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Aerobic respiration indicates high metabolic activity in bacteria in acute lower respiratory tract infections

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INTRODUCTION

While oxygen (O_2) is restricted in the endobronchial mucus of chronic lung infections as seen in cystic fibrosis, it has not been established whether restriction of O_2 applies to acute lower respiratory tract infections (LRTI). O_2 restriction is suggested to be caused by O_2 consumption by the polymorphonuclear leukocytes (PMNs) surrounding the bacteria. The clinically most important aspect of O_2 restriction may relate to the responsiveness of pathogenic bacteria to antibiotics.

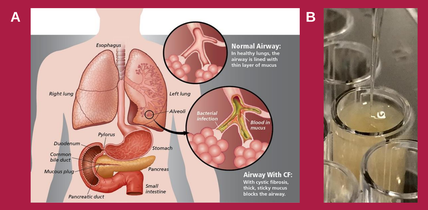

Figure 1: A, a patient with lower respiratory tract infection with mucus plugs in the conductive zone in the respiratory system, B, O_2 profiling with an inserted microsensor in expectorated sputum.

AIM

We aimed to determine if O_2 restriction is present in sputum of patients with LRTI and if it is caused by O_2 consumption by the PMNs or cellular aerobic respiration.

METHOD

In a prospective observational study, we have included 52 patients with LRTI. Expectorated sputum samples (n=69) were collected from admission to discharge. To analyze sputum samples, we used flow cytometry for neutrophil counts (n=61), O_2-profiling (n=42) and micro-respirometry during treatment with potassium cyanide (KCN) for estimation of O_2 consumption by cellular aerobic respiration (n=12). The effect of KCN on the O_2 consumption by cellular aerobic respiration was tested on pure cultures of *Staphylococcus aureus, Escherichia coli, Pseudomonas aeruginosa, Klebsiella pneumoniae, Haemophilus influenzae, Moraxella catarrhalis* and *Streptococcus pneumonia.*

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RESULTS

Tabel 1. Charactheristics of 52 patients with lower respiratory tract infections

Median age in years (IQR[a])	69.5 (56.5-79.5)
Female, n (%)	18 (34.6)
Median body mass Index (kg/m2) (IQR)*	25.8 (23-28.4)
Median CRP (mg/L) on admission (IQR)**	97 (44-132)
Diabetes, n (%)	7 (13.5)
Median blood neutrophil count (10^9/L) on admission (IQR)***	8.4 (6.6-12.5)
Smoking	
Never, n (%)	7 (13.5)
Current, n (%)	16 (30.8)

[a]Interquartile range, *n=46, **n=45, ***n=46, ****n=39

O_2 depletion was observed in 20/42 sputum samples from LRTI patients

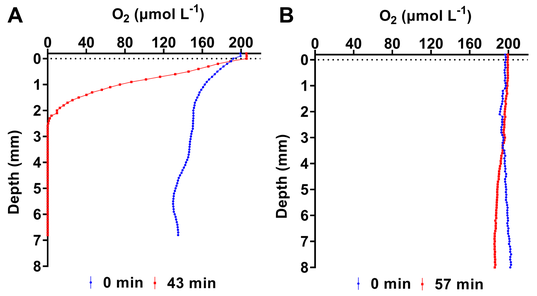

Figure 1. Representative sputum samples from LRTI samples with anoxic (A) and oxic (B) conditions. The blue line indicates the initial O_2 profile through sputum and the red line indicate the last profile trough sputum. Dsahed line indicate the sputum surface.

More neutrophils in anoxic and purulent sputum samples from LRTI patients

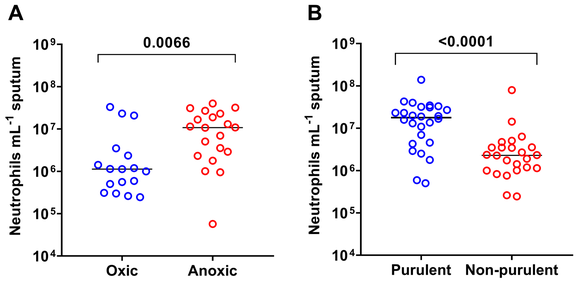

Figure 2. A, Neutrophils mL^{-1} sputum for oxic (blue, n=17)) and anoxic (red, n=20) sputum samples from LRTI patients. B, Neutrophils mL^{-1} sputum for purulent (blue) and non-purulent (red) sputum samples from LRTI patients. Statistical significance was assessed using Mann-Whitney test.

Estimated aerobic respiration in sputum of LRTI patients

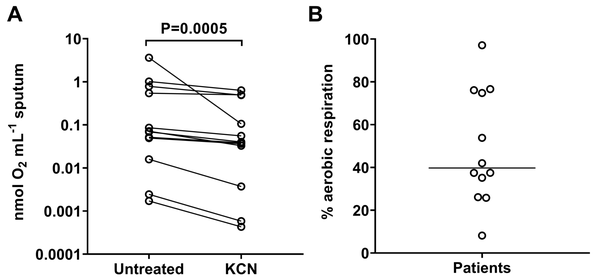

Figure 3 A, The O_2 consumption sec^{-1} mL^{-1} in untreated and KCN treated sputum is shown for individual sputum samples, n=12. B, The estimated aerobic respiration in percentage for individual samples are shown, n=12. Estimated aerobic respiration ranged from 25.8-97.1% with a median of 39.7%, shown as a horizontal line. Statistical significance was assessed using Wilcoxon test.

Inhibition of bacterial aerobic respiration by KCN

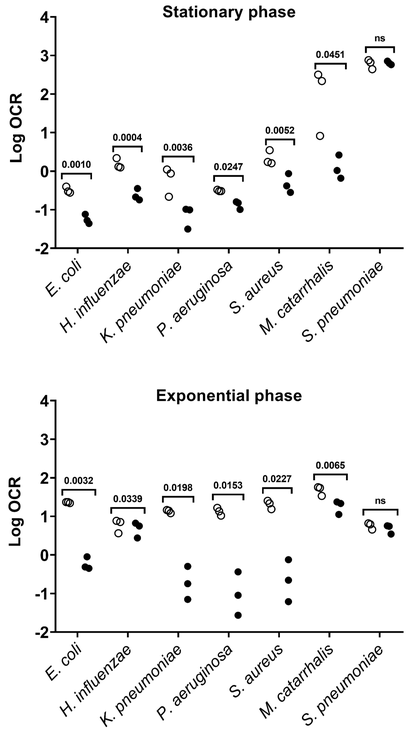

Figure 4. Effect of 2 mM KCN on OCR (O2 consumption rate (amol bacteria^{-1} sec^{-1})) bacteria in stationary phase and in exponential phase. The effect on each species was compared by paired t-test. The OCR was significantly reduced by 2 mM KCN in all species *but S. pneumonia*. 0 mM KCN (open circle) and 2 mM KCN (black circle). Statistical significance was assessed using Student t-test.

CONCLUSIONS

This study has demonstrated that ongoing cellular aerobic respiration is present in acute lung infections using expectorated sputum samples from LRTI patients, indicating the presence of bacteria with sensitive physiology, as bacterial respiration is connected to high susceptibility to antibiotics. These findings indicate variations in bacterial physiology between LRTI patients and CF patients, which can be assumed to, at least partly, explain the variation in treatment success for the two patient groups.