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ORIGINAL

# Intubated patients developing tracheobronchitis or pneumonia have distinctive complement system gene expression signatures in the pre-infection period: A pilot study

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**KEYWORDS**
Ventilator associated pneumonia;
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Sepsis;
RNA;
Biomarker;
VAP;
VAT

**Abstract**
*Introduction:* It remains unknown why some intubated patients remain infection-free while others develop tracheobronchitis (VAT) or pneumonia (VAP).
*Objective:* To identify and compare VAP/VAT gene expression ''signatures'' using genome-wide oligonucleotide microarrays.
*Material and methods:* A prospective translational study of gene expression profiles of VAP and VAT groups was carried out, establishing comparisons in both pre-infection and infection phases. Pathway and functional analyses were performed with Ingenuity Pathway Analysis (IPA). Data analysis and hierarchical clustering of the genes involved in the signalling pathways expressed differentially in the two groups were performed with GeneSpring GX 11.0.
*Results:* Eight patients developing respiratory infections (3 VAP and 5 VAT) after 4 days of mechanical ventilation were assessed. Comparison of gene expression profiles in the pre-infection period revealed 5595 genes expressed differentially between VAP and VAT ($p < 0.01$, fold change >2). Comparative IPA analysis identified a significant depression of the complement system signalling pathway in the VAP group, affecting the classical pathway along with the final common pathway ($p < 0.05$). In addition, the cAMP and calcium signalling pathways were also significantly depressed in the VAP group during the pre-infection phase also.
*Conclusion:* Intubated patients complicated with pneumonia developed immune impairment in the pre-infection period, manifesting as a relatively lower expression of genes involved in the complement system that differed from patients developing tracheobronchitis. These findings suggest that a significant proportion of VAP episodes cannot be prevented, but might be treatable through pre-emptive therapy.


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**PALABRAS CLAVE**
Neumonía asociada a ventilador;
Expresión genética;
Traqueobronquitis asociada a ventilador;
Genes;
Infección;
ARN;
Biomarcador;
NAV;
TAV

**Los pacientes intubados que presentan traqueobronquitis o neumonía tienen patrones distintivos de expresión genética del sistema del complemento en el período previo a la infección: un estudio piloto**

**Resumen**
*Introducción:* Seguimos sin saber por qué algunos pacientes intubados no sufren infecciones mientras que otros presentan traqueobronquitis (TAV) o neumonía (NAV).
*Objetivo:* Identificar y comparar los patrones de la expresión genética de la NAV/TAV usando micromatrices multigénicas oligonucleotídicas.
*Material y métodos:* Se realizó un estudio aplicado prospectivo de los patrones de la expresión genética de los grupos con NAV y TAV, estableciendo comparaciones tanto en la fase previa a la infección como en la fase infecciosa. Se realizaron análisis de vías y funcionales con Ingenuity Pathway (IPA). Los análisis de datos y el agrupamiento jerárquico de los genes implicados en las vías de señalización expresados de forma diferenciada en ambos grupos se realizaron con GeneSpring GX 11.0.
*Resultados:* Se evaluaron ocho pacientes que presentaron infecciones respiratorias (3 NAV y 5 TAV) después de 4 días con la ventilación mecánica. La comparación de los perfiles de la expresión genética durante el período previo a la infección reveló 5.595 genes expresados de forma diferenciada entre la NAV y la TAV ($p < 0,01$, cambio múltiplo > 2). Los análisis comparativos de los IPA identificaron una depresión importante de la vía de señalización del sistema del complemento en el grupo con NAV, que afectó a la vía clásica además de a la vía común final ($p < 0,05$). Por otra parte, el monofosfato cíclico de adenosina y las vías de señalización del calcio también se vieron muy deprimidos en el grupo con NAV durante la fase previa a la infección.
*Conclusión:* Los pacientes intubados que presentaron complicaciones por la neumonía desarrollaron un trastorno inmunitario durante el período previo a la infección, que se manifestó como una expresión relativamente menor de genes implicados en el sistema del complemento diferente de la de los pacientes que presentaron traqueobronquitis. Estos resultados sugieren que no se puede evitar una importante proporción de los casos de NAV, aunque podrían ser tratables mediante un tratamiento preventivo.


## Introduction

Respiratory infections including pneumonia and tracheobronchitis remain the leading nosocomial infections in the ICU.[1] Ventilator-associated tracheobronchitis (VAT) is manifested by purulent respiratory secretions.[1] Differentiating VAT from ventilator-associated pneumonia (VAP) is mainly based on presence/absence of radiographic opacities, but the potential continuum of VAT to VAP is unknown.[2,3] The incidence of VAT has been reported to be more common in surgical patients.[4] Some reports suggest that patients with VAT also have increased length of ICU stay and a prolonged need for mechanical ventilation.[5,6]

To gain a better understanding of the molecular mechanisms that underlie the lung phenotypes in VAT vs VAP, we sought to identify and compare their gene expression ''signatures'' using genome-wide oligonucleotide microarrays. We hypothesized that VAP/VAT would exhibit different gene expression signatures which could help to distinguish between the two conditions and would also shed light on their pathogenesis. We also expected that specific signatures could lead to the identification of new biomarkers to aid in the diagnosis and classification of these diseases.

## Methods

### Study design

This is a cohort study including ICU patients with VAP/VAT prospectively recorded. The protocol was approved by the ethical committee and informed consent was obtained from the next of kind prior to enrolment in the study. All patients enrolled were recorded anonymously in the registry.

Immunocompetent patients who were intubated and ventilated for more than 48 h were eligible for the study and were followed up for a week. Patients, who developed nosocomial pneumonia before or after the occurrence of VAT, were excluded. Patients with chronic respiratory failure, patients who were not ventilated or ventilated for less than 48 h, patients who received only noninvasive pressure ventilation, patients with tracheostomy at ICU admission, patients who had been treated with corticosteroids/immunosuppressive drugs and patients who were immunocompromised were not eligible and not included in the present study.

VAT was defined as the presence of all of the following in a patient endotracheally intubated and receiving mechanical ventilation for >48 h: body temperature > 38.3 °C or <36.0 °C, new or increased purulent tracheal secretions,[7]