 215 | | | 130 | | | 345 |
| Third line | 11 | | | 13 | | | 24 |
| 3 time positive | 88 | | | 59 | | | 147 |
| First line | 70 | | | 44 | | | 114 |
| Second line | 17 | | | 13 | | | 30 |
| Third line | 1 | | | 2 | | | 3 |
| Overall total | 2,659 | | | 1,501 | | | 4,160 |

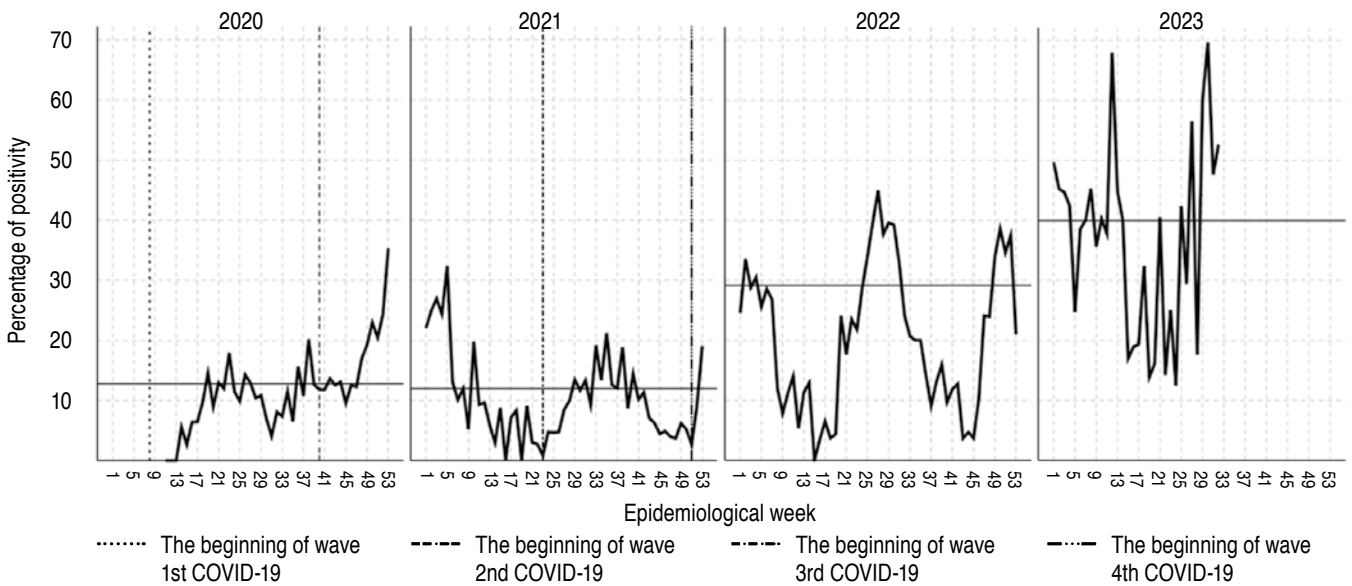


**Figure 1:** Percentage of positivity according to the epidemiological week, each column corresponds to a different year. The gray line of the X axis corresponds to the median of the percentage of annual positivity (2020: 12.82%; 2021: 11.96%; 2022: 29.17%; 2023: 39.93%).

In this context, the Occupational Health Coordination implemented a protocol to carry out frequent diagnostic tests and post-contact monitoring of symptomatic patients and their contacts in order to avoid high infection rates and a decrease in personnel due to multiple isolations. Oster et al. reported in Israel a low rate of positivity among health personnel, with nursing staff and the doctor being mostly affected,[14] this approach of testing asymptomatic contacts early allowed detecting cases without symptoms or slightly symptomatic, which led to early isolation and avoid outbreaks in the services.

Of the 33,780 tests carried out on 4,772 active workers, 4,160 were positive, which meant 12.31% of all tests carried out. The staff with the highest number of positives was the first line (1,106 nursing), which has been reported in other studies;[15-17] however, the above may be due to the fact that it was the one with the highest number of members. Fortunately, the cases presented with mild to moderate symptoms, probably due to the fact that they were young health personnel and the vast majority had no comorbidities.

When a proactive epidemiological questionnaire was applied, it resulted in the majority of infections being acquired in the community. The highest number of cases occurred in January and July 2022, due to the appearance of the omicron variant of the SARS-CoV-2 virus, which occurred in December 2021 and caused high levels of cases from that date, having its highest peaks in the community in those months. This was reported by the United States CDC.[18]

In total there were only 30 hospitalized workers, three of them with multiple comorbidities, who died at the beginning of the pandemic. All three acquired the infection in the community and arrived at the hospital in a very serious way.

## CONCLUSIONS

The results of this project, protecting the health and well-being of health personnel, were successful; it should be noted that the number of infections was low, the vast majority being a product of community transmission. Personal protective equipment, training, and testing were consistently shown to be effective in protecting workers within the hospital.

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To Dra. Silvia Pérez Pulido.

To the teacher Viridiana López Rodríguez.

But, above all, to the staff of the Institute for their work during the pandemic.

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# Serum CD26 levels and fibroblast phenotypic markers in patients with tracheal stenosis secondary to orotracheal intubation

## Niveles séricos de CD26 y marcadores fenotípicos de fibroblastos en pacientes con estenosis traqueal por intubación

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**ABSTRACT. Introduction:** orotracheal intubation stenosis (OTIS) is the most common cause of benign tracheal stenosis. It is the result of a deregulated healing response, a process in which dipeptidyl peptidase-4 (DPP-4 or CD26) has been proposed as one of the molecules with a possible regulatory role. This work aims to evaluate the relationship between serum levels and tissue expression of CD26 with OTIS and its complications, as well as to describe the expression of myofibroblasts by immunofluorescence. **Material and methods:** a case-control study was carried out; serum and tissue CD26 levels were measured. The characteristics of the cases (patients with tracheal stenosis) versus controls (healthy) were compared, as well as associations between serum DPP-4 and surgical variables (bleeding, complications, type of anastomosis, etc.); subsequently, a logistic regression model was performed to evaluate the association of DPP-4-S and the presence of OTIS. The expression of DPP-4 and myofibroblasts in tracheal tissue was also qualitatively evaluated. **Results:** 22 cases and 22 controls were analyzed. In the analysis of the cases, no differences were found between pre-surgical and three months post-surgical DPP-4-S levels. In the logistic regression analysis, DPP-4-S levels did not show adequate sensitivity and specificity to discriminate OTIS; the expression of myofibroblasts in the tracheal tissue analyzed by immunofluorescence revealed an increase in their expression. **Conclusions:** under the conditions of this study, DPP-4-S levels did not adequately discriminate cases of OTIS, although its expression was found to increase in tracheal

**RESUMEN. Introducción:** la estenosis traqueal secundaria a intubación orotraqueal es la causa más común de estenosis traqueal benigna. Es el resultado de una respuesta desregulada de cicatrización, proceso en el cual la dipeptidil peptidasa-4 (DPP-4 o CD26) se ha propuesto como una de las moléculas con posible papel regulatorio. El trabajo tiene por objetivo evaluar la relación entre los niveles séricos y la expresión tisular de CD26 con la estenosis traqueal secundaria a intubación orotraqueal y sus complicaciones, así como describir la expresión de miofibroblastos mediante inmunofluorescencia. **Material y métodos:** se realizó un estudio de casos y controles; se midieron niveles séricos y tisulares de CD26. Se compararon las características de los casos (pacientes con estenosis traqueal) versus controles (sanos), así como asociaciones entre DPP-4 sérica y las variables quirúrgicas (sangrado, complicaciones, tipo de anastomosis, etcétera); posteriormente, se realizó un modelo de regresión logística para evaluar la asociación de DPP-4-S y la presencia de estenosis traqueal secundaria a intubación orotraqueal. Además, de manera cualitativa, se evaluó la expresión de DPP-4 y miofibroblastos en tejido traqueal. **Resultados:** se analizaron 22 casos y 22 controles. En el análisis de los casos no se encontraron diferencias entre niveles de DPP-4-S prequirúrgicos y tres meses posquirúrgicos. En el análisis de regresión logística los niveles de DPP-4-S no mostraron una adecuada sensibilidad y especificidad para discriminar estenosis traqueal secundaria a intubación orotraqueal; la expresión de miofibroblastos en el tejido traqueal analizado por inmunofluorescencia reveló un aumento en la

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tissue, and it cannot be ruled out that it may serve as a therapeutic target in the early stages of tracheal stenosis secondary to orotracheal intubation or before its formation.

**Keywords:** tracheal stenosis, CD26, tracheoplasty, DPP-4, myofibroblasts.

expresión de éstos. **Conclusiones:** bajo las condiciones de realización de este estudio, los niveles de DPP-4-S no discriminaron adecuadamente los casos de estenosis traqueal secundaria a intubación orotraqueal, aunque su expresión se encontró incrementada en tejido traqueal; y no se descarta que pueda fungir como blanco terapéutico en etapas tempranas de la estenosis traqueal secundaria a intubación orotraqueal o previo a su formación.

**Palabras clave:** estenosis traqueal, CD26, traqueoplastía, DPP-4, miofibroblastos.

**Abbreviations:**

DPP-4 or CD26 = dipeptidil peptidasa-4.
Serum-DPP-4 = serum dipeptidyl peptidase-4.
TSSOI = tracheal stenosis secondary to orotracheal intubation.
FAP = fibroblast activation protein.

## INTRODUCTION

Stenosis caused by prolonged intubation is the most common cause of benign tracheal stenosis. Its incidence has been reported between 0.3-11% and up to 20% of people undergoing tracheal intubation in some studies.[1,2] Severe acute respiratory syndrome due to coronavirus 2 (SARS-CoV-2) and the associated COVID-19 pandemic have caused an increase in critical patients requiring prolonged mechanical ventilation,[3] with an expected increase in the frequency of tracheal pathologies, including tracheal stenosis, in the coming years.[4]

Surgical treatment is the first choice and although it is usually successful in most cases, the recurrence of the disease remains a major obstacle, which has motivated much of the research on the pathogenesis of tracheal stenosis. In this regard, several studies have demonstrated the role of inflammation signaling pathways and infectious processes in the development of laryngotracheal stenosis.[5] Thus, it has been seen in murine fibroblasts and human dermal fibroblasts that soluble dipeptidyl peptidase-4 (DPP-4 or CD26) activates NF-κB and SMAD signaling through PAR2, which leads to the activation of dermal fibroblasts, so it has been suggested that elevated levels of circulating soluble DPP-4 could function as one of the mediators that induce fibrosis in patients.

Currently, there is no universally validated classification that includes a specific therapeutic recommendation and an associated prognosis, however, an integrative classification of the main airway has been proposed that considers the cause of stenosis, magnitude of obstruction, involvement of the mucosa and wall, number of stenotic lesions, presence of fistulas, among other characteristics.[6] This work focuses on tracheal stenosis secondary to orotracheal intubation, which is the most frequent and responsible cause of 48 to 55% of cases.[7] It is often considered that the duration of intubation is the most important risk factor for the development of tracheal stenosis secondary to orotracheal intubation (TSSOI), in both adults and children;[4] although it has also been documented that tracheal stenosis is common even in patients intubated for short periods of time.[8] It is currently believed that aberrant scarring leads to the onset of TSSOI. The normal functional process of wound healing goes through four programmed phases: hemostasis, inflammation, proliferation, and epithelialization or remodeling. These phases are synchronized, temporally controlled, and involve a complex interaction between different cell types, cytokines, mediators, and the vasculature. Phases 1-3 typically last up to three weeks, while the remodeling phase lasts from weeks to years.[9]

Multiple mechanisms involved in the formation of TSSOI have been described, including: the TGF-β superfamily, mucosal trauma, ischemia, biomechanical stress, bacterial translocation, and fibrosis.[10] We particularly focus on the role of myofibroblasts and DPP-4. Current evidence demonstrates that fibroblasts undergo a shift towards myofibroblastic phenotype in response to hypoxia suffered by fibroblasts in TSSOI. This supports the role of hypoxia in the initial pathogenesis of TSSOI, leading to a transdifferentiation of resident fibroblasts into contractile and profibrotic myofibroblasts.[11]

The importance of the CD26 gene family in the regulation of critical biochemical pathways continues to be evident. The two most studied members of the family, CD26 and fibroblast activation protein (FAP), have been investigated as both disease therapeutic targets and diagnostic biomarkers. Their interest as potential biomarkers has been driven mainly by the observation of altered expression profiles in inflammatory diseases and cancer. In addition, the stability and persistence of these soluble proteins in serum make them an attractive proposition as serological markers.[12] The DPP-4 inhibitor linagliptin has been shown to abrogate the expression of fibrotic proteins (such as elastin and a-SMA), and prevent DPP-4-induced activation of transcription factor signaling pathways.[13]

The current management of patients with tracheal stenosis is surgical (tracheal resection with anastomosis),[14] provided that the clinical and anatomical conditions of the patient allow it. Otherwise, there are other alternatives such as laser resection or endoscopic dilation, placement

of stents, interposition grafts, Montgomery splints and, as a last option, tracheostomy.[7,15] Multiple medical interventions have been used with the aim of intervening in the inflammatory process inherent to tracheal stenosis, in order to decrease the rate of restenosis and thereby offer the best alternatives to the patient, these range from the use of non-steroidal anti-inflammatory drugs and steroids, mitomycin C, antibiotics, PPAR receptor agonists (such as lanifibranor), among other therapies that are under investigation.[7,16,17] So a better understanding of the mechanisms underlying the inflammatory and healing process in patients with TSSOI is a relevant field, due to its potential to allow the development of targeted anti-inflammatory therapies. CD26 shows promise in various organs and in various forms of acute and chronic fibrosis.[18] Inhibition of enzyme activity with diprotin A has been shown to result in decreased scarring, making DPP-4 an attractive molecule as a potential therapeutic target or biomarker in TSSOI.[19]

This research work aims to evaluate the relationship between serum levels and tissue expression of CD26 with TSSOI and its complications, as well as to describe the expression of myofibroblasts by immunofluorescence, with the present project being the first in the literature to seek to demonstrate both relationships. Thus, this research seeks to contribute to the understanding of the molecular mechanisms involved in the development of TSSOI.

## MATERIAL AND METHODS

Case-control study 1:1. All post-operative tracheoplasty patients diagnosed with TSSOI were included in the National Institute of Respiratory Illness Ismael Cosío Villegas (INER). Non-probability sampling was performed for convenience. Information was collected from 22 cases and 22 controls. The recruitment period was from November 2021 to March 2023.

**Table 1:** Surgical variables of post-operative patients for tracheal stenosis secondary to orotracheal intubation. N = 22.

| Qualitative variables | n (%) |
|---|---|
| Tracheostomy | |
| Yes | 6 (27) |
| No | 16 (73) |
| Type of anastomosis | |
| C-T | 6 (27) |
| T-T | 16 (73) |
| She/He presented complications | |
| Yes | 2 (9) |
| No | 20 (91) |
| **Quantitative variables** | **Median [p25-p75]** |
| Intubation days | 14.5 [9-18] |
| Number of previous dilations | 1 [1-2] |
| Number of tracheal rings resected | 4 [3-5] |
| Bleeding, (mL) | 100 [70-120] |
| Surgical time, (min) | 190 [160-210] |

C-T= crico-tracheal anastomosis. T-T= tracheo-tracheal anastomosis.

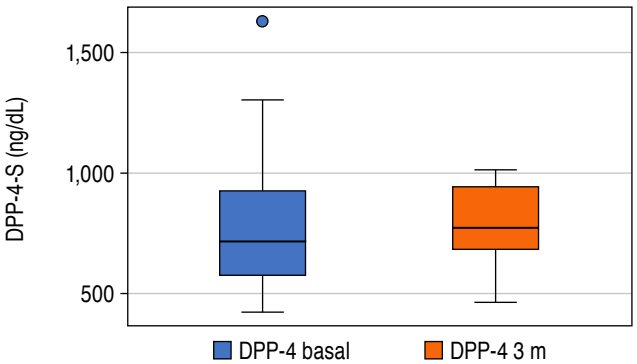


**Figure 1:** Pre- and post-surgical serum dipeptidyl peptidase-4 levels in cases (patients with tracheal stenosis secondary to orotracheal intubation).

The inclusion criteria for the cases were: patients diagnosed with TSSOI, over 18 years of age, tracheoplasty candidates who had signed informed consent and CRP for SARS-CoV-2 negative upon admission. For controls: INER resident doctors or people who come to the Blood Bank Area to donate blood components, over 18 years of age and who signed the informed consent. Exclusion criteria: patients diagnosed with kidney, autoimmune, liver disease or cancer in the five years prior to their surgical procedure, intake of antibiotics, steroids or anti-inflammatories up to seven days before the surgical procedure or use of DPP-4 inhibitors. Elimination criteria: loss at follow-up, alteration in initial inflammatory markers (CRP > 2 mg/dL, procalcitonin > 1 ng/mL) or patient request for withdrawal from the study.

The following clinical laboratory studies were performed: complete blood biometry, blood chemistry, lipid profile, glycosylated hemoglobin, C-reactive protein, and procalcitonin, as well as base line measurement of serum CD26 on the day of hospital admission. In the surgical procedure, a representative part of the resected surgical piece (stenotic tracheal rings) was taken for the qualitative determination of membrane CD26 staining and the determination of myofibroblast expression by immunofluorescence (FAP, SMA, vimentin and alpha smooth muscle actin). Complications were followed up in the first three months and control serum CD26 was determined between eight and 2 weeks after the surgical procedure.

Serum from patients was obtained in a tube without anticoagulant, centrifuged at 1.800 rpm for 15 minutes at 4 °C (Eppendorf 5810R); subsequently, they were

stored in polypropylene tubes at -20 °C. Quantification of plasma CD26 levels was performed by Enzyme-Linked Immunosorbent Assay (ELISA) MyBioSource 96-well MBS2882455 (San Diego; California, United States).

For the immunofluorescence protocol the tissue was cryoprotected with Tissue-Tek and stored at –80 °C. 8 µm thick sections were made on the cryostat, fixed with 4% paraformaldehyde and washed twice with PBS. Sections were washed with 1% blocking serum in PBS-T (PBS with 0.4% Triton X-100). Nonspecific binding was blocked by incubating tissue sections with 5% serum in PBS-T for 30 minutes at room temperature.

Primary antibody diluted in PBS-T from 1% animal serum (CD26 Recombinant Rabbit Monoclonal Antibody [JM11-42], 100 µL) was added. Invitrogen; anti-vimentin antibody [RV202] - Cytoskeleton Marker, 100 µg. Abcam; anti-alpha smooth muscle actin antibody [1A4], 100 µgand Abcam FAPA Polyclonal Antibody, 100 µL. Bioss antibodies). The recommended dilution of the antibody specified in the data sheet was used.

Sections were washed with 1% PBS-T serum, and was added secondary antibody diluted in 1% PBS-T serum (goat anti-Rabbit IgG [H+L] Cross-Adsorbed Secondary Antibody, Alexa Fluor™ 55, 1 mg. Invitrogen and goat anti-Mouse IgG [H+L] Cross-Adsorbed Secondary Antibody, Alexa Fluor™ 488, 1 mg invitrogen), was incubated at room temperature for one to two hours, with the recommended dilution of the antibody specified in the data sheet. After application of all primary antibodies, DAPI DNA binding dyes were applied and control was performed in fibroblast culture. Evaluation was performed with a ZEISS Axio Vert A1 microscope.

Healthy controls over 18 years of age who signed informed consent were also included, blood samples were taken for serum CD26 measurement, as well as complementary laboratories for their study (blood biometry, blood chemistry, CRP, procalcitonin, lipid profile and glycosylated hemoglobin).

**Table 2:** Median difference of serum dipeptidyl peptidase-4 values in categorical surgical variables.

| DPP-4-S (ng/dL) | Median [p25-p75] | p |
|---|---|---|
| Complications | | 0.6478 |
| Yes | 791 [558-1,023] | |
| No | 708 [528-908] | |
| Tracheostomy | | 0.1845 |
| Yes | 569 [509-722] | |
| No | 752 [537-980] | |
| Type of anastomosis | | 0.507 |
| C-T | 822 [538-937] | |
| T-T | 663 [528-841] | |

C-T= crico-tracheal anastomosis. T-T= tracheo-tracheal anastomosis.

**Table 3:** Correlation of serum dipeptidyl peptidase-4 values and quantitative surgical variables.

| Variable | Correlation coefficient | p |
|---|---|---|
| Intubation days | -0.2154 | 0.3358 |
| Days with tracheostomy | -0.3201 | 0.1464 |
| Number of dilations | -0.0799 | 0.7236 |
| Number of rings resected | -0.1595 | 0.4784 |
| Bleeding | 0.4893 | **0.0208** |
| Surgical time | -0.061 | 0.7874 |

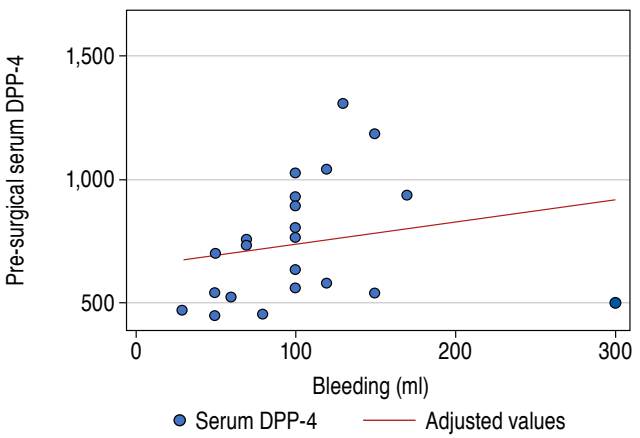


**Figure 2:** Serum dipeptidyl peptidase-4 levels and transoperative bleeding.

All data was collected in an Excel spreadsheet. The information collected was analyzed with the Stata® version14 program for Mac. Simple frequencies and percentages were calculated for qualitative variables and central tendency and dispersion measures were calculated for quantitative variables. The type of graph was chosen according to the nature of the variables according to whether they were quantitative or qualitative.

Statistical analysis: Differences in DPP-4 levels were analyzed according to the different surgical variables (surgical time, type of anastomosis, bleeding, complications, type of suture). A description of the characteristics of the controls was made and finally the comparison of the characteristics between the cases and the controls was made with the Fisher's exact test for the case of qualitative variables and with the Mann-Whitney or Kruskal Wallis U tests (according to the number of categories of the variable) for quantitative variables. Wilcoxon's sign range test for paired data was also performed, and whether changes in pre and post-surgical Serum DPP-4 levels were statistically significant was evaluated. Additionally, a logistic regression model was performed to evaluate whether Serum DPP-4

**Table 4:** Qualitative variables of controls divided by subgroup. N = 22.

| Variable (category) | Residents (N = 11) n (%) | Non-residents (N = 11) n (%) | p |
|---|---|---|---|
| Gender | | | 0.99 |
| Female | 2 (18) | 1 (9) | |
| Male | 9 (82) | 10 (91) | |
| Eage | | | 0.99 |
| ≤ 33 | 7 (64) | 6 (55) | |
| > 33 | 4 (36) | 5 (45) | |
| Weight, (BMI) | | | 0.562 |
| Normal | 5 (46) | 3 (27) | |
| Overweight | 4 (36) | 3 (27) | |
| Obese | 2 (18) | 5 (46) | |
| Comorbidities* | | | 0.476 |
| Yes | 2 (18) | 0 (0) | |
| No | 9 (82) | 11 (100) | |
| Tobacco use | | | 0.99 |
| Yes | 1 (9) | 0 (0) | |
| No | 10 (91) | 11 (100) | |
| Alcohol consumption | | | **0.04** |
| Yes | 7 (64) | 2 (18) | |
| No | 4 (36) | 9 (82) | |
| History of COVID-19 | | | **0.002** |
| Yes | 10 (91) | 2 (18) | |
| No | 1 (9) | 9 (82) | |

BMI = body mass index. COVID-19 = coronavirus disease 2019.
* They include: diabetes, systemic high blood pressure, drug addiction, thyroid disease, epilepsy, and asthma.

could be a predictor of tracheal stenosis and a sensitivity and specificity analysis of the test was performed.

The study was approved by the INER Ethics and Research Committee, with approval number C 14-22. Healthy patients and controls were asked to sign the informed consent letter. The study followed the rules of the General Health Law for Research and the Declaration of Helsinki.

## RESULTS

A total of 22 cases and 22 controls were included in the study. *Table 1* shows the characteristics of the surgical procedure in patients with TSSOI surgical treatment. About 73% of patients underwent tracheo-tracheal anastomosis and most had no complications. As part of the detailed case analysis, differences in pre-surgical and post-surgical Serum DPP-4 levels were evaluated *(Figure 1)*; no statistically significant differences were found (p = 0.9738). Finally, we evaluated whether one or more surgical features were related to DPP-4 levels. This was performed on both categorical *(Table 2)* and continuous *(Table 3)* variables. The only variable that showed correlation with serum DPP-4levels was transoperative bleeding *(Figure 2)*.

Given the differences in recruitment, in the first part of this research work,19 in which INER resident physicians were recruited as controls (they were recruited from INER Blood Bank donors), a sub-analysis of the controls was performed, comparing residents and non-residentsin search of statistical differences that could point to a selection and information bias in the controls. When analyzing the primary dependent variable, non-significant differences in Serum DPP-4 levels were found between residents and

**Table 5:** Quantitative variables of the controls divided by subgroup. N = 22.

| Variable | Residents (N = 11) Median [p25-p75] | Non residents (N = 11) Median [p25-p75] | p |
|---|---|---|---|
| Serum DPP-4 (ng/dL) | 677 [577-700] | 927 [771-1,179] | 0.3981 |
| Leukocytes (cels/µL) | 6.4 [4.9-7.3] | 6.8 [5.8-8.3] | 0.0956 |
| Linfocitos (cels/µL) | 2.2 [1.6-2.8] | 2.4 [1.9-2.7] | 0.8233 |
| PMN (cels/µL) | 2.8 [2.3-4.4] | 3.1 [3.7-4.5] | 0.0527 |
| Glucose (mg/dL) | 93 [90-96] | 81 [78-84] | 0.1805 |
| HbA1c (%) | 5.3 [5.2-5.5] | 5.5 [5.3-5.7] | 0.9159 |
| Total cholesterol (mg/dL) | 183 [143-215] | 161 [152-200] | 0.4455 |
| Triglycerides (mg/dL) | 107 [86-144] | 167 [76-211] | **0.05** |
| Atherogenic index | 3.7 [2.5-4.2] | 3.5 [2.9-4.9] | 0.2358 |
| CRP (mg/L) | 0.11 [0.04-0.2] | 0.17 [0.1-0.2] | **0.0001** |
| Procalcitonin (ng/mL) | 0.02 [0.01-0.03] | 0.02 [0.01-0.02] | 0.344 |

Serum DPP-4 = serum dipeptidyl peptidase-4. PMN = polymorphonuclear. CRP = C-reactive protein.

**Table 6:** Quantitative variables of cases versus controls. N = 22.

| Variables | Cases median [p25-p75] | Controls median [p25-p75] | p |
|---|---|---|---|
| Serum DPP-4 | 708 [536-922] | 503 [10-679] | 0.3981 |
| Leukocytes | 6.9 [6.3-8.5] | 6.5 [5.4-7.3] | 0.0956 |
| PMN | 4.3 [3.7-5.1] | 3.6 [2.7-4.4] | 0.0527 |
| Glucose | 93 [82-104] | 87 [81-96] | 0.1805 |
| HbA1c | 5.4 [5.2-5.8] | 5.4 [5.2-5.6] | 0.9159 |
| Total cholesterol | 166 [138-190] | 170 [146-200] | 0.4455 |
| Triglycerides | 187 [124-212] | 121 [78-181] | **0.05** |
| Atherogenic index | 4.2 [3.1-5] | 3.7 [2.7-4.6] | 0.2358 |
| CRP | 0.35 [0.3-0.4] | 0.115 [0.1-0.2] | **0.0001** |
| Procalcitonin | 0.02 [0.01-0.03] | 0.02 [0.01-0.02] | 0.344 |

Serum DPP-4 = serum dipeptidyl peptidase-4. PMN = polymorphonuclear. CRP = C-reactive protein.

non-residents. The comparison of the categorical variables is shown in *Tables 4 and 5*.

In the statistical analysis of cases versus controls, a higher frequency of comorbidities was observed among patients post-operated by TSSOI, who also showed statistically higher levels of triglycerides and C-reactive protein *(Table 6)*, which corroborated the absence of active inflammatory processes in the controls. In contrast, no statistical differences in serum DPP-4 levels were observed between the groups *(Figure 3)*.

A univariate logistic regression model was performed to evaluate whether Serum DPP-4 could be a predictor of tracheal stenosis, where an OR very close to 1 is observed, and a pseudo R2 of just 1.4%, implying that there is virtually no difference in Serum DPP-4 levels between cases and controls, and that the likely contribution of Serum DPP-4 as a predictor of stenosis is less than 2%.

The detection of FAP-α, CD26, α-SMA and vimentin was performed in trachea tissue, as well as its control. Phase contrast photographs (PH-C) were taken of the markings of each and the images of both markings were spliced (MERGE). Subsequently, the area positive to DAPI, FAP-α *(Figure 4A)*, CD26 *(Figure 4B)*, α-SMA and vimentin labels was quantified. An increase in the area percentage of markers CD26 and FAP-α was observed, suggesting architecture formation of the fibrotic microenvironment in the tissue. The increase in the area percentage of α-SMA suggests the presence of myofibroblasts in the tissue, cells associated with fibrotic tissue formation.

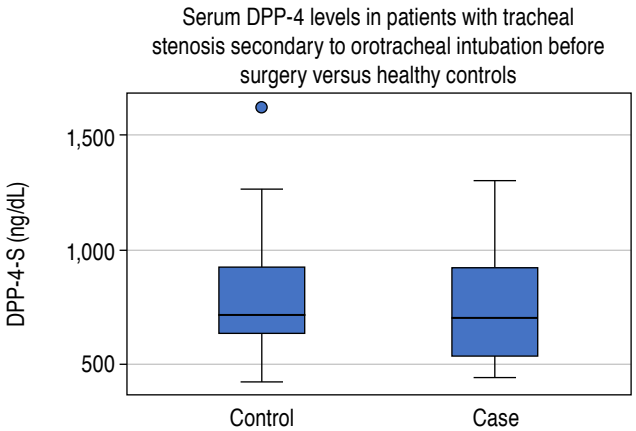


**Figure 3:** Dipeptidyl peptidase-4 levels in cases (N = 22) and controls (N = 22).

## DISCUSSION

The levels of Serum DPP-4 did not show adequate sensitivity and specificity to discriminate the disease, although CD26 tissue expression was found to be increased in the cases with respect to the control tissue.

In the analysis of the cases, no statistically significant differences were found between pre and post-surgical levels, a result that contrasts with what was identified in the first part of this study,[19] which can be explained by the significantly lower levels of DPP-4 in the controls included in said work. However, we must consider that the action of DPP-4 is complex and involves multiple signaling pathways and mechanisms that are still poorly understood,[13] while the effects of Serum DPP-4 could be independent of its enzymatic activity, which also plays an important role in inflammation.[20] In addition, tracheal stenosis is a pathology of very heterogeneous etiology,[21] in which the role of multiple pathophysiogenic mechanisms has been described.[22] It has been seen, for example, the clinical impact can vary depending on the type of stenosis,[23] for

which we cannot affirm that the simple values of Serum DPP-4 are the only marker of its activity, and we do not rule out its potential usefulness in other contexts of inflammation and scarring.

Not surprisingly, the positive correlation found between Serum DPP-4 levels and transoperative bleeding *(Figure 2)*, given the high frequency of DPP-4 expression in the endothelium and its recognized role in hemostasis.[11] In the case of the airways, Johnson et al.[24] found that several monoclonal antibodies that recognize CD26/Serum DPP-4 intensely stained the endothelium of the pulmonary capillaries, but not those of other types of large-caliber blood vessels,[20] leading us to think that the airway could have particularities that make it especially susceptible to

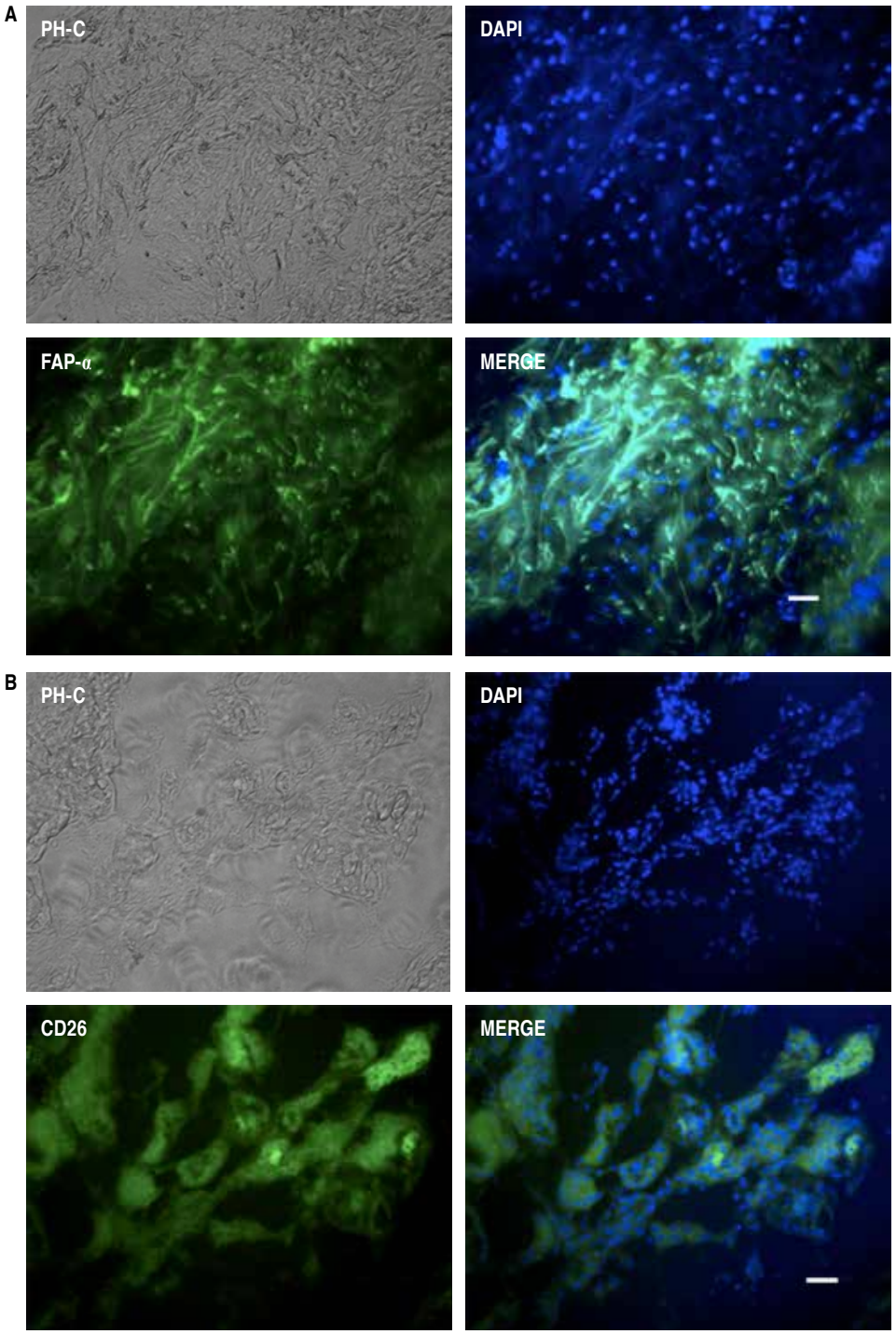


**Figure 4:**

**A)** Detection of FAP-α in tracheal tissue. Scale bar 20 μm. **B)** Detection of CD26 in tracheal tissue. Scale bar 20 μm.

altered healing processes, as has been hypothesized in the etiology of trachealstenosis.[18]

Intra-control analysis revealed differences in Serum DPP-4 levels between residents and non-residents. Likewise, when comparing other characteristics between both subgroups, residents had higher blood glucose and higher frequency of alcohol consumption and a history of COVID-19 (all with $p < 0.05$). To explore these results, we performed complementary exploratory linear regression models, in which an inverse relationship was observed between the history of COVID-19 and Serum DPP-4 ($p < 0.001$), while the significance of blood glucose and alcohol consumption was lost when controlling for other variables.

Thus, it is evident that there could be a relationship between SARS-CoV-2 infection and Serum DPP-4 activity. Although this relationship has not been fully elucidated, in one study it was observed that in patients infected with MERS-CoV, the concentration of Serum DPP-4 in plasma decreased significantly and correlated with the severity of the disease, while in 2020 a similar result was reported in patients with severe COVID-19.[25]

The results of this analysis showed no significant differences in sociodemographic and clinical characteristics between cases and controls *(Table 6)*. There was also no difference in the primary end point, Serum DPP-4 levels. However, there was a difference in CD26 expression in tracheal tissue. This could be due to several factors, including the half-life of Serum DPP-4,[20] the stage of tracheal stenosis in which it could have the greatest impact (acute versus chronic phase), or the time when its levels were measured in the cases, which was done prior to surgery, a time that does not necessarily correspond to the stages of stenosis formation.

This study does have its limitations. First, given that most of the patients referred for surgery came from other institutions, the interval between the onset of the pathology and the performance of the surgery is unknown, which has implications for the potential usefulness of the detection of DPP-4 in the initial stages of the pathogenesis of TSSOI. Finally, the immunofluorescence results are descriptive, so the relationship between serum and tissue levels of DPP-4 could not be statistically evaluated.

The investigation of other molecules as possible diagnostic, prognostic or therapeutic targets, especially in humans, is extremely valuable for the progress of scientific knowledge in this field, even if there are no significant differences since, as we know, «the lack of evidence is also evidence» and serves as a guide for future research, increasing the potential for the development of specific immunological, genomic and proteomic therapies for the treatment of inflammatory/fibrotic conditions, including TSSOI.[10,26]

## CONCLUSIONS

Under the conditions of this study, Serum DPP-4 levels did not adequately discriminate cases of TSSOI, although its expression was increased in tracheal tissue, so it is not ruled out that it may serve as a diagnostic marker or therapeutic target in early stages of TSSOI or prior to its formation, for example, at times close to intubation.

This article is one of the few studies in humans where serum and tissue levels of CD26 are evaluated, as well as the description of the transdifferentiation of fibroblasts to myofibroblasts in the diseased tissue of patients with ETSIO, serving as a precedent and making an invaluable contribution to the study of its pathophysiology, which opens the way to future research in the search for biomarkers in tracheal pathology.

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Review
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# Neutrophils as defense cells? Immunobiology and pathophysiology in human respiratory infectious diseases

## ¿Los neutrófilos como células de defensa? Inmunobiología y fisiopatología en las enfermedades infecciosas respiratorias humanas

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**ABSTRACT.** The immune system protects us from infections and the entry of any pathogen activates innate immunity. Neutrophils are part of this type of response and are the most abundant in the blood with a short half-life that increases when they are activated. They are generated in the bone marrow during granulopoiesis and their release into the blood depends on the binding of CXCR4-CXCL12. They are the first cells to reach the site of infection or inflammation, and their bactericidal mechanisms are phagocytosis, degranulation, production of ROS, NET, cytokines, and chemokines. During infections, they carry out phagocytosis characterized by direct phagosome-granule fusion, and the pathogens are killed by the action of toxic granule proteins and oxidant molecules (ROS and hypochlorous acid). Pathogens or cytokines promote degranulation which, together with the production of ROS and hypochlorous acid, act on proteins, DNA, and bacterial membranes favoring their elimination. Neutrophils produce NET to trap pathogens and prevent their spread, and they are also a source of cytokines and chemokines, which is why they participate in the regulation of the immune response. In human infectious diseases, their participation can help, or contribute to a poor prognosis, causing tissue damage. This review aims to know the generalities of neutrophils and their participation in human respiratory diseases such as COVID-19 and influenza, tuberculosis, and histoplasmosis.

**Keywords:** neutrophils, COVID-19, influenza, tuberculosis, histoplasmosis.

**RESUMEN.** El sistema inmunológico nos protege de las infecciones y la entrada de cualquier patógeno activa la inmunidad innata. Los neutrófilos son parte de este tipo de respuesta y son los más abundantes en la sangre con una vida media corta que se incrementa cuando están activados. Se generan en la médula ósea durante la granulopoyesis y su liberación a la sangre depende de la unión de CXCR4-CXCL12. Son las primeras células en llegar al sitio de infección o inflamación, y sus mecanismos bactericidas son la fagocitosis, la desgranulación, la producción de especies reactivas de oxígeno, trampas extracelulares de neutrófilos, citocinas y quimiocinas. Durante las infecciones, llevan a cabo la fagocitosis caracterizada por la fusión directa fagosoma-gránulo, y los patógenos mueren por la acción de proteínas granulares tóxicas y moléculas oxidantes (especies reactivas de oxígeno y ácido hipocloroso). Los patógenos o citocinas favorecen la desgranulación que, junto con la producción de especies reactivas de oxígeno y ácido hipocloroso, actúan sobre las proteínas, ADN y las membranas bacterianas favoreciendo su eliminación. Los neutrófilos producen trampas extracelulares de neutrófilos para atrapar los patógenos y evitar su propagación y, además, son fuente de citocinas y quimiocinas, por lo que participan en la regulación de la respuesta inmune. En las enfermedades infecciosas humanas su participación puede ayudar, o contribuir a un mal pronóstico, provocando daño tisular. Esta revisión tiene como objetivo conocer las generalidades de los neutrófilos y su participación en enfermedades respiratorias humanas como COVID-19, influenza, tuberculosis e histoplasmosis.

**Palabras clave:** neutrófilos, COVID-19, influenza, tuberculosis, histoplasmosis.

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**Abbreviations:**
cit-H3 = citrullinated histone 3.
G-CSF = granulocyte-colony stimulated factor.
HMGB1 = High mobility group box 1.
$HOCl^-$ = hypochlorous acid.
MCP-3 = monocyte chemotactic protein-3.
MPO = myeloperoxidase.
NAR = neutrophil-albumin ratio.
NE = neutrophil elastase.
NET = neutrophil extracellular traps.
NLR = neutrophil-lymphocyte ratio.
PKC = protein kinase C.
ROS = reactive oxygen species.
PTB = pulmonary tuberculosis.
IAV = influenza type A virus.

## INTRODUCTION

The immune system includes cells involved in innate immunity or acquired immunity to maintain body homeostasis. The entry of any pathogen triggers an innate response that is quick to eliminate the pathogen and prevent disease. This is mediated by the recognition of pathogen-associated molecular patterns and cellular damage-associated molecular patterns without generating immunological memory. Neutrophils are part of this response and their bactericidal mechanisms are phagocytosis, degranulation, production of reactive oxygen species (ROS), neutrophil extracellular traps (NET), cytokines and chemokines.

### *1. Neutrophils*

1.1 Origin and characteristics: they are generated in the bone marrow by granulopoiesis from a myeloid precursor and it has been estimated that a healthy adult produces 1-2 $\times 10^{11}$. Hematopoietic stem cells are located in the spaces created by osteoblasts and endothelial cells characterized by low blood flow and lower oxygen tension, while the more mature and cell-dividing cells are near the abluminal side of the sinusoids, a special vascular structure of the bone marrow.[1] After maturation, neutrophils are released into the blood and this process depends on the interaction of their chemokine receptor CXCR4 and the chemokine CXCL12 produced by stromal cells in the bone marrow.[2]

Their homeostasis is regulated by phagocytosis of apoptotic neutrophils by macrophages and dendritic cells in tissues, reducing their proliferation in an IL-23/IL-17A/G-CSF axis-dependent manner.[3] Phagocytosis decreases the production of interleukin 23 (IL-23), causing decreased production of IL-17A by neutrophil regulatory T lymphocytes or Th17 (Tn/Th17), which are located in mesenteric lymphoid nodules.[4] Consequently, low levels of IL-17A decrease the production of granulocyte-colony stimulated factor (G-CSF) by fibroblasts and endothelial cells reducing the production of mature neutrophils. On the other hand, inflammation or infection causes an increase in G-CSF favoring granulopoiesis, production and recruitment of neutrophils.[2,5] In addition, neutrophil homestoasis involves their cell death by necrosis, necroptosis, NET and pyroptosis *(Figure 1)*.[6,7]

They constitute 50-70% of circulating leukocytes, with a diameter of 7-10 $\mu$m, a segmented nucleus, high granule content and the presence of secretory vesicles in their cytoplasm.[2,5] Its half-life is eight to 20 hours without stimulus, although after its migration to tissues it lasts 1-4 days.[6]

They recognize pathogens through their membrane receptors such as: scavenger receptors, mannose receptors, Dectin 1, CD14, FcγR, CiqR, CR1, CR3, colectins, Toll-like receptors, NOD-like receptors.[7]

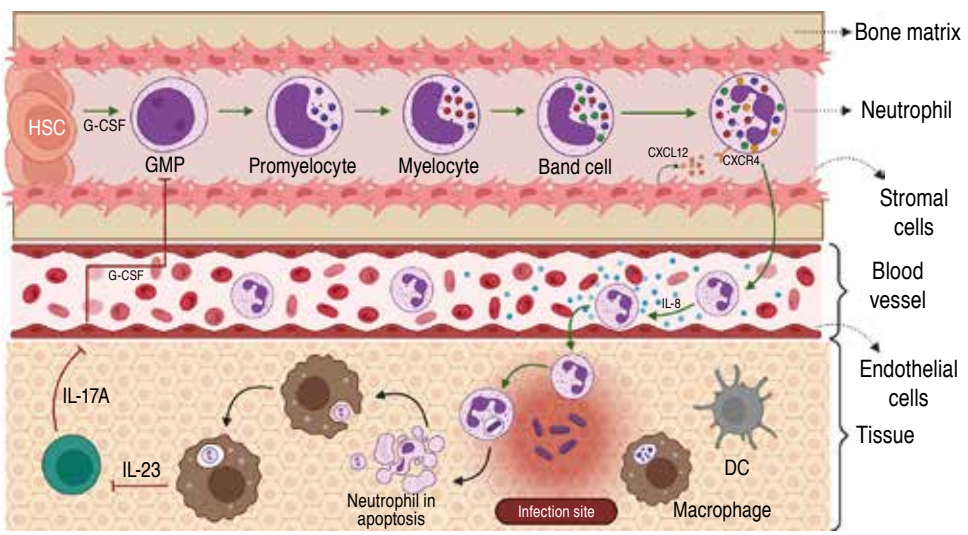


**Figure 1:**

**The origin, maturation and homeostasis of human neutrophils.** Neutrophils are generated in the bone marrow and during their maturation acquire cytoplasmic granules and are released into the bloodstream in an IL-23/IL-17A/G-CSF axis-dependent mechanism. These are directed to the site of infection in response to the chemotactic chemokine IL-8. (CXCL8) (Created by BioRender.com). DC = dendritic cell. G-CSF = granulocyte-colony stimulating factor. GMP = granulocyte/monocyte progenitor. HSC = hematopoietic stem cells.

Their cytoplasmic granules are classified into: Primary/azurophil granules, containing myeloperoxidase (MPO), serine proteases, neutrophil elastase (NE), proteinase 3, cathepsin G, azurocidin, α-defensins (HNP-1, HNP-2, and HNP-3), serprocidins, and Bactericidal-permeability-increasing protein (BPi). Secondary/specific granules, containing matrix metalloproteinase 8 (MMP8), lactoferrin, LL-37, lipocalin 2, haptoglobin, Pentraxin 3 and olfactomedin 4. Tertiary granules/gelatinase contain gelatinase B, MMP8, MMP9, Arginase-1, LL-37 and lysozyme, and secretory vesicles containing albumin, cytokines, membrane receptors (CR1, CR3, C1qR, FcγR, CD14, FPR1), cell adhesion molecules (CD11b/CD18, CD67) and part of the nicotinamide adenine dinucleotide phosphate oxidase (NADPH) complex. The granule proteins are acquired during granulopoiesis.[2,5,8,9]

## 2. Bactericidal mechanisms

2.1 Phagocytosis: is a process of ingestion and elimination of particles or pathogens that enter the organism larger than 0.5 $\mu$m, including apoptotic bodies.[10,11] Neutrophils, macrophages, monocytes and dendritic cells are classified as professional phagocytes, as they perform this task with great efficiency,[10] while fibroblasts, epithelial and endothelial cells are considered non-professional phagocytes in charge of eliminating dead cells to maintain homeostasis.[10,11]

It starts with ligand binding to phagocytic receptors, which are divided into opsonic receptors such as: IgG crystallizable fragment receptors (FcγR) and complement receptors (CiqR, CR1, CR3); and non-opsonic receptors such as: mannose receptors, Dectin 1, CD14, collectins, TLRs, C-type lectin and scavenger receptors.[10,12] Compared to macrophages, phagocytosis by neutrophils is rapid as the phagosome directly fuses with the cytoplasmic granules in less than 60 seconds, allowing rapid clearance of pathogens *(Figure 2A)*.[13,14]

Binding of the pathogen or particle causes a rapid oxidative and non-oxidative response by the assembly of the NADPH oxidase complex on the phagosome membrane.[9]

2.2 Degranulation: these cells also combat extracellular pathogens by releasing their cytoplasmic granules. Two types of signals are required: a) β2 integrin-dependent; and b) activation of receptors such as Mac1/CR3, FcγR and G Protein-Coupled Receptors (GPCR). This process involves the Rab and Soluble N-ethylmaleimide-sensitive factor attachment protein receptor (SNARE) proteins involved in the control of vesicular trafficking *(Figure 2B)*.

Degranulation of secretory vesicles and tertiary granules is rapid; however, primary granules require neutrophils preactivated with proinflammatory cytokines, chemokines or microbial components. Neutrophils prevent excessive degranulation of tertiary granules by increasing ROS production, as the dysregulated process can cause tissue damage.[2]

Phagocytosis and degranulation cause the assembly of the NADPH oxidase complex at the membrane, causing the production of ROS.[9,15]

2.3 Production of reactive oxygen species (ROS): the interaction of neutrophils with the pathogen triggers the oxidative burst involving the NADPH oxidase-NOX2 enzyme system, which assembles on the cell membrane and generates two $\bullet O_2^-$ molecules and the enzyme superoxide dismutase generates $H_2O_2$ that acts as an antimicrobial by reacting with the thiol groups of enzymes, proteins, DNA and bacterial membranes.[11,16-18]

In addition, MPO utilizes $H_2O_2$ and catalyzes the reaction with chloride ions, forming a hypochlorous acid (HOCl-) that is highly reactive with thiol groups and methionine residues *(Figure 2C)*.[11,19]

It is important to mention that some pathogens have generated a defense against ROS, but neutrophils possess other alternative bactericidal mechanisms.[20]

2.4 Production of neutrophil extracellular traps (NETs): neutrophils are killed by NETosis where NETs are generated, which are extracellular fibers composed of DNA, cytosolic proteins and antimicrobial granules that trap, neutralize and eliminate pathogens. This process begins with the loss of the lobular shape of the nucleus and disassembly of the nuclear membrane, loss of the permeability of the granular membranes, inactivation of histones by the action of NE that degrades the central histone H1 causing the decondensation of chromatin, causing the mixing of chromatin in the cytosol with the cytosolic and granular components, as well as the loss of the permeability of the cell membrane that allows the release of NETs into the extracellular space *(Figure 2D)*.[15,21,22]

It has been reported that the proteins identified in NETs may vary depending on the stimulus, since with the stimulus Phytohemagglutinin (PMA, Phorbol-Myristate-Acetate) 24 proteins were identified, and with *Pseudomonas aeruginosa* 80 proteins were identified;[15,21] although histones, NE, MPO, calprotectin, cathelicidins, α-defensins and actin are always found.[21]

NETs are formed by two pathways: a) NADPH oxidase-dependent (lytic, most studied) which is activated by antibodies, microorganisms, cholesterol and mitogens (PMA, concanavalin A).[23,24] The stimuli activate protein kinase C (PKC), which activates the assembly of NADPH oxidase to the membrane, initiating the production of ROS, which disintegrate the membranes of the nucleus and granules, allowing NE and MPO to interact with histones to facilitate chromatin decondensation.[23,24]

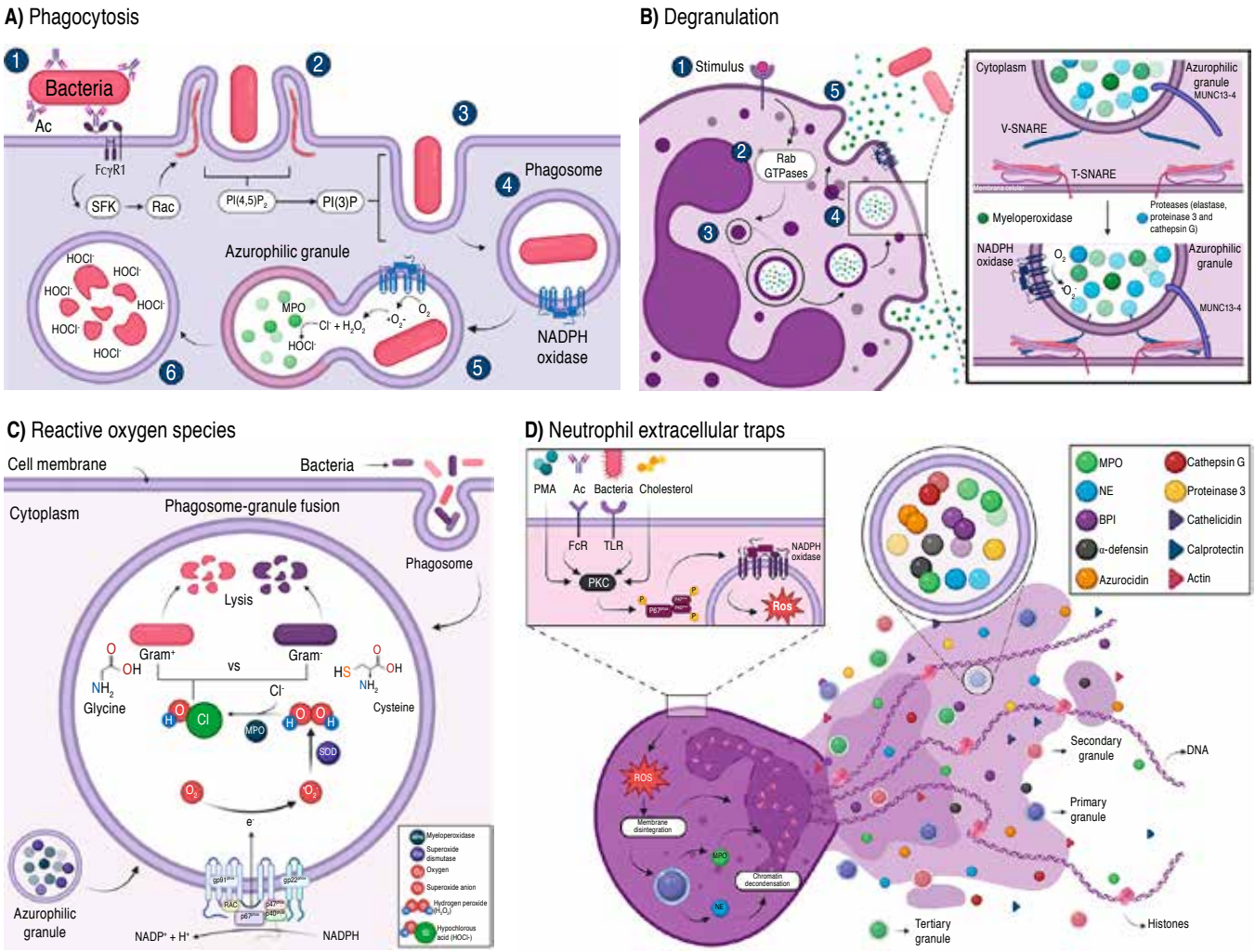


**Figure 2:** Bactericidal mechanisms of neutrophils. **A)** Phagocytosis. 1) Bacteria opsonized with IgG antibodies bind to FcγR1 receptors on the neutrophil membrane activating signaling (SFK-Rac), uncoupling the activation of Rab GTPases. 2) Actin fibers reorganize and form pseudopodia, associated with changes in membrane phospholipids ($PI(4,5)P_2$ to PI(3)P ) to envelop the bacterium. 3) The membrane is enveloped with the bacterium. 4) The phagosome is released into the cytoplasm and the NADPH oxidase complex is assembled to the membrane. 5) The phagosome fuses with the azurophilic granules, where NADPH oxidase produces ROS and MPO in the granules acts on $H_2O_2$ to produce hypochlorous acid (HOCl-). 6) Finally, HOCl-, ROS and proteases act to eliminate pathogens. **B)** Degranulation. Neutrophils stimulated with pathogens, microbial components cytokines or chemokines and respond by secreting their granules containing bactericidal molecules. 1) Stimulation of the neutrophil is accomplished by the interaction of the ligand with the FcγR receptor on the membrane. 2) Rab GTPases-mediated signaling is triggered. 3) The granules move through the actin fibers by the Rab. 4) Rab triggers the response of Munc13-4 molecules that interact with SNARE proteins, facilitating the binding of V-SNAREs (from granules) to T-SNAREs (from the membrane). In addition, NADPH oxidase is coupled to produce ROS. 5) The fusion of the membranes (cell and granule) allows the azurophilic granules to discharge their contents and ROS to the outside of the cell to act against extracellular pathogens. **C)** Reactive oxygen species (ROS). After phagocytosis of the pathogen the phagosome fuses with the azurophil granule and NADPH oxidase assembles to the membrane. NADPH oxidase catalyzes the reaction that generates one molecule of $NADP^+$ and one $H^+$, allowing two electrons to be released into the phagosome-granule that reduce oxygen ($O_2$) to superoxide anion($\bullet O_2^-$), and superoxide dismutase catalyzes the dismutation of $\bullet O_2^-$ to hydrogen peroxide ($H_2O_2$). From $H_2O_2$, MPO catalyzes the reaction that produces hypochlorous acid ($HOCl^-$), which has antimicrobial properties and acts on the cysteine or glycine of Gram-negative and Gram-positive bacteria, respectively. **D)** Neutrophil extracellular traps (NET). Some pathogens have the ability to escape phagocytosis or neutrophil degranulation; however, one of the alternatives for neutrophils to die is programmed death called NETosis, where NETs are produced. The NADPH oxidase-dependent or lytic pathway is triggered by stimulations (pathogens, antibodies, cholesterol and mitogens) that cause PKC activation and promote the assembly of the NADPH oxidase complex to the membrane and the production of ROS. The nuclear and granule membranes are disintegrated by ROS and chromatin is decondensed by the action of NE and MPO. DNA fibers are launched outward to trap pathogens and carry with them granules and components with bactericidal activity.

[Created with BioRender.com].

MPO = myeloperoxidase. NADPH = nicotinamide adenine dinucleotide phosphate oxidase. NE = neutrophil elastase. NET = neutrophil extracellular traps. PKC = protein kinase C. ROS = reactive oxygen species. SFK = Src family kinases. SNARE = Soluble N-ethylmaleimide sensitive factor -NSF– **A**ttachment protein **R**eceptor.

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On the other hand, b) in NADPH oxidase-independent (non-lytic, less studied) the nucleus condenses and the nuclear membranes separate, forming vesicles with DNA that are expelled into the extracellular medium, releasing chromatin.[25] This mechanism prevents the spread of pathogens, although it also has direct bactericidal properties. For example, NE acts on outer membrane proteins and virulence factors of enterobacteria.[15] The presence of NET is implicated in inflammatory and autoimmune disorders, such as acute respiratory distress syndrome, thrombosis in COVID-19, and in rheumatoid arthritis.[22]

2.5 Cytokine and chemokine production: neutrophils are a source of cytokines and chemokines to interact with T lymphocytes, B lymphocytes, macrophages and dendritic cells; participating in the regulation of innate and acquired immunity.[26] They produce pro- and anti-inflammatory, immunoregulatory cytokines, G-CSF and important CXC-type chemokines in cell migration into the tissue and vice versa *(Table 1)*.[27-34]

## 3. Neutrophils in infectious diseases

3.1 COVID-19: in December 2019, a new form of coronavirus called SARS-CoV-2 (severe acute respiratory syndrome coronavirus 2), a single-stranded, positive-sense enveloped RNA virus belonging to the β-coronaviruses, spread in Wuhan, China. The World Health Organization (WHO) declared the pandemic on March 11, 2020.[35] Until November 16, 2023, there were 772,011,164 confirmed cases and 6,979,786 deaths worldwide.[36] In patients with COVID-19, the presence of NE, MPO, citrullinated histone 3 (cit-H3), NET and platelets have been shown to be associated with vascular occlusion, necroinflammation and oxidative stress.[37]

In the severe form of COVID-19, inflammation and «cytokine storm» (IL-1β, IL-2, IL-6, IL-7, IL-8, IL-17, TNF, IFN-γ, IP-10, GM-CSF, MCP-1 and IL-10) have been described leading to the development of acute respiratory distress syndrome.[38,39] Neutrophil recruitments to the site of infection and the formation of NETs have been described as contributing to thrombus formation and respiratory distress *(Figure 3)*.[39]

The neutrophil-lymphocyte ratio (NLR) based on the number of neutrophils and lymphocytes in the blood together with the neutrophil-albumin ratio (NAR) are considered as biomarkers of infection and systemic inflammation. NLR values are useful for prognosis, as values less than 3 indicate mild systemic inflammation, 3 to 5 moderate inflammation, and greater than 5 are indicative of severe inflammation,[40] acute respiratory failure syndrome is the primary cause of death in COVID-19 patients. Together, neutrophilia, NLR and NAR in the early stages of infection correlate with the severity of infection.[41]

In the severe form of COVID-19 there is neutrophilia in blood and lung tissue with increased IL-1β, IL-6 and D-dimer; while NETs have the potential to propagate inflammation, microvascular thrombosis and cytokine storm in the lungs.[42,43]

In vascular occlusion NLR, MCP-3 (monocyte chemotactic protein-3) and IL-8 promote neutrophilia in patients with mild and severe COVID-19, forming aggregates of neutrophils and thrombocytes that target mainly the pulmonary vessels,[44] platelet-fibrin complexes target small pulmonary arteries and thrombi target pulmonary capillaries.[42] Neutrophils and NETs favor necroinflammation,[45] by infiltration of NET aggregates that form thrombi in pulmonary vessels, inducing vasculitis and finally necrosis that favors cytokine storm, causing further inflammation.[46]

Among the critical complications of COVID-19 is thrombosis which has been associated with elevated levels of free DNA, cit-H3, MPO-DNA complex and NET identified in arteriolar microthrombi. Released DNA, NET, MPO and cathepsin G have cytotoxic effects on pulmonary epithelium and endothelial cells *(Table 2)*.

Potential markers of NET associated with symptoms have been described, for example: elevated levels of DNA, citH3, NE and the MPO-DNA complex are associated with admission to intensive care, mechanical ventilation and short-term mortality. The MPO-DNA complex has been associated with sequential organ failure, NE and Histone-DNA associated with lung damage, renal failure, body temperature and MPO associated with days with severe hypoxia.[41] The release of NETs and ROS causes imbalance between ROS production and antioxidant mechanisms, increasing tissue injury.[47]

3.2 Influenza: caused by viruses of the genus Influenzavirus, belonging to the *Orthomyxoviridae* family, a negative-sense monocaterial RNA virus, which are transmitted by aerosols affecting cells of the respiratory tract and type II pneumocytes.[48,49] Annual epidemics result in 3 to 5 million severe cases and 290,000 to 650,000 deaths.[50]

Influenza A, B, and C viruses affect humans, but only A and B are of medical importance.[49,51] influenza type A virus (IAV) is the most common virus and has several subtypes, which are classified according to their antigenic variation in their surface proteins: hemagglutinin and neuraminidase.[49]

It causes seasonal epidemics and manifests as an acute illness with mild to severe symptoms; however, it can become complicated leading to hospitalization or death.[48,49] Complications affect at-risk groups (children or older adults) and those with comorbidities (chronic heart or lung disease, diabetes mellitus and immunosuppression).[48]

**Table 1:** Cytokines and chemokines secreted by neutrophils.

| | Function | Target cell | References |
|---|---|---|---|
| **Cytokines** | | | |
| IL-1α | - Proinflammatory effect<br>- Promotes proliferation and differentiation<br>- Endogenous pyrogen | T and B lymphocytes, MN, eosinophils, DC and fibroblasts | 27,30 |
| IL-1β | - Increases differentiation and IL-9, RORγt and IRF4 expression | Subpopulations of T lymphocyte subpopulations: $T_H9$ and $T_H17$ | 27,30 |
| IL-6 | - Promotes inflammation<br>- Hematopoiesis<br>- Differentiation | T and B lymphocytes | 27,30 |
| IL-17 | - Proinflammatory effect<br>- Increases production of IL-1, IL-6, TNF-α, G-CSF, GM-CSF and chemokines that attract MN and neutrophils | Endothelial cells, epithelial cells and fibroblasts | 27,30 |
| IL-18 | - Promotes $T_H1$ lymphocyte differentiation<br>- Induces IFN-γ production by T lymphocytes<br>- Increases cytotoxic activity of NK lymphocytes | T lymphocytes and NK cells | 30 |
| TNF-α | - Regulates the growth and differentiation of various cell types<br>- Promotes angiogenesis, bone resorption and thrombotic processes<br>- Suppresses lipogenic metabolism | Neutrophils, macrophages, fibroblasts and T and B lymphocytes | 27,30 |
| MIF | - Promotes activation<br>- Inhibits macrophage migration | Macrophages | 30 |
| IL-1 Ra | - Anti-inflammatory activity<br>- IL-1 antagonist, blocking its binding to the receptor, preventing a proinflammatory response | MN, lymphocytes, fibroblasts and endothelial cells | 31 |
| TGF-β | - Anti-inflammatory effect<br>- Inhibits growth, differentiation and functions of various cell types<br>- Promotes angiogenesis and tissue repair<br>- Stimulates the production of IgA antibodies | T and B lymphocytes, MN, macrophages and fibroblasts | 27,30 |
| IL-22 | - Proinflammatory and anti-inflammatory effect<br>- Stimulates transcription of protein genes with microbicidal activity | Keratinocytes | 27,30 |
| IL-23 | - Promotes differentiation<br>- Induces IL-17A and IL-17B synthesis | $T_H17$ lymphocytes | 27,30 |
| G-CSF | - Growth and differentiation of neutrophil precursors | Neutrophils | 27,30 |
| **Chemokines** | | | |
| CXCL1, CXCL2, CXCL3, CXCL5, CXCL6, CXCL8 (IL-8) | - Proinflammatory activity<br>- Neutrophil recruitment | Neutrophils | 18,32 |
| CXCL4 | - Proinflammatory activity<br>- Platelet aggregation | Platelets | 27 |
| CXCL9, CXCL10, CXCL11 | - Proinflammatory activity<br>- Recruitment of effector T-lymphocytes | Effector T lymphocytes | 23,27 |
| CCL2 | - Proinflammatory activity<br>- Leukocyte recruitment | MN and basophils | 32 |
| CCL3, CCL4 | - Proinflammatory activity<br>- Leukocyte recruitment<br>- T-lymphocyte-DC interaction | Macrophages, NK lymphocytes, T-lymphocytes and DCs | 27,33 |

Contents

**Continue Table 1:** Cytokines and chemokines secreted by neutrophils.

| | Function | Target cell | References |
|---|---|---|---|
| **Chemokines** | | | |
| CCL17, CCL22 | - Migration and activation | $T_H2$ lymphocytes, regulatory T lymphocytes and basophils. | 27,33 |
| CCL18 | - Activation | Lymphocytes $T_H2$ | 33 |
| CCL19 | - Migration to lymph nodes | DC and T lymphocytes | 22,32 |
| CCL20 | - Migration to intestinal lymphoid tissue | $T_H17$ lymphocytes, B lymphocytes and DCs | 33 |
| CCL23 | - Chemoattractive activity | T lymphocytes, MN and neutrophils | 34 |

DC = dendritic cell. G-CSF = granulocyte-stimulating factor. GM-CSF = granulocyte-monocyte-stimulating factor. IFN = interferon. IgA = immunoglobulin A. IL = interleukin. IRF4 = interferon regulatory factor 4. Reg T lymphocytes = regulatory T lymphocytes. MIF = macrophage migration inhibitory factor. MN = monocyte. NK = natural killers. RORγt = retinoid orphan receptor gamma t. TGF-β = transforming growth factor-beta. Th = T helper lymphocyte. TNF = tumor necrosis factor.

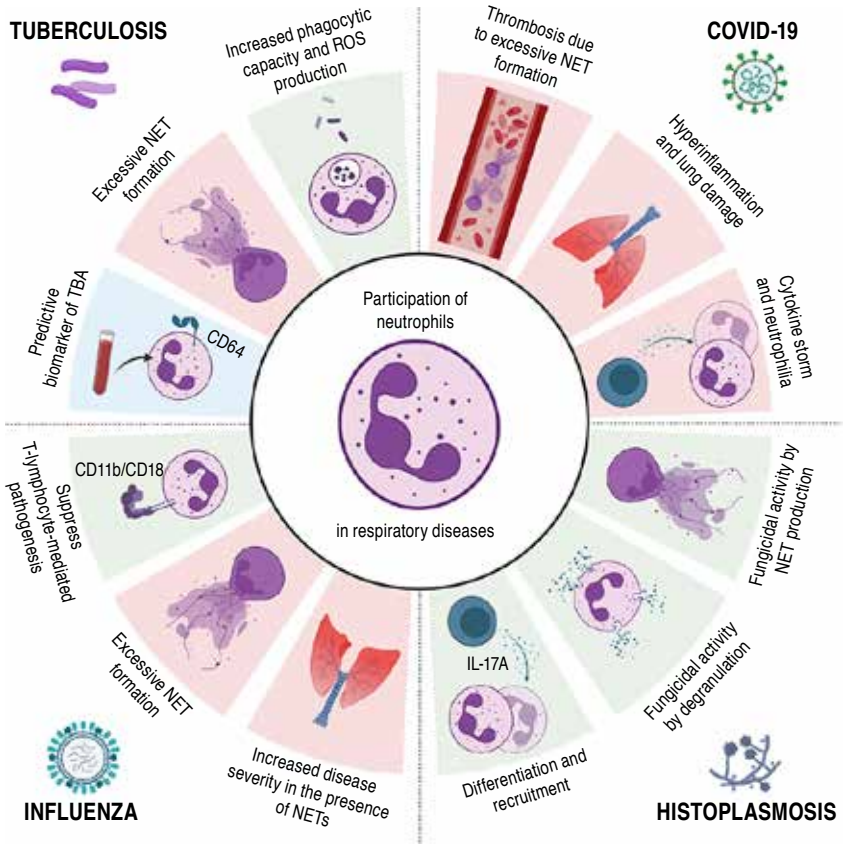


**Figure 3:**

**Participation of neutrophils in respiratory diseases.** Neutrophils participate in the elimination of the causative agents of different infectious diseases; however, their rapid response bactericidal mechanisms can cause damage to the organism. In viral diseases such as COVID-19 and influenza, neutrophilia, excessive production of NETs and proinflammatory cytokines cause severe lung damage with poor prognosis, even in COVID-19 excess NETs cause thrombosis with fatal outcomes. Whereas in tuberculosis and histoplasmosis neutrophilia, NET production, phagocytosis, and degranulation are bactericidal mechanisms involved in the pathogenesis of the disease, without causing severe lung damage that is directly responsible for a poor prognosis.
NET = neutrophil extracellular traps ROS = reactive oxygen species. AT = active tuberculosis.

Neutrophils contribute to disease control, and in IAV infection in the murine model, neutrophils and their adhesion molecules (CD11b/CD18) are important in limiting T cell-mediated pathology.[52] However, although the virus induces an innate immune response characterized by neutrophil infiltrate in the lungs and mechanisms that promote resolution of the infection, they also contribute to the pathogenesis of severe disease.[53] Despite tissue damage, lack of neutrophils is associated with increased lung damage.[53,54]

In patients with influenza, neutrophils and NETs are associated with greater severity.[55] In mice co-infected with IAV and *Staphylococcus aureus* there is excessive recruitment of neutrophils to the lungs and NETs, which contribute to severe pulmonary inflammation.[56] Similarly, patients with

severe IAV H1N1 and H7N9 infection have elevated levels of NETs associated with disease severity and poor prognosis.[57]

3.3 Tuberculosis: WHO reported 10.6 million of new cases with tuberculosis and 1.6 million deaths in 2021.[58] *M. tuberculosis* causes tuberculosis (TB) and is transmitted by aerosols. It causes asymptomatic infection (latent TB) in 90-95% and active TB in 5-10% of infected persons, causing mainly pulmonary TB (PTB).[58-61]

Protection depends on the innate and acquired immunity generated. Neutrophils participate in the response to *M. tuberculosis* during early infection, carrying out phagocytosis, production of ROS, cytokines and chemokines.[62] However, although they participate in the immune response, they are not very crucial in the resolution of the disease, probably because they are cells with a short half-life.[63] They participate in macrophage recruitment and promote inflammation and granuloma formation to contain infection.[60,62-64]

They contribute to TB resistance by producing antimicrobial peptides (LL-37 and lipocalin 2) that participate in the elimination of mycobacteria.[65,66] Macrophages phagocytose NETs generated by infection with *M. tuberculosis* and produce IL-1β, IL-6, TNF-α and IL-10, evidencing their participation in the modulation of the immune response.[67] NETs are in the plasma of TB patients and the increase correlates with the severity of the disease.[68,69]

In the search for biomarkers, the FcγR1 receptor (CD64) has been found to be increased on neutrophils and monocytes of patients with active TB, and may be a predictive biomarker of disease.[59,70]

3.4 Pulmonary histoplasmosis: caused by the inhalation of *Histoplasma capsulatum* microconidia or mycelial fragments, it is an endemic mycosis that affects more than 60 countries.[71] With high incidence in North America and in tropical areas of Latin America with temperate, subtropical or humid tropical climates.[72]

**Table 2:** Toxic effects of NETs on lung epithelium and epithelial cells.

| NET components | Cytotoxic effect |
|---|---|
| DNA fibers | Diffuse alveolar damage and hemorrhage |
| Histones | Increase the permeability of the endothelium |
| NE | Destruction of the cytoskeleton of endothelial cells.<br>Affects the integrity of the alveolar barrier.<br>Associated with inflammation and thrombosis by the release of proinflammatory cytokines. |
| MPO | Involved in epithelial cell apoptosis by the release of ROS |

DNA = deoxyribonucleic acid. MPO = myeloperoxidase. NE = neutrophil elastase. NET = neutrophil extracellular traps.

The disease is benign and asymptomatic in immunocompetent individuals, but can progress to acute lung disease, and the severity depends on immune status, time of exposure and virulence of the strain.[73] In the pulmonary alveoli, microconidia develop into yeast and are phagocytosed by macrophages through the CR3 receptor. However, *Histoplasma capsulatum* multiplies and induces apoptosis to spread to other cells.[74] Neutrophils phagocytize opsonized yeasts through their CR1 and CR3 receptors, while non-opsonized yeasts are recognized by CD18 with the release of NETs.[75] Components of azurophilic granules, such as BPI and cathepsin G inhibit yeast growth.[8,76]

Protection depends on cellular immunity; however, IL-17A production promotes granulopoiesis, production and recruitment of neutrophils to the site of infection.[3,4,77] Study of neutrophil subcellular fractions has shown that yeasts promote NETs release and reduce their viability.[78]

## THERAPEUTIC STRATEGIES IN COVID-19

Mortality of patients with COVID-19 (with mechanical ventilation) was 24-53%, in part, due to the interference of mucopurulent secretions in ventilation. Neutrophil NETs contribute to the viscosity of secretions and are also present in serum.

There are almost 100 clinical studies involving the use of drugs to inhibit NET formation although only 19 have been completed.[41] Studies have shown that the use of aerosolized **dornase alfa** (Pulmozyme, human recombinant DNAsa 1 and mucolytic) and albuterol decrease the viscosity of secretions by degrading DNA, improving oxygenation and reducing respiratory support in patients.[79-82] In addition, complications are due to the presence of antigen-antibody complexes in the plasma, which interact with FcγRIIA receptors (CD32a) on the neutrophil membrane and favor the formation of NETs. However, **fostamatinib** is a drug that inhibits the Spleen Tyrosine Kinase (SYK) activation pathway associated with these receptors, reducing the formation of NETs.[83] Also, there are controlled studies in COVID-19 patients using **colchicine**, since it interferes in inflammatory pathways inhibiting neutrophil adhesion, mobilization, degranulation and chemotaxis. It decreases cell adhesion molecules, consequently, it reduces migration and interaction of neutrophils with endothelial cells and their recruitment to the site of inflammation. It also prevents microtubule polymerization by inhibiting the formation of the NLRP3 inflammasome by reducing the production of IL-1β which prevents the production of IL-6 and TNF-α, and also inhibits the production of ROS and Nitric Oxide (NO).[84,85]

Additionally, drugs have been proposed to block NET components such as **alvelestat** which inhibits NE involved in inflammation,[86] **metformin** which binds to HMGB1 (High

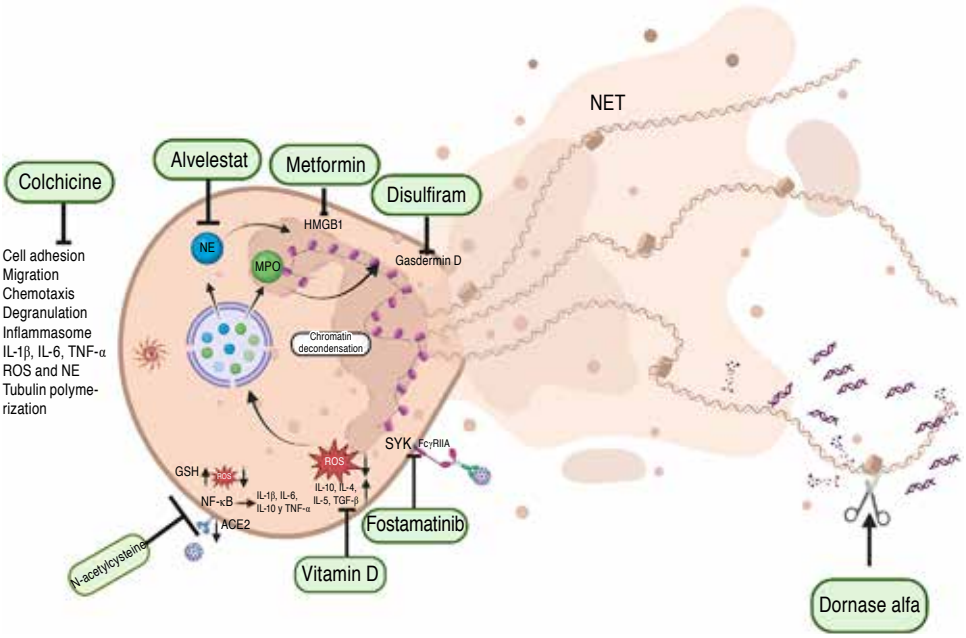


**Figure 4:**

**Therapeutic strategies in patients with COVID-19.** Neutrophils are involved in inflammation and thrombosis in patients with COVID-19, and drugs that intervene in different stages of activation have been used to reduce host damage.
(Created with BioRender.com).
GSH = reduced glutathione.
HMGB1 = High Mobility Group Box 1.
MPO = myeloperoxidase. NE = neutrophil elastase. NF-κB = nuclear factor kappa B.
ROS = reactive oxygen species.
TNF-$\alpha$ = tumor necrosis factor alfa.

mobility group box 1) inhibiting inflammation,[87] **disulfiram** which binds to gasdermin D inhibiting its ability to cause membrane pores and promote NET formation in COVID-19 patients reducing inflammation and tissue damage.[88]

The production of ROS is critical as it favors the production of NETs, and the use of **N-acetylcysteine**, which has an antioxidant function favoring the production of reduced glutathione that decreases ROS; and an anti-inflammatory effect by preventing the binding of the SARS-CoV-2 virus to the ACE2 receptor and inhibits the activation of the transcriptional factor NF-κB reducing the production of inflammatory cytokines, have been proposed.[89] **Vitamin D** has been proposed for use because it has anti-inflammatory actions by increasing the production of IL-10, IL-4, IL-5, and TGF-β, and because through antioxidant mechanisms it reduces oxidative stress and ROS production *(Figure 4)*.[90]

## CONCLUSIONS

Neutrophils are important in host defense; however, in viral infections such as COVID-19 they are associated with inflammatory events and thrombosis. The knowledge of their mechanisms of action has allowed proposing therapeutic alternatives that can provide a better prognosis to patients with COVID-19. While in tuberculosis and histoplasmosis they have limited participation in the resolution of the disease.

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Review
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# Use of antioxidants in patients with tuberculosis

## Uso de antioxidantes en pacientes con tuberculosis

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**ABSTRACT.** Tuberculosis is a public health problem, statistics from the World Health Organization mentions that a quarter of the population is infected, but only 5 to 10% may develop the active tuberculosis. This infection mainly affects the lungs but can spread to other organs. When the pathogen enters the host, defense mechanisms are induced which activates the immune system and promotes the elimination of mycobacteria. The interaction of immune cells with the pathogen activates the release of cytokines and the production of reactive oxygen and nitrogen species. However, a continuous and exacerbated response leads to oxidative stress and chronic inflammation in the lung tissue, producing cellular and tissue damage. Despite having established anti-tuberculosis treatment, mycobacteria have defensive mechanisms to evade the host's immune response that permits them to survive for decades. The persistence of the pathogen contributes to the development of resistance to first-line drugs, increasing the mortality rate. Currently, several studies have shown that the use of antioxidants such as: vitamins A, C, and E, other than N-acetyl cysteine and some trace elements such as zinc and selenium used as complementary therapy together with anti-tuberculosis drugs improve the patient health. Antioxidants counteract the oxidation state, reduce the exacerbated inflammatory response in the host and enhance the effectiveness of first-line antibiotics. The objective of this review is to demonstrate the beneficial effect of various antioxidants in clinical studies to propose their use as complementary therapy.

**Keywords:** tuberculosis, oxidant stress, inflammation, antioxidants.

**RESUMEN.** La tuberculosis es un problema de salud pública, datos de la Organización Mundial de la Salud mencionan que una cuarta parte de la población está infectada, pero sólo de 5 a 10% desarrollará la tuberculosis activa. Cuando el patógeno entra al hospedero se inducen los mecanismos de defensa activando el sistema inmune, promoviendo la eliminación de las micobacterias. La interacción de las células con el patógeno activa la liberación de citocinas y se induce la producción de especies reactivas de oxígeno y nitrógeno. Sin embargo, una respuesta continua y exacerbada conlleva estrés oxidante e inflamación crónica en el tejido pulmonar, produciendo daño celular y tisular. A pesar de tener una terapia antituberculosis establecida, las micobacterias presentan mecanismos de defensa para evadir la respuesta inmune del hospedero, lo que le permite sobrevivir por décadas. La persistencia del patógeno contribuye al desarrollo de resistencia a los fármacos de primera línea, aumentando la tasa de mortalidad. Actualmente varios estudios han demostrado que el uso de antioxidantes como las vitaminas A, C y E, así como el N-acetilcisteína y algunos oligoelementos como el zinc y el selenio son utilizados como terapia complementaria junto con los fármacos antituberculosos, mejorando la salud del paciente. Los antioxidantes contrarrestan el estado de oxidación, disminuyen la respuesta inflamatoria exacerbada en el hospedero y potencian la eficacia de los antibióticos de primera línea. El objetivo de esta revisión es evidenciar el efecto benéfico de diversos antioxidantes en estudios clínicos, con el fin de proponer su uso como terapia complementaria.

**Palabras clave:** tuberculosis, estrés oxidante, inflamación, antioxidantes.

**Abbreviations:**

ATRA = transretinoic acid.
ERN = reactive nitrogen species.
GSH = glutathione.
GSNO = S-nitrosoglutathione.
IFN-γ = interferon gamma.
IL = interleukins.
IU = international units.
MDA = malondialdehyde.
NAC = N-acetylcysteine.
NF-κB = nuclear factor kappa core.
NK = natural killer.
PKC = protein kinase C.
ROS = reactive oxygen species.
TNF-α = tumor necrosis factor-alpha.

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Contents

## INTRODUCTION

Tuberculosis is an infectious disease caused by *Mycobacterium tuberculosis*, which is transmitted by airborne transmission when a person with tuberculosis coughs, sneezes or spits, because it generates Flügge droplets containing mycobacteria. According to data reported by the World Health Organization, it is estimated that a quarter of the world's population is infected by this bacterium, but only 5-10% will develop signs and symptoms. In 2021, 10.6 million cases were reported worldwide.

Tuberculosis primarily affects the lungs, but can affect other sites in the body, when the mycobacteria enter the lung tissue, antigen presenting cells release interleukins (IL)-6 and -12, followed by the production of interferon gamma (IFN-γ), IL-23, IL-1β and IL-17 by lymphocytes, leading to activation of macrophages, which in turn produce tumor necrosis factor-alpha (TNF-α), and promote intracellular mycobacterial clearance. Macrophages induce the production of antimicrobial peptides and proteins, as well as reactive oxygen and nitrogen species (ROS and RNA) as a defense mechanism.[1] When ROS and RNS production is continuous and exacerbated, it leads to chronic inflammation in lung tissue creating oxidative stress, necrosis and cavitations in lung tissue.[2] Mycobacteria that manage to evade the immune response penetrate uninfected macrophages and replicate within them, forming a granuloma in the lungs.[3]

In most parts of the world, the standard treatment for pulmonary tuberculosis consists of a two-month intensive phase of isoniazid, rifampicin, pyrazinamide and ethambutol.[4] This is followed by a four-month maintenance phase of isoniazid and rifampicin. In this phase, the three-day-a-week schedule is taken.[5] Despite being an effective treatment for sensitive tuberculosis, it has been shown that these drugs can also produce oxidative stress.[6,7]

It has been shown that patients with tuberculosis present a chronic oxidative and inflammatory state before and during anti-tuberculosis therapy at least in the first two months of treatment. Various oxidation products have been detected in tuberculosis patients, for example hydrogen peroxide (H2O2)[8] and lipoperoxides such as malondialdehyde (MDA) and 8-isoprostane.[2] Decreased antioxidant enzymes such as superoxide dismutase, catalase, glutathione peroxidase and glutathione reductase,[9] as well as antioxidant molecules such as glutathione (GSH),[10] vitamin A, C and E, have also been observed.[11]

It has been shown that high levels of ROS and RNA in patients with pulmonary tuberculosis result in decreased immune response and antioxidant capacity, contributing to exacerbated inflammation leading to pulmonary dysfunction.[9] In addition, increased lipoperoxidation has been reported to alter the lipid profile of patients, resulting in decreased total cholesterol levels. Cholesterol is essential for the proper functioning of the immune system.[12] Lymphocytes require cholesterol to properly carry out their function (it induces differentiation and their cytotoxic effect).[13] Macrophages also require cholesterol for phagocytic function, cell motility, exocytosis and endocytosis.[14] Thus, cholesterol depletion by lipoperoxidation leads to immune cell dysfunction during tuberculosis.

The main effectors of this increase in ROS and ERN are infected macrophages that generate respiratory burst; subsequently, as these molecules are not adequately neutralized, they lead to oxidative stress that leads to cellular damage affecting DNA, lipid membrane and proteins.[9] Antioxidants are substances that can counteract oxidative stress and prevent cellular and tissue damage.

The aim of this review is to demonstrate in clinical studies the synergistic/potentiating effects of six main antioxidants such as vitamin A, C, E, N-acetylcysteine (NAC), selenium (Se) and zinc (Zn), as complementary therapy to the antituberculosis scheme *(Figure 1)*.

## ANTIOXIDANTS DECREASE LUNG DAMAGE IN TUBERCULOSIS PATIENTS

### Use of vitamins with antioxidant activity

#### *Vitamin A*

Vitamin A is a fat-soluble molecule, which is obtained by the diet from animal products in the form of vitamin A (retinol and its derivatives) or as provitamin A (carotenoids) of plant origin, characterized by an unsaturated isoprenoid chain structure.[15] It has several functions in the human body; one of them is to maintain the homeostasis of epithelial tissues and mucous membranes, through its metabolite, retinoic acid formed from retinol[16] (circulating form of vitamin A). The main bioactive molecules are the oxidized 11-cis-retinal derivatives and transretinoic acid (ATRA).[15] ATRA favors the immune system, promoting the differentiation and function of immune cells (mainly T and B cells).[17]

Vitamin A deficiency is associated with a high rate of infections, particularly respiratory infections, which contributes to morbidity and mortality.[18] In relation to this, it was demonstrated in an *in vivo* model that epithelial cells obtained from nasopharyngeal secretions of pediatric patients with vitamin A deficiency show increased bacterial adherence on the cell surface after 15 hours of co-incubation of the epithelial cells with *Klebsiella pneumoniae*, which represents a risk factor for acquiring a respiratory infection.[18]

In a clinical study carried out in Indonesia, two groups of 15 patients each were formed, the control group only received anti-tuberculosis treatment, while the other group additionally received complementary therapy with

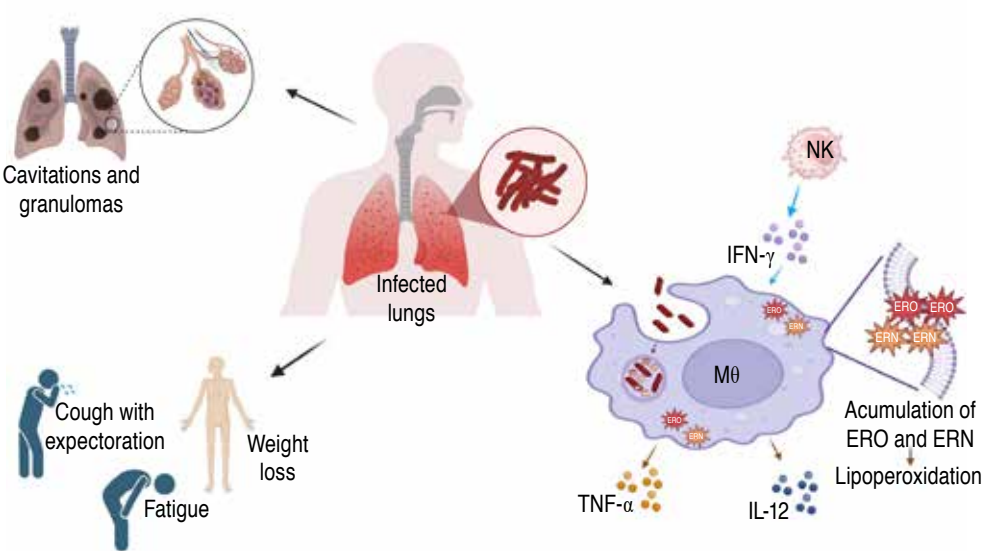


**Figure 1:**
Effects of tuberculosis on humans. Image created at BioRender.com.

vitamin A. It was demonstrated that the patients with complementary therapy decreased the bacterial load since, in the analysis of the smear tests, the patients became negative in 2.4 weeks after supplementation with vitamin A, compared to the placebo group, which became negative up to 4.1 weeks on average.[19]

In another study, a comparison was made between the placebo group and the group receiving supplemental therapy with vitamin A at a daily dose of 5,000 IU (international units) orally and Zn at a daily dose of 15 mg orally for six months. The use of these antioxidants was shown to improve the efficacy of antituberculosis drugs. In addition, the patients with complementary therapy at two weeks had negative smear microscopy in up to 23% (n = 40 patients) compared to the placebo group with 13%.[20] Another aspect that improved after two months was the decrease in the size of cavitations analyzed in the chest X-Ray, in addition to the 5% increase in body weight. Additionally, at six months of complementary treatment they had a gain of 16.7% of their initial body weight 20 *(Figure 2)*.

### *Vitamin C*

Vitamin C or ascorbic acid is a water-soluble molecule, a six-carbon lactone of 2-keto-L-gluconic acid, with an endiol grouping (two hydroxyl groups at the ends of a double bond between C2 and C3).[21]

Vitamin C has an important role in the immune system, as it induces microbicidal activity, cell migration, phagocytosis and the generation of extracellular traps in neutrophils, induces the differentiation of natural killer cells,[22] increases the proliferation of lymphocytes.[23]

In *in vivo* studies, vitamin C maintains intracellular redox balance by using ascorbate as an electron acceptor, neutralizing various ROS such as superoxides, hydroxyl radicals, singlet oxygen and hypochlorous acid, generated by metabolic respiration and mitochondrial oxidative phosphorylation.[23] Its antioxidant capacity is achieved at a physiological dose between 75-120 mg/day.[24]

Vitamin C and E levels as well as glutathione reductase have been shown to be decreased during tuberculosis infection.[11] In a study conducted in India, they demonstrated that when using vitamin C as an adjuvant, patients showed significant increase in hemoglobin percentage (% Hb) with 53.5% at the end of six months, they also gained weight by 5% at two months and 13.1% at six months with respect to the initial mean. Likewise, baseline vitamin C and cholesterol levels increased compared to the group that only received anti-tuberculosis treatment at the end of six months of therapy.[25] In fact, it has been shown that the combined administration of vitamins C and E with anti-tuberculosis drugs enhances the efficacy of treatment[26] *(Figure 3)*.

### *Vitamin E*

Vitamin E is a fat-soluble molecule, containing eight isomers: four tocopherols (α-, β-, γ-, and δ-tocopherol) and four tocotrienols (α-, β-, γ-, and δ-tocotrienols), its name depends on the number and location of the methyl groups on the chromanol ring, tocotrienols have an isoprenoid side chain, whereas tocopherols have a phytyl chain.[27]

Vitamin E is an important antioxidant, found mainly in cell membranes, it has a protective function against lipoperoxidation, since oxidation of low-density lipoproteins leads to increased cholesterol uptake by macrophages, increasing lipoperoxidation products and stimulating atherosclerotic processes.[27] Thus, the protective function of

vitamin E plays its essential role by decreasing membrane free radicals, preventing efflux to the cytoplasm and intracellular damage.[28] Vitamin E has been shown to inhibit protein kinase C (PKC) activity by increasing PKC-α dephosphorylation through activation of protein phosphatase A2, resulting in inhibition of platelet aggregation, reduced proliferation of macrophages, neutrophils, and vascular smooth muscle cells. A decrease in superoxide production in neutrophils and macrophages has also been observed.[29]

In a study carried out in India, the beneficial effect of vitamin E as an adjunctive therapy in patients with tuberculosis for six months was determined, demonstrating that the use of vitamin E as an adjunct significantly increased the percentage of hemoglobin (% Hb) with 40.7% at the end of six months. Patients gained weight 6.6% at two months and 16.6% at six months with respect to the initial mean. They also increased baseline vitamin E and cholesterol levels compared to the control group that received only anti-tuberculosis treatment at the end of six months of therapy.[25]

In another case-control study, the group treated with anti-tuberculosis therapy that received vitamin E as adjunctive therapy, at a dose of 140 mg orally daily for two months, showed a significant decrease of 63% in the levels

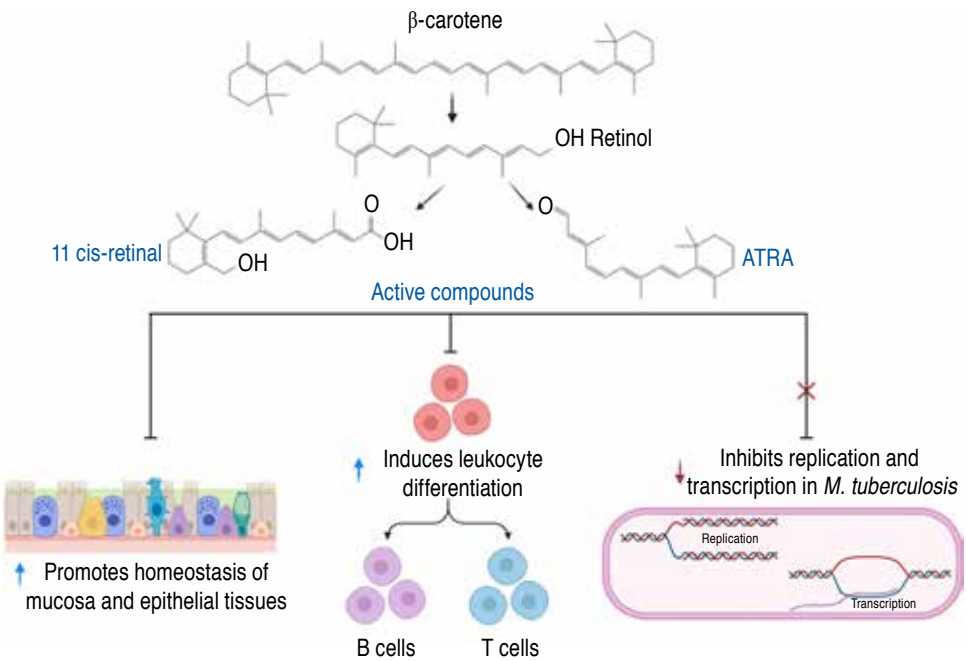


**Figure 2:**

Effect of vitamin A during tuberculosis. 11 cis-retinal and ATRA (all-trans retinoic acid) are active compounds derived from retinol. These compounds allow homeostasis of mucosa and epithelial tissues during an infection, promote lymphocyte differentiation and inhibit bacterial replication and transcription.
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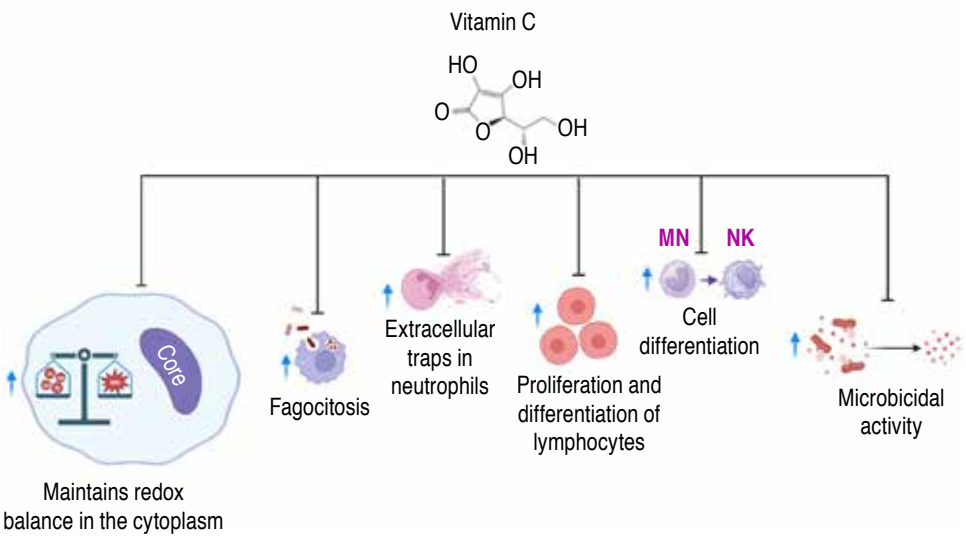


**Figure 3:**

Cellular effect of vitamin C during tuberculosis. Vitamin C maintains intracellular redox balance, induces phagocytosis and the generation of extracellular DNA traps in neutrophils. In addition, it promotes NK cell differentiation and inhibits bacterial growth.
Image from BioRender.com.

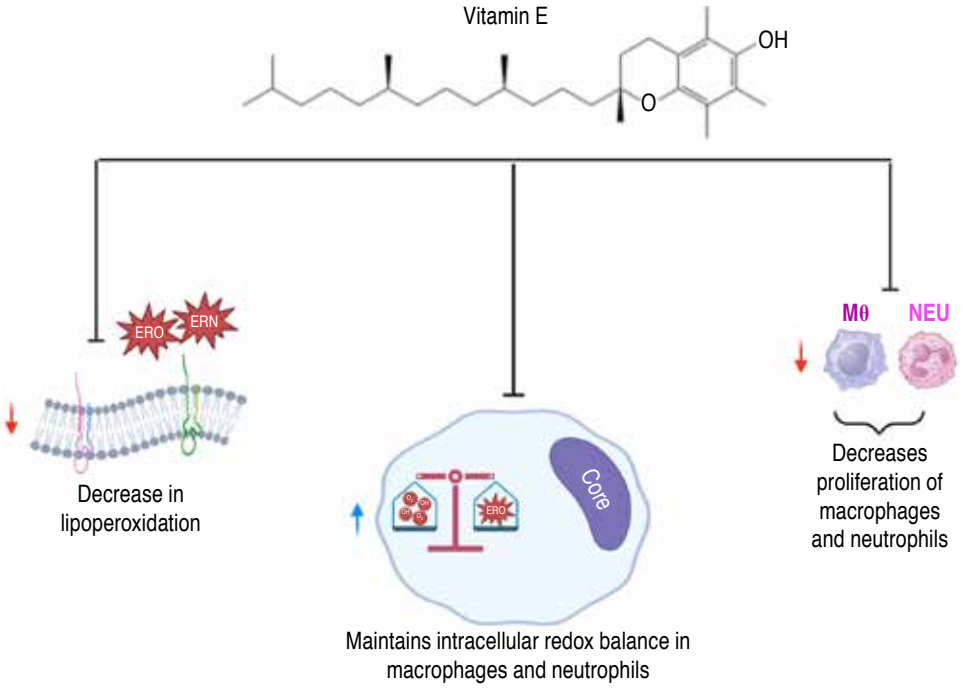


**Figure 4:**

Antioxidant mode of action of vitamin E. It is capable of neutralizing ROS and ERN, prevents lipoperoxidation of cell membranes, maintains redox balance and decreases the proliferation of neutrophils (NEU) and macrophages (Mθ) avoiding exacerbated inflammation.
Image made in BioRender.com.

of globular segmental velocity, with respect to their initial value, compared to the control group, which decreased by 34.5%. There was also a significant increase of 29% in serum vitamin E levels with respect to their initial value, while the control group increased by 4.9%. On the other hand, to determine the levels of oxidation in the patients, they analyzed the concentration of MDA, showing that after two months of treatment there was a 40% decrease with respect to its initial value, compared to the control group, which decreased to 13.3%.[28]

It is important to mention that the dosage of vitamin C and E has not been standardized based on their toxicity, so it is necessary to establish the optimal dose for their administration in patients with tuberculosis, so that they can be applied in a standardized manner as complementary therapy *(Figure 4)*.

### *N-acetylcysteine (NAC)*

It is an antioxidant that directly neutralizes ROS and is a precursor and inducer of endogenous GSH.[30] It plays a crucial role in cellular defense against oxidative stress by stimulating GSH. NAC increases GSH levels in macrophages, this induces the formation of S-nitrosoglutathione (GSNO).[31] GSNO is an endogenous generator of nitric oxide. The latter has a direct antibacterial effect, generating oxidative stress in mycobacteria and damaging their cellular constituents.[32]

NAC has been shown to decrease the TH2-type cytokine response,[31] reduce IL-4 production and the humoral response.[33] In another study, NAC enhances the TH1 response, induces IFN-γ production, contributes to the control of mycobacterial replication, and improves the efficacy of antituberculosis drugs.[31]

In a study conducted in India in patients with tuberculosis with adjuvant therapy with NAC, at a daily dose of 600 mg orally for two months, it was shown that after three weeks the patients showed a significant recovery, since 95.83% were negative in the bacilloscopies and there was a decrease of 87% in pulmonary infiltration compared to the placebo group with 58.35 and 33%, respectively.[31]

In another study conducted in Brazil, using NAC as adjunctive therapy at a dose of 600 mg orally twice a day for eight weeks, showed that 61.1% of patients with NAC had negative smear tests compared to 33.3% in the control group. Regarding radiological analysis, 45% of the group that received NAC improved the radiological image compared to 30.8% of the control group, with no significant adverse effects.[34]

In a study conducted in Iran, they evaluated the hepatoprotective effect of NAC in two groups of patients: the control, which only received anti-tuberculosis therapy, and the other group with NAC supplementation, at a dose of 600 mg orally twice a day for two weeks. They measured two liver damage marker enzymes: alanine aminotransferase and aspartate aminotransferase. At baseline there were no significant differences, but at weeks one and two the control group significantly increased both enzymes. This group developed 37.5% hepatotoxicity due to the anti-tuberculosis therapy, while the group supplemented with NAC maintained constant values of both enzymes, avoiding liver damage[35] *(Figure 5)*.

### *Selenium (Se)*

It is an essential trace element, since it interacts with proteins in the form of cofactors; selenium is cotranslationally incorporated into the polypeptide chain as part of the amino acid selenocysteine, forming selenoproteins, which have essential functions in the cell.[36]

Various selenoproteins are involved in the activation, proliferation and differentiation of cells involved in the innate and adaptive immune response. They are also involved in immune regulation, which is crucial to prevent exacerbated inflammation.[37] Several antioxidant enzymes have been shown to contain selenocysteine, such as glutathione peroxidase and thioredoxin synthetases, and their function is dependent on Se levels. In fact, a relationship between Se deficiency and increased susceptibility to tuberculosis has been suggested.[38]

In new strategies to eradicate multidrug-resistant strains of *Mycobacterium tuberculosis*, nanomedicine has been used, mainly the administration of metallic nanoparticles containing selenium.[39] Selenium has been proposed as a complementary therapy, as it has several mechanisms of action, it has been suggested that it has a bactericidal effect, as it affects the cell envelope of the mycobacteria, which facilitates the incorporation of antibiotics into the cytoplasm, prevents permanence in the phagosome and facilitates phagosome-lysosomal fusion of *Mycobacterium tuberculosis*, activating the macrophage bactericidal system and inducing autophagy through PI3K/Akt/mTOR signaling.[39]

In a double-blind clinical study conducted in Tanzania, 887 patients with pulmonary tuberculosis were enrolled and assigned to two groups: the placebo group comprising 471 patients and the complementary therapy group comprising 416 patients, where a dose of 100 $\mu$g of Se was used; after one month of treatment, patients significantly reduced the risk of reinfection by 45% during the rest of the treatment. In addition, treatment failure was reduced by up to 34%.[40] In another study in Pakistan, 80 patients with newly diagnosed pulmonary tuberculosis (not older than two months) were randomly divided into two groups: the control and the supplemented group with 40 patients each. The group supplemented with Se, at a dose of 100 $\mu$g/day orally for six months, showed a significant decrease in the leukocyte count compared to the placebo group, demonstrating control of the infection. In addition, it was evidenced that the concentration of Se in blood increased significantly up to 106% in comparison with the control group that only increased 6.1%. With respect to oxidation status, MDA levels decreased by up to 25.3% in the supplemented group, compared to the placebo group which decreased serum levels by 17.8%.[41] Based on the above, Se is a suitable supplement during the treatment of tuberculosis.

### *Zinc (Zn)*

It is an essential trace element that plays a fundamental role in several physiological processes, such as immune function and wound healing. It is known to be involved in the development and function of immune cells, participating in the survival, proliferation and differentiation of monocytes, polymorphonuclear cells, natural killer (NK) cells, as well as T and B lymphocytes.[42]

Zn is an essential component of signal transduction pathways that eliminate pathogens and lead to the formation of neutrophil extracellular traps, it is also involved in modulating the proinflammatory response by inhibiting the translocation to the nucleus of nuclear factor kappa (NF-

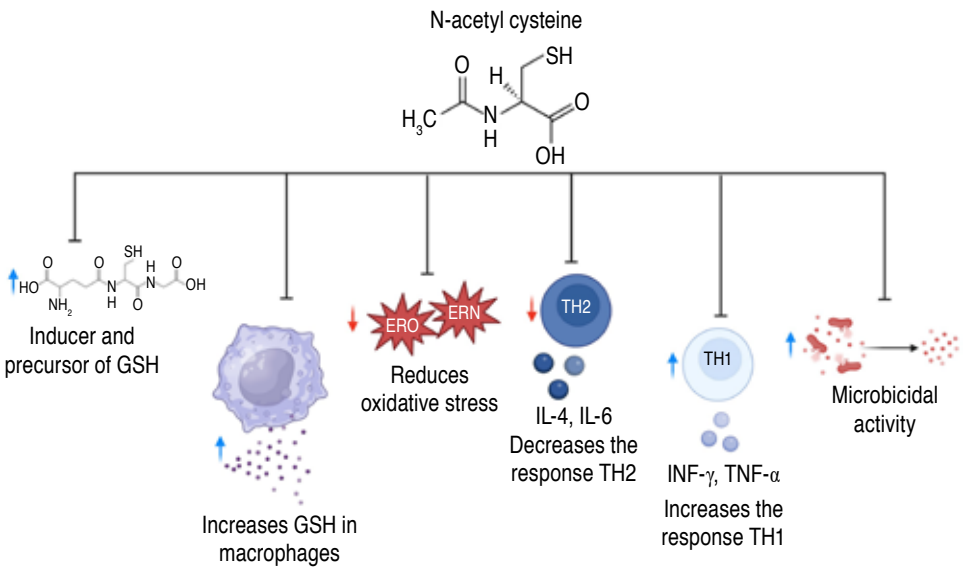


**Figure 5:**

Mode of antioxidant action of N-acetylcysteine. The main mechanism is to induce the production of endogenous glutathione in macrophages and directly neutralize ROS and ERN. It decreases the TH2 response and induces the TH1 response, generating a bactericidal effect and inducing INF-γ and TNF-α. Image made at BioRender.com.

κB), a transcription factor that induces the proinflammatory response.[43]

Several studies have shown that Zn plays an essential role in the metabolism of vitamin A, since in patients with tuberculosis, serum Zn levels are decreased, affecting the intestinal absorption of retinol.[44] However, when receiving vitamin A (5,000 IU) and Zn (15 mg) as adjunctive therapy in patients with tuberculosis after two months of treatment, the patients´ clinical status improved significantly, the number and size of cavitations decreased significantly.[45] In a study in India, it was shown that when receiving adjunctive therapy with Zn there was clinical improvement, the patients gained weight and there was a negative conversion in the sputum smear test.[46]

## CONCLUSIONS

Based on the studies carried out in different countries, it was demonstrated that patients who use antioxidants improve their symptomatology, gain weight significantly and improve their health compared to patients who only receive their antituberculosis therapy. The use of antioxidants decreases lung damage, since it is possible to reduce the area and size of cavitations generated by the infection. In addition, the patient ceases to be bacilliferous more quickly compared to patients who do not receive antioxidants; this prevents the spread of mycobacteria to people who are in close contact with the patient.

Regarding the proinflammatory state and the imbalance in the redox state, it was shown that antioxidants contribute to homeostasis early, since they decrease the exacerbated inflammatory state, as well as the oxidation state, considerably decrease the concentration of lipoperoxides and increase the level of endogenous antioxidants such as glutathione.

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# Sternoclavicular joint osteomyelitis as a cause of descending necrotizing mediastinitis

## Osteomielitis de la articulación esternoclavicular como causa de mediastinitis necrosante descendente

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**ABSTRACT.** Descending necrotizing mediastinitis is a rare entity with a high morbimortality; the most common non-surgical causes are infections coming from oral or cervical region that spread through cervical fascias to mediastinum. However, there are other uncommon etiologies that affects by proximity mediastinum as sternoclavicular joint infection does; sternoclavicular joint infections (SCJI) constitute less than 1% of all joint infections and even 0.5% in healthy patients. We describe the case of a female 76 years old, diabetic patient with descending necrotizing mediastinitis associated with left SCJI, who unfortunately died, despite treatment provided. Likewise, a review of the current literature is made due to low frequency of this entity as a cause of mediastinitis.

**Keywords:** descending necrotizing mediastinitis, osteomyelitis, septic arthritis, sternoclavicular joint, case report.

**RESUMEN.** La mediastinitis necrosante descendente es una entidad poco frecuente y está asociada a una alta mortalidad; las etiologías más frecuentes de origen no quirúrgico son el odontogénico y el absceso cervical. Sin embargo, se han reportado algunas otras causas menos comunes, por afecciones a otras estructuras que, por su cercanía, contaminan el mediastino superior. La articulación esternoclavicular es un sitio inusual de artritis séptica y está involucrada en sólo 0.5-1% de todas las infecciones articulares y en menos de 0.5% de los pacientes inmunocompetentes. Presentamos el caso de una paciente diabética de 76 años de edad con diagnóstico de mediastinitis necrosante descendente y con evidencia de artritis séptica de la articulación esternoclavicular izquierda, la cual falleció a pesar del manejo médico-quirúrgico proporcionado. De igual manera, se realiza una revisión de la literatura reportada debido a la baja frecuencia de esta entidad como causa de mediastinitis.

**Palabras clave:** mediastinitis necrosante descendente, osteomielitis, artritis séptica, articulación esternoclavicular, reporte de caso.

## INTRODUCTION

Descending necrotizing mediastinitis is an urgent condition associated with high morbidity and mortality, and therefore requires a multidisciplinary approach. There are several classifications for the diagnosis and management of mediastinitis, one of the most popular is the one by Endo,[1] who proposed that according to the tomographic extension it should be classified as type I when it is located in the superior mediastinum above the tracheal bifurcation, which may not require aggressive mediastinal drainage; type IIA when it extends to the lower anterior mediastinum and type IIB when it also includes the posterior mediastinum, requiring complete mediastinal drainage. The most common cause is an infection from the oral or cervical region that spreads through the fasciae to the superior mediastinum.

Treatment depends largely on the etiology, as well as the extent of the disease; in Mexico, an extensive review

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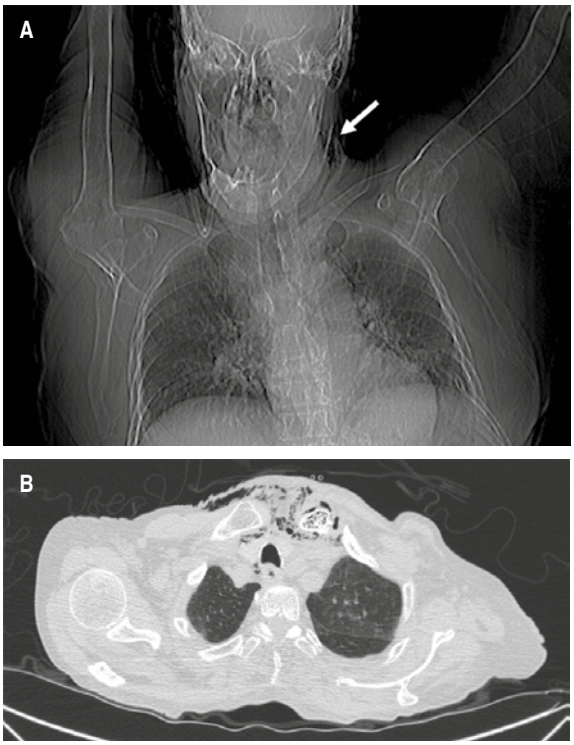


**Figure 1: A)** Topogram showing the presence of air in the left cervical region (white arrow). **B)** Computed tomography showing pulmonary window with evidence of retrotracheal air and subcutaneous emphysema.

of this condition was carried out, seeking to standardize treatment from the surgical point of view, for a condition that represents a health problem in certain segments of our population.[2] However, there are less common causes that affect the mediastinum by proximity, such as an infection in the sternoclavicular joint; this type of infection constitutes less than 1% of all joint infections. Due to the uncertain nature of its presentation, as well as its low prevalence, the diagnosis of a sternoclavicular joint infection is often delayed.[3] Suppurative infections involving this joint are especially difficult to treat because of its proximity to major vascular structures and the absence of a sufficient amount of peripheral tissue to limit damage. Patient-specific factors as well as infection by resistant microorganisms often complicate the picture;[4] because of these factors and the low incidence of such infections, surgical treatment has not been standardized.

We present the case of a patient with descending necrotizing mediastinitis associated with infection of the left sternoclavicular joint, as well as a review of the reported literature.

## CASE PRESENTATION

We present the case of a 76-year-old woman with long-standing diabetes with irregular insulin-based control, systemic arterial hypertension treated with captopril and losartan, as well as chronic renal failure treated with peritoneal dialysis; she was admitted to the emergency department by her relatives, with an approximate evolution of three days with general malaise, progressive neurological deterioration and abdominal pain. Based on the patient's medical records, she was approached with suspected dialysis catheter-associated peritonitis, meeting clinical and biochemical criteria of systemic inflammatory response, with a partially compensated metabolic acidosis with lactate of 6.1 mmol/L.

During the initial approach in the emergency department, it was decided to place a urinary catheter (the patient still had spontaneous diuresis), with evidence of pyuria; an attempt was made to place a central venous access, palpating the presence of subcutaneous emphysema, so it was requested to perform a computed tomography scan; with report of an inflammatory process located in the cervical region with extension to the upper mediastinum, as well as the presence of intramedullary gas in the left clavicle and sternal manubrium, suggestive of osteomyelitis *(Figures 1 and 2)*. The study of the peritoneal fluid obtained through the dialysis catheter was found to be within reference parameters, so in view of the evidence of septic shock associated with radiological evidence of Endo IIA mediastinitis, urgent surgical treatment consisting of exploration and cervical drainage was proposed, which was not accepted by the patient's relatives until 12 hours after hospital admission. Broad-spectrum antibiotic treatment (carbapenemics)

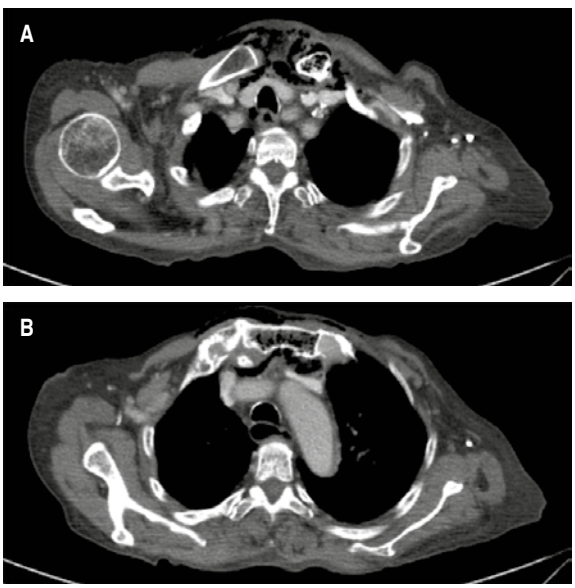


**Figure 2: A)** Pneumomediastinum with bone destruction and air in the sternal border of the left clavicle. **B)** Pneumomediastinum with bone destruction and air in sternal manubrium.

**Table 1:** Summary of previously reported cases of mediastinitis and sternoclavicular joint infection.

| Authors | Year | Patients (n) | Joint | Treatment | Comorbidity | Isolated pathogen |
|---|---|---|---|---|---|---|
| Pollack MS[8] | 1990 | 3 | Sternoclavicular | | | *S. aureus* |
| Tabib W, et al[9] | 1996 | 1 | Sternum | Antibiotic + surgery | | |
| Sonobe M, et al[10] | 1999 | 1 | Left sternoclavicular | Antibiotic + surgery | Diabetes *mellitus* | *S. aureus* |
| Dajer Fadel WL, et al[11] | 2012 | 1 | Right sternoclavivular | Antibiotic + surgery | Diabetes *mellitus* | *S. aureus* and *E. coli* |

was started from hospital admission and during surgery a cervical collar approach was performed, 1 cm above the sternal notch, dissected in planes and the presence of purulent fluid from the left sternoclavicular joint was identified; Due to the hemodynamic instability of the patient, manifested by persistent hypotension, use of pressor amines with progressive doses and presence of ventricular extrasystoles, it was decided to limit surgery to temporary control of the infectious focus with drainage, irrigation and partial debridement of the affected area.

The patient was admitted to the Intensive Care Unit post-surgery, with a torpid evolution and progressive deterioration despite the established medical-surgical treatment; she died eight hours after surgery. The final report of the culture and Gram stain of peritoneal fluid requested was negative for the presence of microorganisms, urine culture with growth of more than three Gram (-) microorganisms, bone culture and cervical abscess positive for *K. pneumoniae*.

## DISCUSSION

Descending necrotizing mediastinitis is an urgent condition that demands an opportune diagnosis with aggressive treatment. Although there are frequent and well-established causes of this entity, infection of the sternoclavicular joint is not one of them, with few reports in the literature. Infection of this joint is rare, but when it does occur, it results in abscess formation in 20% of patients; because the joint capsule has no ability to distend, the infection quickly spreads beyond the joint, evolving into fistulas, abscesses or mediastinitis (as the least common form).[5]

Osteomyelitis of a bone can be secondary to direct trauma or manipulation of the bone, sometimes it can originate from hematogenous dissemination from a distant site, especially in immunocompromised patients. In our case, the patient had no previous traumatic record, suffered from long-standing diabetes with poor adherence to treatment, chronic kidney disease and evidence of urinary tract infection, according to what has been reported in the literature (dissemination of distant infectious focus).[6] Due to the advanced stage of the disease at the time of admission to the emergency department, and an added delay in urgent surgical care due to the decision of the family members, the outcome was negative. It is noteworthy that the cervical abscess culture was positive for *K. pneumoniae*, when the vast majority of these cases are associated with the presence of Gram (+) microorganisms, unfortunately the urine culture was inconclusive.

Although the treatment of sternoclavicular joint infection is a topic of controversy,[7] and is not yet standardized *(Table 1)*, it is important to note that because of the proximity of this joint to the mediastinum, thoracic surgeons may be consulted for the care of such patients and should be familiar with the surgical management of sternoclavicular joint infection, which is a rare but potentially lethal cause of descending necrotizing mediastinitis that should be considered in this group of patients with multiple comorbidities.

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# Disseminated cryptococcosis in an immunocompetent patient: a case report and review of the literature

## Criptococosis diseminada en un paciente inmunocompetente: reporte de un caso y revisión de la literatura

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**ABSTRACT.** Cryptococcosis is a disease that is caused by the infection of encapsulated yeasts of the species *Cryptococcus sp.*, mainly *C. neoformans* and less frequently *C. gattii*; it is considered an opportunistic infection due to its greater impact on patients with risk factors. Transmission occurs mainly through spores or dried cells of the fungus, which are present in the environment and penetrate through the respiratory route into the body. It rarely occurs through accidental inoculation or penetration through wounds or lesions. We report the case of a 52-year-old man, without known immunocompromise (human immunodeficiency virus, type 2 diabetes, cancer or transplant) with disseminated cryptococcosis; with clinical symptoms of 2 years of evolution with cough and expectoration, hemoptysis, weight loss, headache and loss of alertness; he was hospitalized for extension studies concluding in disseminated cryptococcosis.

**Keywords:** cryptococcosis, disseminated cryptococcosis, *Cryptococcus gattii*, immunocompetent.

**RESUMEN.** La criptococosis es una enfermedad que se produce por la infección de levaduras encapsuladas de las especies *Cryptococcus sp.,* principalmente *C. neoformans* y con menor frecuencia *C. gattii*; se considera una infección oportunista debido a su mayor afección en pacientes con factores de riesgo. La transmisión ocurre principalmente por medio de las esporas o células desecadas del hongo que se encuentran presentes en el ambiente y penetran por vía respiratoria al interior del organismo; rara vez se produce por inoculación accidental o por la penetración a través de heridas o lesiones. Reportamos el caso de un varón de 52 años, sin inmunocompromiso conocido (virus de la inmunodeficiencia humana, diabetes *mellitus* tipo 2, cáncer o trasplante) con criptococosis diseminada; con cuadro clínico de dos años de evolución con tos y expectoración, hemoptoicos, pérdida ponderal, cefalea y pérdida del estado de alerta. Se hospitalizó para estudios de extensión concluyendo en criptococosis diseminada.

**Palabras clave:** criptococosis, criptococosis diseminada, *Cryptococcus gattii*, inmunocompetente.

## INTRODUCTION

Cryptococcosis is a fungal infection caused primarily by *Cryptococcus neoformans* and *gattii*,[1] commonly associated with a compromised immune system. A disseminated infection in an immunocompetent patient is extremely rare.[2]

We present the case of a 52-year-old Mexican patient with cryptococcosis in the lung and, subsequently, in the brain, mediastinal lymphadenopathy and pericardium.

## PRESENTATION OF THE CASE

Male, 52 years old, resident of Chimalhuacán, State of Mexico, bricklayer, history of visiting caves in Veracruz; no chronic degenerative diseases, cancer, use of drugs, denies smoking and ethylism. He started two years before, with cough with yellowish expectoration, occasional hemoptoics without hemodynamic or respiratory repercussions; weight loss of 22 kilos in six months, asthenia, adynamia, dysphagia to solids, as well as constant holocranial headache without

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identifying exacerbating or exacerbating factors. With loss of alertness 20 days before his admission, he went to the doctor who requested a tomographic study in which a lymph node conglomerate was observed at paratracheal 4R and subcarinal 7, at the level of the middle mediastinum a heterogeneous image of 4.7 × 4.8 cm with 75 HU and contrast enhancement, extrinsic compression of the esophagus and alveolar filling pattern in the middle lobe associated with solid and semi-solid spiculated nodules was observed *(Figure 1)*.

At the outpatient clinic, bronchoscopy was performed with findings of irregular mucosa with nodular lesions in the right main bronchus extending to the bronchus intermedius, cryobiopsy of bronchus intermedius and bronchioalveolar lavage of the right lower lobe, with positive Grocott and Schiff's periodic acid, compatible with *Cryptococcus sp.*, without presence of malignant cells, negative GeneXpert. Panendoscopy was performed with extrinsic compression of the middle esophagus, retentive stomach and erosive gastropathy, being negative for microorganisms and neoplastic cells in histopathology. He was hospitalized to continue the approach and start antifungal treatment. Physical examination showed no neurological alterations, chest with diminished respiratory sounds in the right subscapular region; hemogram, blood count, blood sugar, glycated hemoglobin in normal ranges; no transaminasemia or hyperbilirubinemia, negative inflammatory markers, serology for human immunodeficiency virus (HIV), hepatitis B virus (HBV) and hepatitis C virus (HCV) were not reactive.

During hospitalization, a tomographic study of the brain was performed, but no alterations were found; a lumbar puncture was performed with positive results in the meningitis panel for *Cryptococcus*, positive Chinese ink and Grocott, positive culture result for *Cryptococcus gattii* *(Figure 2)*. A week after admission, a lung biopsy was performed by videothoracoscopic surgery (VATS) of the lower lobe lesion in segment 6 and upper lobe in segment 3, with negative trans-operative result for malignancy, pericardial infiltration of the mediastinal lesion was found, and a pericardial window was performed. Histopathology in lung parenchyma sections showed pneumonia in various stages of organization without evidence of microorganisms, pericardium and mediastinum sections with spherical structures of reinforced wall, with pseudohypha and clear peripheral halo positive for Schiff and Grocott periodic acid stains, concluding cryptococcosis, Ziehl-Neelsen stain negative. After 20 days he presented again with pericardial effusion with 28 mm separation and pleural effusion, washing and drainage of the thoracic cavity plus pericardial window was performed, stains, pericardial biopsy culture and pericardial fluid were negative. Treatment with fluconazole and liposomal amphotericin B was maintained. Outpatient follow-up is planned to rule out primary immunodeficiency or other added factors.

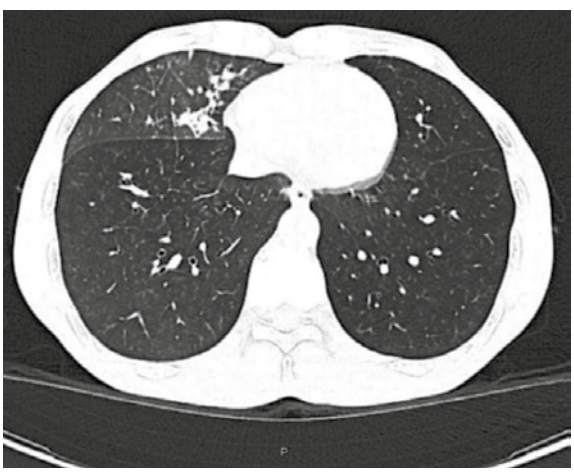

**Figure 1:** Parenchymal window chest CT scan with alveolar filling pattern in the middle lobe associated with solid and semi-solid spiculated nodules.

## DISCUSSION

*Cryptococcus* is a genus of encapsulated yeasts belonging to the phylum *Basidiomycota* and is widely distributed worldwide. Several forms of presentation have been described, the most frequent being pulmonary in its localized form and meningeal in its disseminated form. The main route of entry is inhalation, with easy dissemination by the hematogenous route with great tropism for the central nervous system.[3]

Pulmonary cryptococcosis in an immunocompetent patient is rare. However, a retrospective review in China reported that 60% of pulmonary cryptococcosis cases were diagnosed in immunocompetent patients.[4] Its spread is even rarer, which can lead to fatal complications, with mortality being even higher than those with underlying HIV infection, making early diagnosis critical.[5] A previous study reported that 67% of pulmonary cryptococcosis in immunocompetent patients spread to the central nervous system causing cryptococcal meningitis.[6]

Both the innate and adaptive immune systems participate in the host response against *Cryptococcus* infection. Alveolar macrophages phagocytize *Cryptococcus*, and a granulomatous immune response may follow with the formation of a subpleural nodule or primary lymph node, with pulmonary nodules being a common manifestation on radiological examination. The fate of *Cryptococcus* within macrophages is determined by the immune status of the host, if compromised it can lead to reduced pulmonary clearance and possible dissemination.[7] In those who are immunocompetent, it has been reported that those with

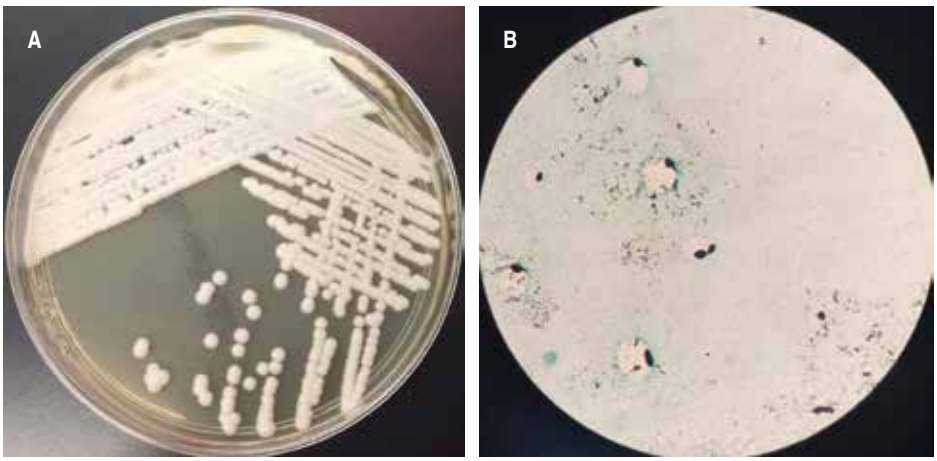


**Figure 2:**

**A)** Growth of colonies on Sabouraud agar, smooth, whitish, mucous appearance, which with time may appear dry. **B)** Grocott staining of tissue sample with presence of 6 to 15 microns thick-walled rounded yeast with isolated right base twins.

pulmonary cryptococcosis have a defect in their immune system, such as the polymorphism in Dectin-2, studied by Hu in China.[8]

Recognition of the disease in immunocompetent patients can be difficult, as the presentation is often more indolent and subtle, like the presenting patient. This can result in delayed diagnosis and initiation of treatment, which can lead to complications and the development of more severe disease. Some studies have reported higher mortality in non-HIV-infected patients than in HIV-infected patients.[9] Initiation of antifungal therapy is important for prevention of complications, which can be particularly challenging in this population.

## CONCLUSIONS

This report documents a rare case of disseminated cryptococcosis due to *Cryptococcus gattii* in an immunocompetent patient. We emphasize the importance of knowing the different forms of presentation, as well as early diagnosis and timely treatment to reduce the risk of complications and sequelae.

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Letter to the Editor
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# Use of inhaled corticosteroids in bronchiectasis

## Uso de corticosteroides inhalados en bronquiectasias

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Dear Editor:

We are writing to you regarding the article entitled «Recommendations for diagnostic approach and treatment of bronchiectasis»[1] recently published in this prestigious journal, in order to address the point of use of inhaled corticosteroids in patients with bronchiectasis.

Bronchiectasis presents itself as a disease of heterogeneous nature, exhibiting diverse etiologies that converge in a pernicious, persistent, progressive and irreversible cycle.[2] Its complexity is manifested in the variability of the severity of all the elements that make up its pathophysiology, such as mucociliary dysfunction, the microbiology involved and the diverse patterns of inflammation.[2] For this reason, the current and most recent research suggests directing attention to clinical phenotypes, inflammatory endotypes and disease overlap, with the aim of providing personalized and targeted therapies, focusing on treatable features.[2,3] With the goal of preserving lung function, halting disease progression, improving quality of life, preventing hospitalization and decreasing mortality.[2,3]

At present, international guidelines agree on the lack of indication for inhaled corticosteroids as routine treatment in patients with bronchiectasis; however, they recognize that the existing scientific evidence supporting this recommendation is limited and that more than 50% of patients with this disease are treated with inhaled corticosteroids.[3]

In this review, the authors refer to the meta-analysis published by Kapur N, *et al*.[4] The latter covered a set of seven clinical trials, highlighting the presence of improvement in the clinical parameters of dyspnea as well as a reduction in sputum production in the group of patients who received inhaled corticosteroids. These results could translate into an improvement in quality of life. In addition, in the cohort of patients treated with inhaled corticosteroids for a period of six months or less, two studies did not identify a significantly increased risk of *Pseudomona aeruginosa* colonization compared to the placebo group.[4]

Recently, Shoemark A, *et al*.[5] demonstrated that blood eosinophil counts of approximately 300 cells/μL are common in bronchiectasis and are found in a subgroup representing approximately 20% of patients with this condition. It was also established that elevated blood eosinophil counts are a risk factor for exacerbation in patients with bronchiectasis.

This study added to recent data showing clinically significant improvement in quality of life with high-dose inhaled corticosteroid therapy in patients with bronchiectasis who had blood eosinophil counts > 3% or 150 cells/μL. This identifies a specific population of patients who may respond to inhaled corticosteroids.[5]

Finally, Miguel Á. Martínez-García and his team detailed that, in individuals affected by bronchiectasis, the presence of both eosinophilia and eosinopenia is associated with greater severity, presumably due to the dual function of eosinophils, derived from their proinflammatory and bactericidal properties. And only in those patients with eosinophilia was there a significant reduction in the number of exacerbations associated with the use of inhaled corticosteroids.[6]

In conclusion, the decision to use inhaled corticosteroids in the treatment of bronchiectasis should be based on an individualized assessment, not limited only to patients with associated comorbidities such as asthma, chronic obstructive pulmonary disease (COPD) or allergic bronchopulmonary aspergillosis (ABPA).[4] It now also extends to individuals with bronchiectasis who lack additional comorbidities and present elevated peripheral eosinophil counts, which represents about 20% of this population.[5]

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# Reply

## Respuesta

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We appreciate the valid and substantiated commentary, limiting the frequent recommendation to limit the use of inhaled corticosteroids in bronchiectasis; this position is present in most guidelines, derived in part from the paucity of information on the management of bronchiectasis phenotypes. The largest cohort of patients with bronchiectasis (EMBARC) shows that up to 53% use inhaled corticosteroids, demonstrating frequent use despite recommendations, and this group has lower lung function, older age, high Bronchiectasis Severity Index (BSI) score, frequent exacerbations and more *Pseudomona aeruginosa* colonization.[1]

The use of inhaled corticosteroids in the presence of eosinophils > 400 cells/μL protects against exacerbations (RR = 0.70) and hospitalizations (RR = 0.56)[2] but, on the other hand, general users of inhaled corticosteroids are at higher risk of exacerbations (RR = 1.21) and hospitalizations (RR = 1.14), although with no increase in mortality;[1] showing a clinically simple and known marker,[3] for example, in patients with chronic obstructive pulmonary disease (COPD), that can guide the selection of patients who benefit from inhaled corticosteroids.[4] Hopefully, information will soon be available to select the most effective inhaled corticosteroid with the greatest benefits and lowest risks.

The benefit provided by inhaled corticosteroids may be associated with induction of apoptosis and eosinophil depletion, with reduction of proinflammatory cytokines from lymphocytes, epithelial cells and macrophages, and perhaps because they also deplete mast cells and dendritic cells, and reduce mucous secretion.[3]

In COPD, which shares certain inflammatory features with bronchiectasis, also only patients with eosinophilia (> 300 cells/mL) improve with inhaled corticosteroids.[4] Interestingly, there is a relationship between *Pseudomona aeruginosa* infection and a Th2 (T Helper Lymphocyte) response[5] and leaves the question of whether there is any relationship between this microbe and the benefit of inhaled corticosteroids. Similarly, other Th2 biomarkers such as exhaled fraction of nitric oxide (FeNO) remain to be explored, but when eosinophils and FeNO are measured the Th2 response increases by 20-30% in patients with bronchiectasis.[6] As in other chronic respiratory diseases, we should be cautious with the use of inhaled corticosteroids in bronchiectasis as they have also been associated with increased risk of pneumonia and mycobacterial infections.[7]

The use of inhaled corticosteroids should be reserved for patients who will reasonably derive clear benefits and few adverse effects; it is encouraging that a marker has been identified in eosinophils that provides guidance in this regard, knowledge that will undoubtedly be incorporated into future versions of the Diagnostic and Treatment Guidelines.

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Letter to the editor

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# Swyer-James-MacLeod syndrome: an uncommon conundrum

## Síndrome de Swyer-James-MacLeod: un enigma poco común

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Dear editor:

The Swyer-James-MacLeod syndrome (SJML) is an uncommon cause of obstructive pulmonary disease with unilateral hyperlucent lung, often in children, with estimated prevalence of 0.01% in over than 17,000 chest X-rays, related to restrictive respiratory tests and lung infections, and the data on the outcomes during pregnancy are scarce.[1-5]

We read the illustrative report in this Journal by Torres-Rodríguez ST *et al.*[5] of a 31-year-old woman who was incidentally diagnosed with SJML when searched medical attention due to a mild insidious retrosternal pain and the imaging studies revealed the hyperlucent left lung that was displacing the mediastinum to the right, and abnormal pulmonary vasculature with hypoperfusion and hypoplasia of the left pulmonary artery. The authors emphasized her childhood and adolescence history of respiratory diseases like common colds, bronchitis, pneumonia; and an uncomplicated COVID-19 infection. They also stressed the diagnostic cornerstones (unilateral lung hyperlucency, reduction of vascularity and perfusion loss), besides the conservative management of this patient.[5] Considering the important role of case studies about uncommon and scarcely reported diseases, it seems opportune add short comments of novel literature data about SJML.[1-4]

Al-Bakri O *et al.*[1] reported a 29-year-old primipara in the prenatal evaluation, who had the diagnosis of SJML seven years before by typical images in the left lung, besides asthma and pneumonia in the early infancy, followed by recurrent respiratory infections. The lung function tests at 29 weeks showed FEV 1.79 L (59% predicted), and FEV1/FVC ratio 64%; and at 34 weeks the FEV1 1.99 L (65% predicted), and FEV1/FVC ratio 66%.[1] She underwent budesonide/formoterol and salbutamol inhalers for the month prior to delivery, and had an uncomplicated vaginal delivery under early epidural control of pain.[1] The authors emphasized the uneventful pregnancy and vaginal labor of a patient with SJMS and moderate obstructive pulmonary disease maintained under a close vigilance.[1] They also stressed the epidural control of pain to reduce oxygen consumption and minute ventilation during the first and second labor stages of women with respiratory diseases.[1] Chlapoutakis S *et al.*[2] described a 63-year-old heavy smoker man with coronaropathy and chronic obstructive lung disease, using beclomethasone, formoterol, and glycopyrronium; and more than three hospitalizations due to infectious exacerbations the last six months. The pulmonary function tests revealed that the FEV1 and the FVC values were of 65%/67% and 99%/102% of predicted values, pre- and post-bronchodilator, respectively.[2] Chest images showed emphysema, bronchiectasis, and an hyperlucent left lower lobe; with diagnosis of SJML he underwent salbutamol/ipratropium, corticosteroid, and antibiotics, besides recommendation for smoking cessation and regular vaccinations.[2] The authors emphasized the uncommon diagnosis of SJML syndrome in adulthood, mainly in absence of antecedent significant pulmonary infection during the childhood.[2] Cheng YH, *et al.*[3] reported a 17-year-old male, who had repeated pneumonia since the childhood, presenting with acute left thoracic pain and dyspnea; and the chest imaging studies showed almost complete atelectasis and bronchiectasis in the right lung, hyperlucency of left lung, mediastinal deviation to the right, and bilateral pneumothorax. The chest X-ray images were very similar to bilateral pneumothorax and due to air leak for over a week a thoracoscopic procedure with chemical pleurodesis was performed.[3] The overexpansion of the left lung by SJMS caused atelectasis of the contralateral lung; the authors stressed the pneumothorax as an ominous complication because of the dysfunction of the other lung, but a closed thoracostomy drainage may be lifesaving.[3] Fontes CP, *et al.*[4] described a 34-year-old male with antecedent of pulmonary tuberculosis in adolescence, who had a prolonged fever, right-sided pleuritic pain and purulent sputum. The chest imaging studies showed a hyperlucent right upper lobe (as seen on ancient lung images), homolateral opacities, besides a reduced pulmonary vasculature and perfusion.[4] Cultures of sputum and bronchoalveolar lavage were negative, and ventilation/perfusion scintigraphy showed a matched ventilation and perfusion defect of the affected areas; these evaluations and the clinical manifestations confirmed the SJMS as final diagnosis.[4] The authors highlighted the role of recognition of this syndrome in adulthood, because of higher possibility of misdiagnosis and adverse effects of the inappropriate management.[4]

Worthy of note, an occasional diagnosis of SJMS during adulthood may constitute a more challenging task, inclusive because some of the differential hypotheses like pneumothorax and pulmonary hypertension are also complications of the syndrome;[2-4] and if accurately followed, affected women who desire can have uneventful pregnancy.[1] The authors believe that the case studies can lessen the underdiagnosis and misdiagnosis.

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# Reply

## Respuesta

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I believe that as more cases are published, the recognition of this entity will be much better understood, recognized and treated. The history of lung disease must be kept in mind, since many architectural changes of the bronchial tree are a consequence of them. Although bronchiolitis is more common in children, it can affect adults. The disease can progress to severe obstructive respiratory failure, especially in the case of constrictive bronchiolitis.[1] The radiological image of a hyperlucent lung should guide us to the suspicion of this syndrome and the treatment is in principle conservative and aimed at preventing recurrent infections.[2] Surgical treatment should be reserved for advanced cases and the magnitude of the type of surgery will be justified by the structural damage to the lung, or by the implications of the hyperinflated lung that can act as a tension pneumothorax by displacing the mediastinum so severely that it can limit healthy lung function; Therefore, surgical intervention is reserved for: a) patients with recurrent lung infections, b) patients who do not respond, or c) patients whose symptoms are not adequately controlled with optimal medical treatment.[3]

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# Institutional Bioethics Committee INER, 20 years

## Comité Institucional de Bioética INER, 20 años

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*After so many years studying ethics, I have come to the conclusion that it can be summed up in three virtues: courage to live, generosity to live together and prudence to survive.*
Fernando Savater

On October 27, 2003, the Bioethics Committee of the National Institute of Respiratory Diseases[1] was established with the participation of the following persons:

- Dr. Fernando Cano Valle: Chief Executive Officer
- Dr. José de Jesús Villalpando Casas: Director of Teaching
- Dr. Fernando Rébora Togno: Chief of Oncologic Pneumology Service
- Dr. Guillermo Carvajal: Deputy Director of Biomedical Research
- Lic. Adriana Martuscelli: Deputy Director of Personnel Administration and Development
- Dr. Ricardo Neri Vela: Head of the Legal Affairs Department
- Dr. Alejandra Gamiño: Head of the Food Department
- Lic. Rocío Buendía Valenzuela: Social Worker
- Lic. Ana María Becerril: Deputy Head of Nursing Department
- Pbro. Lic. Guillermo Gutiérrez Fernández: Bioethics Advisor of the Archbishopric of Mexico.
- Mr. Carlos Vega Alexandre: Patient INER
- Dr. José Antonio Moreno Sánchez: Obstetrician and Gynecologist

Dr. Fernando Rébora was appointed as Chairman of the Committee, who proposed conducting exit surveys of patients and their families at hospital discharge, in which the degree of satisfaction with the care received is preliminarily investigated. In addition, it was proposed to publicize the charter of patients' rights and it was decided to share bibliography related to bioethical issues.

Since that date, the committee has worked uninterruptedly, has been able to deal with two pandemics (influenza and COVID-19) and there has been a rotation of its members; on the other hand, the topics for reflection are increasingly complex, since they are so diverse that they go beyond the interhospital health problem, for example: migration, discrimination, vulnerable population, artificial intelligence, legislation, religion, patient-physician relationship.

It should be noted that the INER committee has a direct relationship with


**Figure 1: A)** Cover of the book Reflexiones Bioéticas Comité Hospitalario de Bioética (INER) and its main author Dr. Jaime Villalba Caloca. **B)** INER Bioethics Committee at the traditional breakfast at the beginning of the year. **C)** Dr. Alejandro Jiménez Chobillon. Head of the INER Institutional Bioethics Committee. Images taken from: https://www.scielo.org.mx/scielo.php?script=sci_arttext&pid=S0028-37462020000100059 https://www.medigraphic.com/cgi-bin/new/resumen.cgi?IDARTICULO=105535 https://www.doctoralia.com.mx/marcos-alejandro-jimenez-chobillon/otorrinolaringologo/ciudad-de-mexico

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the National Bioethics Commission (whose head is Dr. Patricio Santillan Doherty) and has sought to offer recommendations to the needs of the problems that arise, always with due courtesy and fraternity among the various members of the committee.

For many years, Dr. Jaime Villalba Caloca headed the institutional bioethics committee, having a very fruitful activity during his term, in addition to the book of Bioethical Reflections at the end of his term *(Figure 1A)*. The committee is a heterogeneous group, which is recommended and favors the broad vision of the different thoughts and knowledge necessary to approach the different cases.

Personalistic bioethics with its four pillars has been approached:

1. Autonomy
2. Fairness
3. Beneficence
4. Non-maleficence

One of the committee's activities is to disseminate knowledge of bioethics to undergraduate and graduate students. Likewise, recreational activities have been sought, such as the promotion of the so-called film-debate, which has been a great success in each of its exhibitions: a film showing a bioethical dilemma is shown and at the end a debate about it is stimulated.

These first two decades of which I have participated in 14 years, have been of great support for the Institute, resulting in a culture of Ethics and Bioethics in all the staff with a humanistic vision towards patient care *(Figure 1B)*.

At present, under the leadership of Dr. Marcos Alejandro Jiménez Chobillon *(Figure 1C)*, due to the successful appointment of our general director, Dr. Carmen Margarita Hernández Cárdenas -who was president-, the committee has a great future ahead of it.

*In Medio, Virtus…*

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