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# Azithromycin inhibits the production of MUC5AC in the airway mucosa of patients with bronchiectasis induced by *Pseudomonas aeruginosa*

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**Abstract**: To explore the clinical benefits of azithromycin in the treatment of *Pseudomonas aeruginosa* induced bronchiectasis and to evaluate its effect on MUC5AC. From April 2018 to June 2020, 160 patients with bronchiectasis due to *Pseudomonas aeruginosa* infection were selected. The patients were divided into a control groupand an azithromycin group. Statistics of patients' general clinical data, lung function indexes, sputum volume, oxidative stress level, Bhalla score before and after treatment; Western blot analysis of MUC5AC expression; RT-PCR analysis of TNF-α, IL-8, IL- 1β mRNA expression. The mRNA expression of TNF-α, IL-8 and IL-1β in the azithromycin group was lower than that in the control group ($P$<0.05). After treatment, the protein expression of MUC5AC in the azithromycin group was lower than that in the control group ($P$<0.05). The improvement rate in the azithromycin group was significantly higher than that in the control group ($P$<0.05). The azithromycin group had a lower lung infection rate than the control group ($P$<0.05). The azithromycin group had a lower dyspnea rate than the control group ($P$<0.05). Azithromycin treatment has certain clinical benefits for patients with bronchiectasis induced by *Pseudomonas aeruginosa* and inhibits the MUC5AC expression.

**Keywords**: Azithromycin, bronchiectasis, clinical trials, MUC5AC

## INTRODUCTION

Glioblastoma is the most common aggressive tumor in the nervous system. Although in-depth treatment has been carried out by multi drug combined chemotherapy and surgical operation, the prognosis of patients is generally poor and cannot be cured (Kim *et al*., 2018). Therefore, it is still an urgent clinical need to effectively identify and develop new molecular methods for the diagnosis, treatment and prognosis of glioblastoma patients (Boccellato *et al*., 2018). Zinc finger E-box binding homeobox 2 (ZEB2) regulates cell proliferation, migration, invasion and apoptosis in several forms of human cancer (Gonciarz *et al*., 2019). High level of therapeutic resistance, strong cell infiltration and rapid cell growth require active multimodal therapy, including resection, radiotherapy and chemotherapy (Wang *et al*., 2018). Although the treatment strategy including surgical equipment or adjuvant therapy has been improved, the overall survival rate of glioblastoma is still poor, and new therapy is urgently needed (Menzella *et al*., 2018). Epithelial-to-mesenchymal transition (EMT) has also been proved to be related to the occurrence and development of GBM (Lee and Hong, 2019). Members of the zinc finger E-box-binding protein (ZEB) family, including ZEB1 and ZEB2, are transcriptional inhibitors that inhibit E-cadherin transcription and thus promote EMT (Suarez-Cuartin *et al*., 2018). Although ZEB signaling pathway has been proved to regulate the proliferation, migration, invasion and apoptosis of glioma cells *in vitro*, the clinical significance of ZEB in glioma remains controversial (Everaerts *et al*., 2018). For example, a previous study reported that an increase in ZEB1 expression demonstrated a good prognosis in isocitric dehydrogenase (IDH1) mutant low-grade gliomas (Martinez-García, 2018). The adverse effects of *Pseudomonas aeruginosa* on patients with bronchiectasis involve many aspects, including the level of inflammation (local and systemic), lung function injury, acute exacerbation, quality of life and mortality (Bedi *et al*., 2018). This study aims to explore the clinical benefits of azithromycin in the treatment of *Pseudomonas aeruginosa* induced bronchiectasis and to evaluate its effect on MUC5AC.

## MATERIALS AND METHODS

### *Clinical Characteristics*

We retrospectively studied 160 patients with bronchiectasis due to *Pseudomonas aeruginosa* infection in the respiratory department of the third people's Hospital from April 2018 to June 2020. According to the clinical data and high-resolution Computed Tomography scan, they had bronchiectasis in consecutive outpatient clinics. Among them, there were 73 male patients with an average age of 49.31±4.16, 87 female patients with an average age of 51.37±4.68. According to the purpose of the study, all patients were divided randomly into the control group (Piperacillin / sulbactam 3.0g, twice a day, intravenous drip; the course of treatment was 10-14 days) and the azithromycin group (250mg azithromycin oral treatment;

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the course of treatment was 10-14 days). There was no difference in clinical data of all patients.

***Inclusion criteria***

Gender unlimited; 18-70 years old; patient's condition is stable; no history of respiratory failure; lung CT shows bronchiectasis.

***Exclusion criteria***

History of hysterectomy due to uterine tumor and pelvic infection; history of pulmonary surgery; immune deficiency secondary to human immunodeficiency virus; malignant tumor; patients with common variable immune deficiency, emphysema, allergic bronchopulmonary aspergillosis or diffuse interstitial lung disease; history of serious cardiovascular and cerebrovascular diseases; patients who do not agree to sign the informed participation letter.

***Medical ethics***

All patients or their guardians sign informed consent or oral consent informed consent.

***Methods***

*Experimental procedure*

Over a three-month period, both groups continued to receive the same dose of habitual treatment, including inhaled steroids, bronchodilators, myxolysis and physiotherapy.

If severe, steroids or antibiotics are recommended. Medical history, physical examination, high-resolution computed tomography, blood samples, sputum samples, and pulmonary function tests were obtained at the completion of screening visits and treatment. The main results of this study were to change the respiratory oxidative stress indexes (8-Isoprostaglandin, nitrite, etc.). Secondary end points included lung function, sputum color and volume, aggravation, number of hospital admissions, impact on functional capacity and health-related quality of life changes. The reference functional pulmonary function variables in the study were: Forced vital capacity, expiratory capacity within 1 second, expiratory capacity within 1 second after bronchiectasis, expiratory capacity / vital capacity within 1 second and total lung capacity.

***Bhalla Score***

Using the modified Bhalla score, standard high-resolution computed tomography of the lungs was performed to quantify the degree of bronchiectasis (Piazza *et al*., 2015). The score considers the following characteristics of each lung lobe: bronchiectasis, wall thickness, bronchial diameter size, presence of mucus blockage, and presence of attenuation. The highest score is 72. All scores were obtained through an agreement between the two investigators.

***Sputum examination***

In order to analyze the characteristics of sputum, three sterile samples were collected for three consecutive days. Calculate the average value of 3 days and express it in ml / day. Sputum color was scored using a scale developed and validated by our laboratory, ranging from 0 to 15: transparent, 0 white, yellow progressive intensity, 2-7 green 8-10 and brown. The 10-15 color score is in two investigators. Fresh sputum samples from three consecutive days were collected for microbiological analysis. Gram staining and culture of habitual microorganisms, mycobacteria and fungi were carried out. If the blood volume of the sample from at least two samples exceeds 1x103 CFU/ml, the sputum is considered colonized. If the patient needs antibiotic treatment or admission, record the deterioration before and during the study. The quality of life (QOL) was assessed by the dyspnea scale of the Medical Research Council to assess its impact on functional ability and patients' daily life. By measuring the pH value of EBC and 8-Isoprostaglandin, nitrite (NO2-), the oxidative stress and no metabolism in airway were studied.

***Detection of MUC5AC expression***

The sputum of the two groups was collected and processed, using Radio Immunoprecipitation Assay buffer and centrifuged at 13,000 1rpm for 15 min at 4$^{o}$C (Schmitt *et al*., 2018). The total protein concentration was determined using the BioRad protein assay kit (Herakles, California, USA). The protein was separated by twelve alkyl sulphate polyacrylamide gel electrophoresis and transferred to the 0.45nm m nitrocellulose imprinted membrane (GE Medical UK Limited, UK). The Western blotting and anti MUC5AC or anti actin I antibody were immersed in 5% skimmed milk or 5% bovine serum albumin. The detected imprints were then incubated with the second antibody at room temperature for 1 hour. The membrane was washed 3 times with PBS containing 0.05% (v/v) Tween 20, and observed with enhanced chemiluminescence reagent (Thermo Fisher Scientific, Rochford, IL, USA).

***RNA extraction and real-time quantitative PCR (qRT-PCR)***

According to the instructions, RNA was extracted using Qiagen RNeasy Mini kit (Qiagen; Valencia, CA, USA), and cDNA was synthesized from 1mg total RNA using reverse transcriptase kit (Otsu, Japan). SYBR Premix Ex labeling kit (TaKaRa Biotechnology) and ABI 7500 sequencing detection system (Applied Biosystems; California, USA) were used in qPCR. The reaction was performed three times. The primer sequences used in this study are as follows:

***Other biochemical indicators***

The concentration of 8-Isoprostaglandin was measured with commercial kits. NO2/NO3 was measured by Griess

reaction (Cayman chemical, Ann Arbor, Michigan, USA) using colorimetric kit and the results were expressed in mmol. The concentration of 8-Isoprostaglandin was obtained by competitive enzyme immunoassay kit (Cayman chemical company), and its value was expressed in pg/ml. Systemic inflammation was measured by CRP and erythrocyte sedimentation rate.

## STATISTICAL ANALYSIS

All data are expressed as mean ± SD. The difference between the averages was evaluated by using Tukey 5 for Windows (GraphPad Software Inc., La Jolla, CA, United States) using Tukey's multi-range test by one-way ANOVA. $P<0.05$ was considered as statistically significant.

## RESULTS

***Comparison of patients' clinical data***

A total of 160 patients with bronchiectasis induced by *Pseudomonas aeruginosa* were involved in this study. The control group of male patients was 34 (42.5%), with an average age of 49.27±5.46, course of disease 6.58±1.24, lung function score of 25.34±4.16, and history of smoking (20.00%), 39 (48.75%) male patients in the azithromycin group, mean age 51.08±5.63, course 7.23±1.58, lung function score 24.79±4.02, smoking history 14 (17.50%). There was no difference in general statistics between the two groups ($P>0.05$) (table 2).

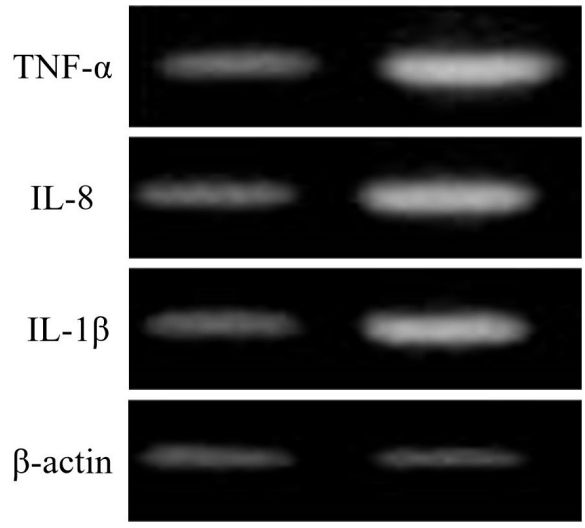


**Fig. 1**: RT-PCR real-time analysis of TNF-α, IL-8, IL-1β mRNA expression

***Real-time analysis of pro-inflammatory factor mRNA expression by RT-PCR***

Three months later, the expression of TNF-α, IL-8, IL-1 β mRNA in the two groups was detected by PCR. The expression of TNF-α, IL-8, IL-1 β mRNA in the azithromycin group was lower than that in the control group ($P<0.05$), indicating that azithromycin can effectively improve the inflammatory reaction in patients with bronchiectasis (fig. 1, table 3).

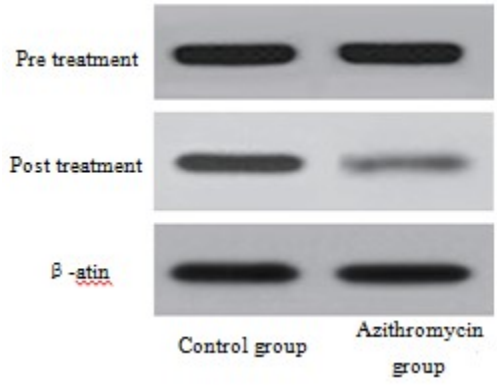


**Fig. 2**: Western blot analysis

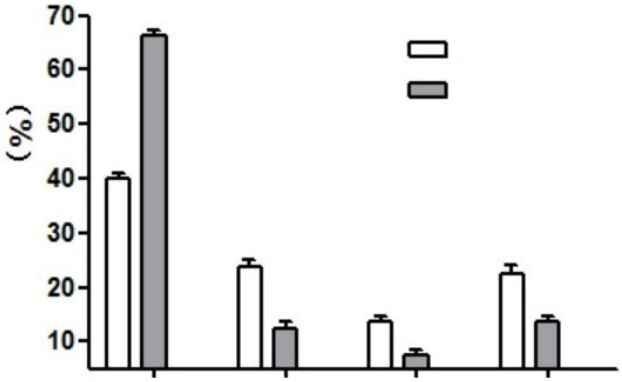


**Fig. 3**: Treatment effect of each group

***Western blot analysis of MUC5AC expression***

Western blot analysis of the protein expression of MUC5AC before and after treatment, there was no difference in protein expression of MUC5AC between the two groups of patients before treatment ($P>0.05$). Reduces the induction of mucin MUC5AC synthesis after inflammatory stimulation (fig. 2, table 4)

***Clinical indicators before and after treatment***

There was no difference in the clinical indicators (talk volume, sputum color score, ESR, CRP, 8-isoprostaglandin, Bhalla score, nitrite) of all patients before treatment ($P>0.05$). In the control group, sputum volume, sputum color score, CRP, 8-isoprostaglandin and other related indexes decreased ($P<0.05$) (table 5)

***Comparison of treatment effect***

The treatment effect of the two groups of patients was counted. The azithromycin group 53 (66.25%) had a significantly improved rate of improvement compared with the control group 32 (40.00%) ($P<0.05$). In the azithromycin group, 10 (12.50%) had a lower lung infection rate than the control group 19 (23.75%) ($P<0.05$). The azithromycin group had no difference in the aggravation rate compared with the control group (P>0.05).

**Table 1**: RT-PCR primer sequence

| Gene | Upstream primer | Downstream primer |
|---|---|---|
| TNF-α | CGAGTATACCATATCGGGCA | CGCGAGTAGTGCTATACTATA |
| IL-8 | GAAGCGCTATCATAATCTTA | CTATCGATAAGCTCGAGGCGC |
| IL-1β | GATATCTCGGCTACGCGTAA | GCGCGCCGTGCCGAATACA |
| β-actin | ACTTTCACCTGGATGACAG | CTAAAAGTTGGGCGACGTGT |

**Table 2**: Summary of patients' clinical data

| Project | Control | Azithromycin | $t/\chi^2$ | *P* value |
|---|---|---|---|---|
| Geder (male: female) | 34:46 | 39:41 | 4.091 | 0.368 |
| Age (years) | 49.27±5.46 | 51.08±5.63 | 5.713 | 0.519 |
| Body Mass Index | 23.62±1.25 | 24.09±1.01 | 5.747 | 0.511 |
| pCO2(mmHg) | 44.23±6.08 | 45.19±6.34 | 6.205 | 0.582 |
| pO2 (mmHg) | 63.29±12.47 | 64.57±15.16 | 4.825 | 0.183 |
| Pulmonary function Score | 25.34±4.16 | 24.79±4.02 | 4.186 | 0.628 |
| Course of disease (year) | 6.58±1.24 | 7.23±1.58 | 5.268 | 0.109 |
| Smoking (%) | 16 (20.00%) | 14 (17.50%) | 4.853 | 0.439 |

**Table 3**: Azithromycin reduces the expression of proinflammatory factors in patients

| Groups | TNF-α | IL-8 | IL-1β |
|---|---|---|---|
| Control group | 1.83±0.24 | 2.12±0.37 | 1.94±0.25 |
| Azithromycin group | 1.06±0.13 | 1.25±0.18 | 1.18±0.16 |
| *t value* | 5.173 | 6.038 | 5.227 |
| *P value* | 0.026 | 0.014 | 0.019 |

**Table 4**: Western blot analysis of MUC5AC expression

| Groups | Pre treatment | Post treatment |
|---|---|---|
| Control group | 2.15±0.32 | 1.63±0.19 |
| Azithromycin group | 1.98±0.21 | 1.04±0.10 |
| *t* value | 4.115 | 5.028 |
| *P* value | 0.218 | 0.016 |

**Table 5**: Comparison of Linchuan indexes in patients with bronchiectasis after 3 months of treatment

| Parameter | | Control | Azithromycin | $t/\chi^2$ | *P* value |
|---|---|---|---|---|---|
| Sputum volume (ml) | Pre treatment | 6.95±1.25 | 7.38±1.54 | 5.138 | 0.172 |
| | Post treatment | 4.14±0.61 | 2.67±0.34 | 4.027 | 0.016 |
| Sputum color score | Pre treatment | 5.27±0.26 | 5.66±0.39 | 6.827 | 0.624 |
| | Post treatment | 2.46±0.15 | 1.35±0.12 | 7.135 | 0.016 |
| Erythrocyte sedimentation rate | Pre treatment | 16.34±3.16 | 14.57±3.62 | 6.233 | 0.267 |
| | Post treatment | 12.06±1.44 | 11.85±1.32 | 4.182 | 0.147 |
| C-reactive protein | Pre treatment | 12.45±4.19 | 12.27±3.88 | 6.274 | 0.572 |
| | Post treatment | 9.58±3.11 | 7.15±2.41 | 5.019 | 0.034 |
| 8-isoprostaglandin (pg/mL) | Pre treatment | 1.34±0.21 | 1.45±0.38 | 5.219 | 0.574 |
| | Post treatment | 1.15±0.13 | 0.86±0.35 | 4.167 | 0.038 |
| Bhalla score | Pre treatment | 42.41±6.49 | 44.55±7.04 | 5.038 | 0.229 |
| | Post treatment | 28.17±4.11 | 18.65±2.66 | 4.288 | 0.015 |
| Nitrite (mmol) | Pre treatment | 14.37±3.168 | 9.37±1.25 | 5.037 | 0.126 |
| | Post treatment | 14.58±2.873 | 8.64±1.14 | 6.117 | 0.273 |

**Table 6**: Comparison of treatment effect

| Groups | Improvement | Pulmonary infection | Aggravation | Dyspnea |
|---|---|---|---|---|
| Control group | 32(40.00%) | 19(23.75%) | 11(13.75%) | 18(22.5%) |
| Azithromycin group | 53(66.25%) | 10(12.50%) | 6(7.50%) | 11(13.75%) |
| *t* value | 5.219 | 4.186 | 5.637 | 5.223 |
| *P* value | 0.027 | 0.013 | 0.246 | 0.035 |

The azithromycin group 11 (13.75%) had a lower dyspnea rate than the control group 18 (22.5) ($P$<0.05) (fig. 3, table 6).

## DISCUSSION

Bronchiectasis is a heterogeneous progressive respiratory disease. It is characterized by repeated coughing, expectoration and recurrent respiratory infections. These patients have been colonized or infected with pathogens for a long time, and their underlying pathological process can be understood as a vicious circle caused by chronic infection. It is reported that the two main pathogens isolated are Haemophilus influenzae and *Pseudomonas aeruginosa* (Schmitt *et al*., 2018). Some studies have shown that 40-67% of the sputum of children with bronchiectasis contains respiratory bacterial pathogens, of which Haemophilus influenzae and Streptococcus pneumoniae are the most common bacteria (Grimwood and Chang, 2018). In the sputum of 12 adult patients with bronchiectasis, the most common infectious organisms isolated were Haemophilus influenzae and Streptococcus pneumoniae (Navaratnam *et al*., 2018). In particular, the content of *Pseudomonas aeruginosa* is higher than that of Haemophilus influenzae. With the growth of individual age, the prevalence of *Pseudomonas aeruginosa* infection increases, and early non Pseudomonas airway colonizers are usually replaced by *Pseudomonas aeruginosa* (Diel *et al*., 2019). The natural history of *Pseudomonas aeruginosa* infection in bronchiectasis is that the bacteria are usually recovered intermittently through the culture of respiratory secretion before the development of chronic infection. Chronic airway diseases, such as diffuse panbronchiolitis and cystic fibrosis, are characterized by airway inflammation and excessive mucus secretion (Malhotra *et al*., 2019). MUC5AC is the main core protein of mucin secreted from airway surface epithelium. MUC5AC expression is upregulated by many factors, such as inflammatory mediators and cytokines (Warrier *et al*., 2019). Clinically, *Pseudomonas aeruginosa* infection in the lung is usually accompanied by excess mucus production. The supernatant of *Pseudomonas aeruginosa* can up regulate the transcription of mucin gene. It has been proved that lipopolysaccharide can activate MUC5AC in supernatant (Pieters *et al*., 2019). Azithromycin was found to reduce MUC5AC expression in patients (Tran *et al*., 2019). The main findings of this study are that in patients with bronchiectasis, azithromycin treatment for 3 months has certain clinical benefits, but does not affect the respiratory tract oxidative stress index (Hsieh *et al*., 2018). In our study, azithromycin treatment for 3 months produced a significant reduction in sputum volume, symptoms and health-related quality of life and frequency of episodes.

This result confirms the data known in other recent publications and is consistent with a randomized cross-over trial in which 11 patients with azithromycin developed aggravation and decreased sputum volume. A recent report showed that azithromycin could reduce the incidence of event aggravation and increase the first aggravation time after 6 months of treatment compared with placebo. These benefits lasted six months after the treatment. In this work, azithromycin does not improve health-related quality of life or lung function. In a bless trial, a patient with at least two exacerbations of infection was treated with erythromycin (400 mg) twice a day for a year, with a significant reduction in the number of exacerbations. Use a treatment similar to this study, but for one year. Another study provides clear evidence of reduced exacerbation and improved lung function, with azithromycin producing significant improvements in health-related quality of life and disease symptoms. Macrolides appear to be beneficial in some patients who have previously worsened their condition. According to the bacterial load or the existence of *Pseudomonas aeruginosa*, some studies have differences. Different from the previous studies, we conducted a post-mortem analysis to compare the therapeutic effect of patients with or without *Pseudomonas aeruginosa*. The mechanism of macrolides to exert their activities is not clear, which may include inhibition of cell chemotaxis, cytokine synthesis, expression of adhesion molecules and production of reactive oxygen species (Sapey *et al*., 2019). Erythromycin has been shown to inhibit neutrophil recruitment and down regulate the expression of adhesion molecules in animal models (Martin *et al*., 2019). However, although the results have been observed *in vitro*, the long-term treatment of macrolides has not yet proved to have a significant effect on these pro-inflammatory drugs (Skike *et al*., 2019).

Studies have shown that fractional exhaled nitric oxide (FeNO) is elevated in patients with bronchiectasis (Padilla-Galo *et al*., 2018), but other researchers have observed that despite a large number of airway inflammation, there is no difference between the expiratory no and the control group (Tliba and Panettieri r, 2018). It is speculated that no in bronchiectasis is not well oxidized because it is metabolized to nitrates (Tagini *et al*., 2018). We have not found a correlation between FeNO and other parameters of airway oxidative stress or inflammation. The effect of azithromycin on oxidative stress marker deficiency may be related to the characteristics of our patients. The sample consisted of relatively stable patients with mild or moderate bronchiectasis. Although it is a common cause of bronchiectasis, patients with primary or secondary immunodeficiency are still excluded, but some of them may interfere with the pathogenesis related to airway oxidative stress (Aizawa *et al*., 2018). In addition, it is well known that bronchiectasis has a high prevalence in emphysema, but in these patients, oxidant burst and neutrophil increase may confuse our findings. Another limitation of our study was that there was no placebo in

the control group. This function can explain differences in subjective variables, such as dyspnea or quality of life. However, it is believed that the changes of objective parameters (such as sputum characteristics or oxidative stress markers) are reasonable.

## CONCLUSION

These results indicate that azithromycin treatment has certain clinical benefits for patients with bronchiectasis induced by *Pseudomonas aeruginosa*, and exerts therapeutic effects by inhibiting the expression of MUC5AC.

## REFERENCES


Aizawa H, Koarai A, Shishikura Y, Yanagisawa S, Yamaya M, Sugiura H, Numakura T, Yamada M, Ichikawa T, Fujino N, Noda M, Okada Y and Ichinose M (2018). Oxidative stress enhances the expression of IL-33 in human airway epithelial cells. *Respir. Res.*, **19**(1): 52.

Boughton JM (2002). The Bretton Woods proposal: A brief look. *Polit. Sci. Q,* **42**(6): 564.

Candice E Van Skike, Jordan B Jahrling, Angela B Olson, Naomi L Sayre, Stacy A Hussong, Zoltan Ungvari, James D Lechleiter and Veronica Galvan (2018). Inhibition of mTOR protects the blood-brain barrier in models of Alzheimer's disease and vascular cognitive impairment. *Am. J. Physiol. Heart Circ. Physiol.,* **314**(4): H693-H703.

Donghai W, Wenlong Fu and Jihong Dai (2018). Meta-analysis of macrolide maintenance therapy for prevention of disease exacerbations in patients with noncystic fibrosis bronchiectasis. *Medicine*, **98**(17): e15285.

Elizabeth S, Jaimin MP, Hannah G, Georgia MW, Frances G, Dhruv P, Rahul YM, Rachel CAD, Sebastian TL, Philip AH, Jon H, Paul N, Aaron S, Peter N, Adam TH and David RT (2019). Simvastatin Improves Neutrophil Function and Clinical Outcomes in Pneumonia. A Pilot Randomized Controlled Clinical Trial. *Am. J. Respir. Crit. Care Med.*, **200**(10): 1282-1293.

Eun L and Soo-Jong H (2019). Pharmacotherapeutic strategies for treating bronchiectasis in pediatric patients. *Expert Opin Pharmacother*, **20**(8): 1025-1036.

Florian T, Trestan P, Antony C, Claire B, Angela K, Alban L and Gilbert G (2018). Distinct Genomic Features Characterize Two Clades of Corynebacterium diphtheriae: Proposal of Corynebacterium diphtheriae Subsp. diphtheriae Subsp. nov. and Corynebacterium diphtheriae Subsp. *Lausannense subsp. Nov. Front Microbiol.*, **9**: 1743.

Francesco B, Sarah W, Imai-Matsushima A, Gabriela S, Christian G, Monika S, Hilmar B, Morey P, Christian, Juergen O and Thomas F Meyer (2018). Polarised epithelial monolayers of the gastric mucosa reveal insights into mucosal homeostasis and defence against infection. *Gut*, pp.2017-314540.

Francesco M, Mirco L, Carla G, Gloria M, Alberto C, Nicola F (2018). Heat-induced necrosis after bronchial thermoplasty: A new concern. *Allergy Asthma Clin. Immunol.*, **14**: 25.

Gregory P, Benjamin H, Michael RJ, Kenneth O, Engelhardt TC, Keith MS, Noah J, John CG, Rohit B, Robert JK, Nilesh G, Kannan N, John R, Immad RS, Stephen KL, Narinder B, Raja ML, Barry SW, JC, Fakhir FE, Mark JG, Mark K, Juan A, Peter S, William K, Ping-Yu L, Samuel ZG and Seattle II (2015). Investigators. A Prospective, Single-Arm, Multicenter Trial of Ultrasound-Facilitated, Catheter-Directed. Low-Dose Fibrinolysis for Acute Massive and Submassive Pulmonary Embolism: The SEATTLE II Study. *JACC Cardiovasc. Interv.*, **8**(10): 1382-1392.

Karam K, Hye JK, Bert B, Jin HK and Yup Chung Il (2018). Inflammatory mediators ATP and S100A12 activate the NLRP3 inflammasome to induce MUC5AC production in airway epithelial cells. *Biochem. Biophys. Res. Commun.*, **503**(2): 657-664.

Keith G and Anne BC (2019). A new dawn: Inhaled antibiotics for patients with bronchiectasis. *Lancet Respir Med*, **7**(3): 188-189.

Kimberly S, Paige C, Milena V, Kinner-Bibeau L, Shuang Hu, James C, Remling-Mulder L, Ken EO, Tawfik A and Ramesh A (2018). Zika viral infection and neutralizing human antibody response in a BLT humanized mouse model. *Virology*, **515**: 235-242.

Martinez-García MA (2018). *Pseudomonas aeruginosa* infection and exacerbations in bronchiectasis: More questions than answers. *Eur. Respir. J.*, **51**(2): 1702497.

Matthew JM, Helen L, Carly C, Kate P, Irshad S, Dominick ES, Tim WH (2019). Idiopathic chronic productive cough and response to open-label macrolide therapy: An observational study. *Respirology*, **24**(6): 558-565.

Meng-Heng H, Chun-Yu L, Chen-Yu W, Yueh-Fu F, Yu-Lun L, Shu-Min L and Horng-Chyuan L (2018). Impact of concomitant nontuberculous mycobacteria and *Pseudomonas aeruginosa* isolates in non-cystic fibrosis bronchiectasis. *Infect Drug Resist*, **11**: 1137-1143.

Navaratnam V, Douglas LF, Kah Peng Eg and Anne BC (2018). Paediatric and adult bronchiectasis: Monitoring, cross-infection, role of multidisciplinary teams and self-management plans. *Respirology*, **24**(2): 115-126.

Padilla-Galo A, Olveira C, de Rota-Garcia LF, Marco-Galve I, Plata AJ, Alvarez A, Rivas-Ruiz F, Carmona-

Olveira A, Cebrian-Gallardo JJ and Martinez-Garcia MA (2018). Factors associated with bronchiectasis in patients with uncontrolled asthma; the NOPES score: A study in 398 patients. *Respir. Res.*, **19**(1): 43.

Pallavi B, Donald JD, Brian JM, Adriano GR and Adam TH (2018). Blood Neutrophils are Reprogrammed in Bronchiectasis. *Am J Respir Crit Care Med*, **198**(7): 880-890.

Pieters A, Bakker M, Hoek RAS, Altenburg J, van Westreenen M, Aerts JGV and van der Eerden MM (2019). The clinical impact of *Pseudomonas aeruginosa* eradication in bronchiectasis in a Dutch referral centre. *Eur. Respir. J.*, **53**(4): 1802081.

Ranjana W, Billy S, Suzanna V, Rosemary C, Ranganathan S (2019). The role of geographical location and climate on recurrent Pseudomonas infection in young children with Cystic Fibrosis. *J Cyst Fibros*, **18**(6): 817-822.

Roland D, Santiago E, Stefan B, Christian J, Fabian J, Korfmann G, Sebastian S, Sivagurunathan S, Rademacher J (2019). Incidence of patients with non-cystic fibrosis bronchiectasis in Germany-A healthcare insurance claims data analysis. *Respir Med*, **151**: 121-127.

Sankalp M, Hayes Jr D, Daniel JW (2019). Mucoid *Pseudomonas aeruginosa* and regional inflammation in the cystic fibrosis lung. *J. Cyst. Fibros*, **18**(6): 796-803.

Stephanie E, Katrien L, Kristina V, Lieven JD, Bart MV and Janssens W (2018). Aspergillus fumigatus Detection and Risk Factors in Patients with COPD–Bronchiectasis Overlap. *Int. J. Mol. Sci.*, **19**(2): 523.

Suarez-Cuartin G, Jordi G, Merino JL, Rodrigo-Troyano A, Anna F, Lidia P, Sanchez-Reus F, Diego C, Vicente P, Chalmers JD and Oriol S (2018). Identification of, *Pseudomonas aeruginosa*, and airway bacterial colonization by an electronic nose in bronchiectasis. *Respir. Med.*, **136**: 111-117.

The-Thien T, Hong Yu, Celine V, Albert YHL, Abisheganaden JA, Chotirmall SH and Hadinoto K (2019). An evaluation of inhaled antibiotic liposome versus antibiotic nanoplex in controlling infection in bronchiectasis. *Int. J. Pharm.*, **559**: 382-392.

Tliba O, Panettieri RA Jr (2019). Paucigranulocytic asthma: Uncoupling of airway obstruction from inflammation. *J Allergy Clin Immunol*, **143**(4): 1287-1294.

Weronika G, Walencka M, Anthony PM, Krzysztof H, Obuchowski M, Magdalena C (2019). Upregulation of MUC5AC production and deposition of LEWIS determinants by Helicobacter Pylori facilitate gastric tissue colonization and the maintenance of infection. *J. Biomed. Sci.*, **26**(1): 23.