Kafkas Universitesi Veteriner Fakultesi Dergisi
ISSN: 1300-6045 e-ISSN: 1309-2251
Journal Home-Page: http://vetdergikafkas.org

Kafkas Univ Vet Fak Derg
28 (1): 87-96, 2022
DOI: 10.9775/kvfd.2021.26491

RESEARCH ARTICLE

Effect of Tocilizumab on *Acinetobacter baumannii* Lung Infection in an Immunosuppressed Rat Model

Demet CELEBI [1,a (*)] Zekai HALICI [2,3,b] Ozgur CELEBI [4,c] Nurullah AKGUN [2,d] Halil KESKIN [5,e]
Irfan CINAR [6,f] Iclal HALICI [7,g] Kagan Tolga CINISLI [4,h] Serkan YILDIRIM [8,i]

[1] Atatürk University, Faculty of Veterinary Medicine, Department of Microbiology, TR-25100 Erzurum - TÜRKİYE
[2] Atatürk University, Faculty of Medicine, Department of Pharmacology, TR-25100 Erzurum - TÜRKİYE
[3] Atatürk University, Clinical Research, Development and Design Application and Research Center, TR-25100 Erzurum - TÜRKİYE
[4] Atatürk University, Regional Education and Research Hospital, Department of Microbiology, TR-25100 Erzurum - TÜRKİYE
[5] Atatürk University, Faculty of Medicine, Department of Pediatrics, TR-25100 Erzurum - TÜRKİYE
[6] Kastamonu University, Faculty of Medicine, Department of Pharmacology, TR-37100 Kastamonu - TÜRKİYE
[7] Atatürk University, Faculty of Medicine, Infectious Community, TR-25100 Erzurum - TÜRKİYE
[8] Atatürk University, Faculty of Veterinary Medicine, Department of Pathology, TR-25100 Erzurum - TÜRKİYE
ORCIDs: [a] 0000-0002-2355-0561; [b] 0000-0001-6854-6059; [c] 0000-0003-4578-9474; [d] 0000-0003-2703-9872; [e] 0000-0003-4491-1327
[f] 0000-0002-9826-2556; [g] 0000-0001-9672-799X; [h] 0000-0003-3909-9637; [i] 0000-0003-2457-3367

Article ID: KVFD-2021-26491 Received: 05.09.2021 Accepted: 17.12.2021 Published Online: 23.12.2021

Abstract

Our study aimed to investigate effect of tocilizumab on the lung tissue in the presence of *Acinetobacter baumannii* infection in immunosuppressed rats. A forty-eight female Wistar albino rats were divided equally into eight groups: Group 1: Healthy (H), Group 2: Immunosuppressed (IM), Group 3: Healthy rats given *A. baumannii* bacteria (H+BAC), Group 4: Immunosuppressed rats given *A. baumannii* bacteria (IM+BAC), Group 5: Healthy rats given tocilizumab (H+TCZ), Group 6: Immunosuppressed rats given tocilizumab (IM+TCZ), Group 7: Healthy rats given *A. baumannii* bacteria and tocilizumab (H+BAC+TCZ), Group 8: Immunosuppressed rats given tocilizumab and *A. baumannii* bacteria (IM+BAC+TCZ). Fourteen days after the immunosuppression of group 2, 4, 6 and 8 with hydrocortisone, group 3, 4, 7 and 8 were *A. baumannii* was dropped into the trachea. One hour after *A. baumannii* application, TCZ was administered to Groups 5, 6, 7 and 8. NF-κB, IL-6 and NLRP3 mRNA expressions were decreased in the IM group compared to the healthy group (P<0.05). Although NF-κB, IL-6 and NLRP3 mRNA expression decreased in the IM+TCZ group compared to the healthy group (P<0.05) NF-κB, IL-6 and NLRP3 mRNA expression increased in the H+TCZ group (P<0.05). Despite decreasing cytokines, *A. baumannii* has been shown to increase infection-related lung injury. This suggests that in patients currently or recently using steroids, tocilizumab may increase organ damage due to opportunistic infection.

Keywords: *Acinetobacter baumannii, Tocilizumab, Immunosuppressed rat*

Tocilizumab'ın İmmünsüprese Rat Modelinde *Acinetobacter baumannii*'nin Akciğer Enfeksiyonu Üzerindeki Etkisi

Öz

Çalışmamızda, immünsüpresyon oluşturulmuş ratlarda *Acinetobacter baumannii* enfeksiyonu varlığında tocilizumabın akciğer dokusundaki etkisini araştırmayı amaçladık. Toplam kırk sekiz dişi Wistar albino rat sekiz eşit gruba ayrıldı: Grup 1: Sağlıklı (H), Grup 2: İmmünsüprese ratlar (IM), Grup 3: *A. baumannii* bakterisi verilen sağlıklı ratlar (H+BAC), Grup 4: *A. baumannii* bakterisi verilen immünsüprese ratlar, Grup 5: Tocilizumab verilen sağlıklı ratlar (H+TCZ), Grup 6: Tocilizumab verilen immünsüprese ratlar (IM+TCZ), Grup 7: *A. baumannii* bakterisi ve tocilizumab verilen sağlıklı ratlar (H+BAC+TCZ), Grup8: Tocilizumab ve *A. baumannii* bakterisi verilen immünsüprese sıçanlar (IM+BAC+TCZ). Grup 2, 4, 6 ve 8'in hidrokortizon ile immünosupresyonundan 14 gün sonra, grup 3, 4, 7 ve 8'e *A. baumannii* suşu trakeaya transtrakeal yolla enjekte edildi. Grup 5, 6, 7 ve 8'e *A. baumannii* uygulamasından bir saat sonra TCZ verildi. İmmünsüprese grupta (IM) sağlıklı gruba (H) göre NF-κB, IL-6 ve NLRP3 mRNA ekspresyonları azaldı (P<0.05). Her ne kadar TCZ verilen IM grubunda (IM+TCZ) NF-κB, IL-6 ve NLRP3 mRNA ekspresyonu sağlıklı grup (H) ile karşılaştırıldığında azalmış olsa da (P<0.05) NF-κB, IL-6 ve NLRP3 mRNA ekspresyonu sadece tocilizumab uygulanan grupta (H+TCZ) arttı (P<0.05). *A. baumannii* enfeksiyonunda tocilizumab kullanımı, steroidlerle immünsüprese edilmiş ratlarda inflamatuar sitokinleri önemli ölçüde azalttı. Azalan sitokinlere rağmen, *A. baumannii*'nin enfeksiyona bağlı akciğer hasarını arttırdığı gösterilmiştir. Bu, steroid kullanan veya yakın zamanda steroid kullanmış hastalarda tocilizumabın fırsatçı enfeksiyon nedeniyle organ hasarını artırabileceğini düşündürmektedir.

Anahtar sözcükler: *Acinetobacter baumannii, Tocilizumab, İmmünsüprese rat*

How to cite this article?

Celebi D, Halici Z, Celebi O, Akgun N, Keskin H, Cinar I, Halici I, Cinisli KT, Yildirim S: Effect of tocilizumab on *Acinetobacter baumannii* lung infection in an immunosuppressed rat model. *Kafkas Univ Vet Fak Derg,* 28 (1): 87-96, 2022.
DOI: 10.9775/kvfd.2021.26491

(*) Corresponding Author

Tel: +90 442 231 7266 Fax: +90 442 236 1301
E-mail: celebiidil@atauni.edu.tr (D. Çelebi)

Introduction

Acinetobacter baumannii is an important pathogen belonging to the family Moraxellaceae, is a gram-negative, non-fermentative, aerobic, oxidase-negative, catalase-positive coccobacillus [1,2]. Despite being harmless in healthy individuals, these bacteria can cause various diseases in immunocompromised individuals [3-5]. Due to increased antibiotic resistance in recent years, *Acinetobacter* infections have become the most common cause of community- and hospital-acquired pneumonia in Asian countries, Latin America and European hospitals [6-8]. *A. baumannii* is also the etiological agent of bacteremia, meningitis, endocarditis, and urinary system, skin and soft tissue infections. The capsule structure, adhesion strength, and biofilm formation and secretion systems are among the virulence factors of *A. baumannii*, which affect its role in clinical conditions [9-15]. These bacteria can adhere well to medical devices and cause pneumonia in patients on mechanical ventilators [12,16,17]. Clinically, immunocompromised patients are particularly susceptible to *A. baumannii* infection, although the cause of this susceptibility has not been yet determined. Therefore, very different strategies should be determined in the treatment of immunocompromised patients with *A. baumannii* infection.

In terms of bacterial resistance and difficulties in treatment, *A. baumannii* is one of the most important infectious agents that has been a major problem in the last decade. Due to its capacity to develop resistance to most antibiotics, *A. baumannii* has recently been classified as a 'red alert' human pathogen [18]. Another issue that needs to be discussed in terms of the current situation is the presence of other drugs used by patients in intensive care units. Although there are many different drugs used in intensive care units, steroids have very strong anti-inflammatory effect which can stop the cytokine storm [19]. Glucocorticoids are widely used in human and veterinary medicine due to their potent non-specific immunosuppressive properties. Due to these properties, it is one of the most suitable drug groups for making immunosuppression models in rats. Steroid use can be considered to cause immune suppression through NF-*κB* and AP-1 or neutrophil phagocytic dysfunction. This can increase the risk of nosocomial infections. However, previous studies have reported conflicting results. It has been determined that the downregulation of inflammation due to glucocorticoid therapy reduces the growth factors of bacteria, which decreases the risk of nosocomial infection [20]. A question that requires furthers investigation is how the risk of nosocomial infection development or progression occurs, especially in intensive care patients using steroids. More important is the question of how to treat *A. baumannii* infection in patients who are given steroids or who are immunosuppressed for any reason. In many studies, susceptibility to and severity of *A. baumannii* infection was significantly higher in immunocompromised mice than in non-imminocompromised mice [21]. *A. baumannii* infection activates the host innate immune responses which leads to the production of proinflammatory cytokines such as IL-6 and IL-1 [22]. Immunomodulators that stimulate host innate immunity have potential as stand-alone treatment or as immune supportive for *A. baumannii* infection [22].

Tocilizumab (TCZ) is an immunomodulator that prevents IL-6 from binding to IL-6R. It has been shown that the use of IL-1 and IL-6 antagonists provides significant benefits in hyperimmune diseases such as SARS-CoV [23]. TCZ was previously indicated for use in autoimmune diseases, and Food and Drud Agency extended its use to cytokine release syndrome in 2017 [24]. However, considering the available studies, it seems very important to empirically show how the use of immunosuppressive drugs such as TCZ for cytokine storm would affect the development and progression of secondary nosocomial infections in patients using that are also using steroids. NF-κB and IL-6 are mediators involved in host defense against *A. baumannii* infections. IL-6 is a multifunctional cytokine with both pro-inflammatory and anti-inflammatory properties. While IL-6 released during infection protects the host against the agent, its irregular and continuous release can cause serious complications and death.

The current study aimed to investigate the effects of TCZ use on the lung tissue in the presence of *A. baumannii* infection in immunosuppressed rats at histo-pathological and molecular levels.

Material and Methods

Ethical Statement

The experiments were conducted according to the ethical norms approved by the Atatürk University Ethics Committee of the Experimental Animal Teaching and Research Center (No: 2017/88).

Animals

The rats were obtained from the Medicinal and Experimental Application and Research Center, and kept in standard laboratory conditions under a natural cycle of light and dark. Forty-eight female Wistar Albino rats weighing 200-220 g were used in the study. During the experiments, the animals were supplied enough water *(ad libitum)* and pellet feed. Animals were housed in groups in typical plastic cages in a well-ventilated room at 22±1°C under specific light conditions (14/10 h light/dark cycle) prior to the experiment.

Experiment Groups

The rats were randomly divided into eight groups with six rats in each group. Group 1, 3, 5 and 7 were comprised of rats with normal immunity. Group 2, 4, 6 and 8 were immunosuppressed groups via hydrocortisone.

Group 1: Healthy (H)

Group 2: Immunosuppressed with hydrocortisone (IM)

Group 3: Healthy rats given *A. baumannii* bacteria (H+BAC)

Group 4: Immunosuppressed rats given *A. baumannii* bacteria (IM+BAC)

Group 5: Healthy rats given tocilizumab (H+TCZ)

Group 6: Immunosuppressed rats given tocilizumab (IM+TCZ)

Group 7: Healthy rats given *A. baumannii* bacteria and tocilizumab (H+BAC+TCZ)

Group 8: Immunosuppressed rats given tocilizumab and *A. baumannii* bacteria (IM+BAC+TCZ)

Acinetobacter baumannii Strain

This bacterial strain was isolated by the the Atatürk University Clinical Microbiology Laboratory and placed in refrigerator at -70°C until the experiment. On the day of the experiment, *A. baumannii* was dissolved using the conventional method and standardized to 1 x 10^8 CFU/mL with sterile physiological saline.

Drug Administrations

- Hydrocortisone Administration

First step of the experiment is that to create immunosuppression. Hydrocort-Liyo® 100 mg IM/IV ampoule (Koçak Farma Pharmaceuticals and Chemical Industry Inc. Türkiye) was dissolved in saline. The rats in immunosuppressed groups (Groups 2, 4, 6 and 8) were subcutaneously given hydrocortisone at a dose of 20 mg/kg for 14 days and hydrocortisone administration continued until the experiment was terminated to the 21st day of experiment [25].

- Tocilizumab Administration

One hour after the *A. baumannii* application, tocilizumab (Actemra 400 mg/20 mL IV Concentrate Vial® The Roche Group) was dissolved in saline, diluted and intraperitoneally injected to groups 5, 6, 7 and 8 at a dose of 2 mg/kg to each rat for seven days (14th day of the experiment to 21st day) [26]. At the end of the seventh day (21st day of experiment), experiment was terminated.

Rat Model of Acinetobacter baumannii Infection

Fourteen days after the immunosuppression of group 2, 4, 6 and 8 with hydrocortisone; Group 3, 4, 7 and 8 were anesthetized using an intraperitoneal injection of 10% chloral hydrate (4 mL/kg). After the rats were placed in the supine position, the skin was cut with a cervical midline incision. In order to minimize tissue damage, fascia, the sternocleidomastoid muscle, and parathyroid glands were removed with cotton swabs; and the trachea was surgically exposed. An insulin syringe with a sterile 26 G needle was positioned intratracheally through the tracheal cartilages. In order to confirm that the needle was positioned correctly in the trachea, the syringe plunger was withdrawn to make sure that only air was observed inside the syringe barrel. 100 µL of 1 x 10^8 CFU/mL suspension of *A. baumannii* was dropped to the trachea. After tracheal instillation, the animals were kept upright for 5 min, and then left until they became conscious [27].

Lung Tissue Collection and Term of Experiment

The mental state, breathing, food and water consumption, exercise, temperature, and hair of the rats were observed daily, and the survival rate was recorded. On the 21st day of the experiment, the rats were sacrificed with diethyl ether, and left lung tissue samples were taken and the experiment was terminated.

Real-Time PCR

- Total RNA Extraction and cDNA Synthesis

The tissues (20 mg) were stabilized in RNA Stabilization Reagent (RNAlater, Qiagen), and then disrupted using TissueLyser II (Qiagen). The total RNA was purified using the RNeasy Mini Kit (Qiagen) in a QIAcube (Qiagen) device according to the manufacturer's instructions [28]. The RNA samples were then reverse-transcribed into complementary DNA with the High Capacity cDNA Reverse Transcription Kit (Applied Biosystems). The cDNA concentration and quality were assessed and quantified using the Epoch Spectrophotometer System and Take3 Plate (BioTek).

- Relative Quantification of Gene Expression

The relative NLRP3 (Rn04244622_m1), IL-6 (Rn01410330_m1) and NF-*κB* (Rn01399565_m1) mRNA expression analyses were performed with StepOnePlus Real Time PCR System technology (Applied Biosystems) using cDNA synthesized from rat lung RNA. The real-time reverse transcriptase-polymerase chain reaction (qPCR) was run using the Primer Perfect Probe mix and the TaqMan Probe-based technology (Primer Design Ltd., Southampton, UK), and the results were expressed as the relative-fold compared to the control animals. The gene expression levels were normalized by β-actin (Rn00667869_m1) as a housekeeping gene. For each tissue, triplicate determinations were performed in a 96-well optical plate for both targets using 9 mL of cDNA (100 ng), 1 mL of Primer Perfect Probe mix, and 10 mL of QuantiTect Probe PCR Master mix (Qiagen, Hilden, Germany) in each 20 mL reaction. The plates were heated for 2 min at 50°C and 10 min at 95°C, and then 40 cycles of 15 s at 94°C and 60 s at 60°C were applied. All data were expressed as fold-changes in expression compared to the expression in other animal groups, using the 2-ΔΔCt method [29,30].

Molecular Statistical Analysis

All data were expressed as mean ± SD in each group. All data were subjected to one-way analysis of variance using

IBM SPSS Statistics 20. All parametric data were analyzed with one-way analyses of variance, Tukey's test. P<0.05 was accepted as significant when compared to healthy group.

Histopathologic Examination

Tissue samples taken for the histopathological evaluation as a result of necropsy were fixed in 10% formalin solution for 48 h. Following tissue processing, the samples were embedded in paraffin blocks. 4-µm thick sections were taken from each block. The preparations prepared for the histopathological examination were stained with hematoxylin-eosin (HE) and examined with a light microscope [31]. In the sections examined, the severity of histopathological findings was evaluated as absent (-), very mild (+), mild (++), moderate (+++), severe (++++), and very severe (+++++) *(Table 1, 2, 3, 4)*.

Statistical Analysis

The Kruskal-Wallis test, one of the non-parametric tests, was used for the analysis of the differences between the groups for the data obtained semi-quantitatively in the histopathological examination, and the Mann-Whitney U test was used for the comparison of paired groups. SPSS 13.0 package program was used for all statistical analyses.

Results

Molecular Analysis

It was found that NF-κB mRNA expressions decreased in the steroid immunosuppressed group (IM) compared to the healthy group (H) (P<0.05). NF-κB mRNA expression decreased in the group of immunosuppressed rats given bacteria (IM+BAC) compared to the healthy rats given bacteria (H+BAC) (P<0.05). It was found that NF-κB mRNA expressions decreased in the immunosuppressed group given tocilizumab (IM+TCZ) compared to the healthy group given tocilizumab (H+TCZ) (P<0.05). NF-κB mRNA expressions decreased in the immunosuppression group given tocilizumab and bacteria together (IM+BAC+TCZ) compared to the healthy group given tocilizumab and bacteria together (H+BAC+TCZ) (P<0.05). NF-κB mRNA expressions considerably increased in the healthy group given tocilizumab and bacteria together (H+BAC+TCZ) and in the healthy group given bacteria (H+BAC) compared to the healthy group (H) (P<0.05). NF-κB mRNA expressions considerably increased in the immunosuppressed group given bacteria (IM+BAC) and in the immunosuppressed group given bacteria and tocilizumab together

Table 1. *Scoring according to the thickening of interstitial tissue in lung tissue sections*

Degree of Positivity	Presence of Interstitial Pneumonia
Absent (-)	No finding of interstitial pneumonia
Very mild (+)	Mild interstitial pneumonia in a very small focus in the lung section
Mild (++)	Mild interstitial pneumonia in multiple very small foci in the lung section
Moderate (+++)	Interstitial pneumonia in the form of a few foci in the lung section
Severe (++++)	Interstitial pneumonia in the form of many foci in the lung section
Very severe (+++++)	Diffuse interstitial pneumonia in the lung section

Table 2. *Scoring according to peribronchiolar cell infiltration in lung tissue sections*

Degree of Positivity	Presence of Peribronchiolar Cell Infiltration
Absent (-)	No mononuclear infiltration
Very mild (+)	1-4 mononuclear infiltrations around one bronchus/bronchiole in the lung section
Mild (++)	5-10 mononuclear infiltrations around one bronchus/bronchiole in the lung section
Moderate (+++)	11-20 mononuclear infiltrations around one bronchus/bronchiole in the lung section
Severe (++++)	20-30 mononuclear infiltrations around one bronchus/bronchiole in the lung section
Very severe (+++++)	More than 30 mononuclear infiltrations around one bronchus/bronchiole in the lung section

Table 3. *Scoring according to the presence of granuloma in lung tissue section*

Degree of Positivity	Presence of Granuloma
Absent (-)	No granuloma
Very mild (+)	1-2 granulomas in the lung section
Mild (++)	1-2 granulomas in the lung section
Moderate (+++)	3-4 granulomas in the lung section
Severe (++++)	More than 5 granulomas in the lung section

Table 4. *Scoring of histopathological findings in the lung tissues of rats treated for seven days*

Groups	Interstitial Pneumonia	Degeneration of Bronchial Epithelia	Peribronchiolar Cell Infiltration	Granuloma
Healthy	-	-	-	-
Immunosuppressed	-	-	-	-
H+BAC	++	++	++	-
IM+BAC	+++	+++	+++	-
H+TCZ	-	-	-	-
IM+TCZ	+	-	-	-
H+BAC+TCZ	++++	+++	++++	++
IM+BAC+TCZ	+++++	++++	+++++	+++

BAC: *A. baumannii;* ***TCZ:*** *Tocilizumab;* ***H:*** *Healthy;* ***IM:*** *Immunosuppressed*

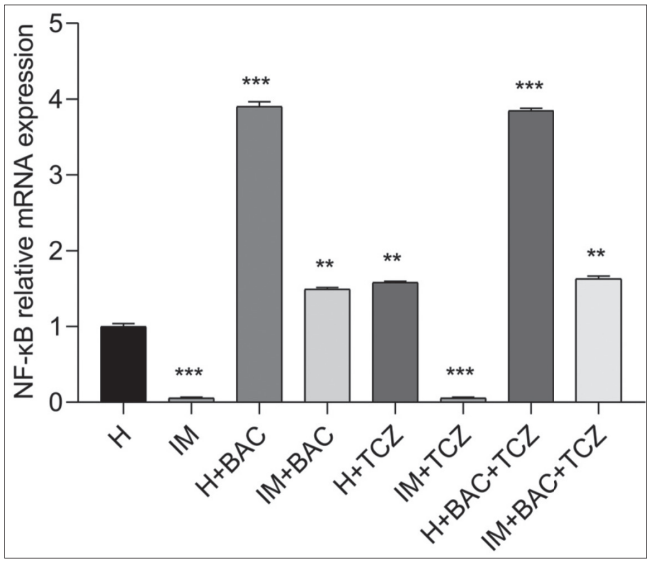

Fig 1. NF-κB relative mRNA expression. NF-*κB* mRNA expression levels in the lung tissues of all the experimental groups. The relative expression levels were calculated by the 2−ΔΔCt method. Each value is mean ± S.D. for six samples in each group. All data were expressed as mean ± SD in each group. All data were subjected to one-way analysis of variance using IBM SPSS Statistics 20. All parametric data were analyzed with one way analyses of variance, Tukey's test. P<0.05 was accepted as significant when compared to healthy group

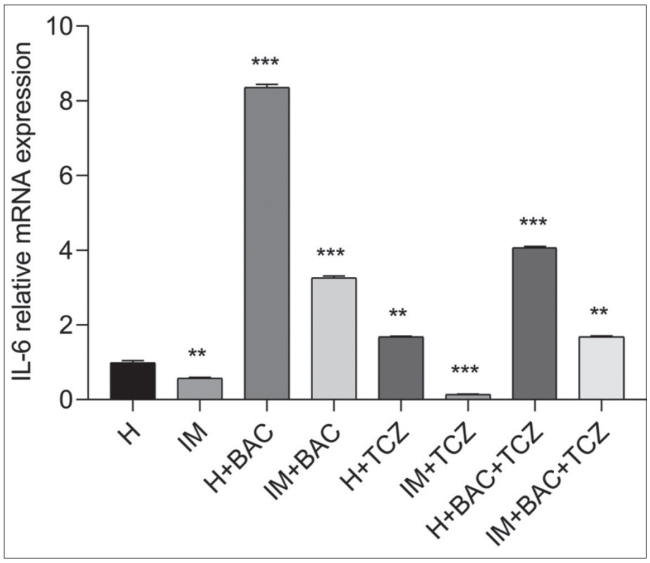

Fig 2. IL-6 relative mRNA expression. IL-6 mRNA expression levels in the lung tissues of all the experimental groups. The relative expression levels were calculated by the 2−ΔΔCt method. Each value is mean ± S.D. for six samples in each group. All data were expressed as mean ± SD in each group. All data were subjected to one-way analysis of variance using IBM SPSS Statistics 20. All parametric data were analyzed with one way analyses of variance, Tukey's test. P<0.05 was accepted as significant when compared to healthy group

(IM+BAC+TCZ) compared to the immunosuppressed group (IM) (P<0.05) *(Fig. 1)*.

It was found that IL-6 mRNA expressions decreased in the steroid immunosuppressed group (IM) compared to the healthy group (H) (P<0.05). IL-6 mRNA expression decreased in the group of immunosuppressed rats given bacteria (IM+BAC) compared to the healthy rats given bacteria (H+BAC) (P<0.05). It was observed that IL-6 mRNA expressions decreased in the immunosuppressed group given tocilizumab (IM+TCZ) compared to the healthy group given tocilizumab (H+TCZ) (P<0.05). IL-6 mRNA expressions decreased in the immunosuppression group given tocilizumab and bacteria together (IM+BAC+TCZ) compared to the healthy group given tocilizumab and bacteria together (H+BAC+TCZ) (P<0.05). IL-6 mRNA expressions considerably increased in the healthy group given tocilizumab and bacteria together (H+BAC+TCZ) and in the healthy group given bacteria (H+BAC) compared to the healthy group (H) (P<0.05). IL-6 mRNA expressions increased in the immunosuppressed group given bacteria (IM+BAC) and in the immunosuppressed group given bacteria and tocilizumab together (IM+BAC+TCZ) compared to the immunosuppressed group (IM) (P<0.05) *(Fig. 2)*.

It was found that NLRP3 mRNA expressions decreased in the steroid immunosuppressed group (IM) compared to the healthy group (H) (P<0.05). NLRP3 mRNA expression decreased in the group of immunosuppressed rats given bacteria (IM+BAC) compared to the healthy rats given bacteria (H+BAC) (P<0.05). It was observed that NLRP3 mRNA expressions decreased in the immunosuppressed

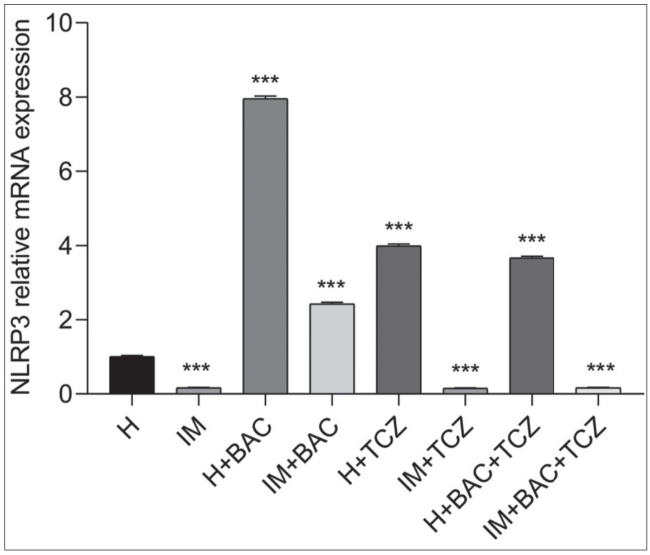

Fig 3. NLRP3 relative mRNA expression. NLRP3 mRNA expression levels in the lung tissues of all the experimental groups. The relative expression levels were calculated by the 2−ΔΔCt method. Each value is mean ± S.D. for six samples in each group. All data were expressed as mean ± SD in each group. All data were subjected to one-way analysis of variance using IBM SPSS Statistics 20. All parametric data were analyzed with one way analyses of variance, Tukey's test. P<0.05 was accepted as significant when compared to healthy group

in the healthy group given bacteria (H+BAC) and in the healthy group given tocilizumab (H+TCZ) compared to the healthy group (H)(P<0.05). NLRP3 mRNA expressions increased in the immunosuppressed group given bacteria (IM+BAC) compared to immunosuppressed group (IM) (P<0.05). About NLRP3 mRNA expressions, there was no statistical difference among the groups of immunosuppressed group given tocilizumab (IM+TCZ), immunosuppressed group given bacteria and tocilizumab together and immunosuppressed group (IM) *(Fig. 3)*.

Histopathological Analysis of Lung Tissues

When the lungs were examined histopathologically, it was determined that healthy group (H) and immunosuppressed group (IM) had a normal histological appearance *(Fig. 4-A,B)*. It was determined that the group of healthy rats given bacteria (H+BAC) had a mild interstitial pneumonia, desquamation of bronchiolar epithelia, lymphocytic cell infiltration surrounding bronchi-bronchioles, and vascular hyperemia *(Fig. 4-C)*. The group of immunosuppressed rats given bacteria (IM+BAC) had a moderate interstitial pneumonia, desquamation of the bronchial-bronchiolar epithelia, surrounded by mononuclear cell infiltration and vascular hyperemia *(Fig. 4-D)*. Statistically significant

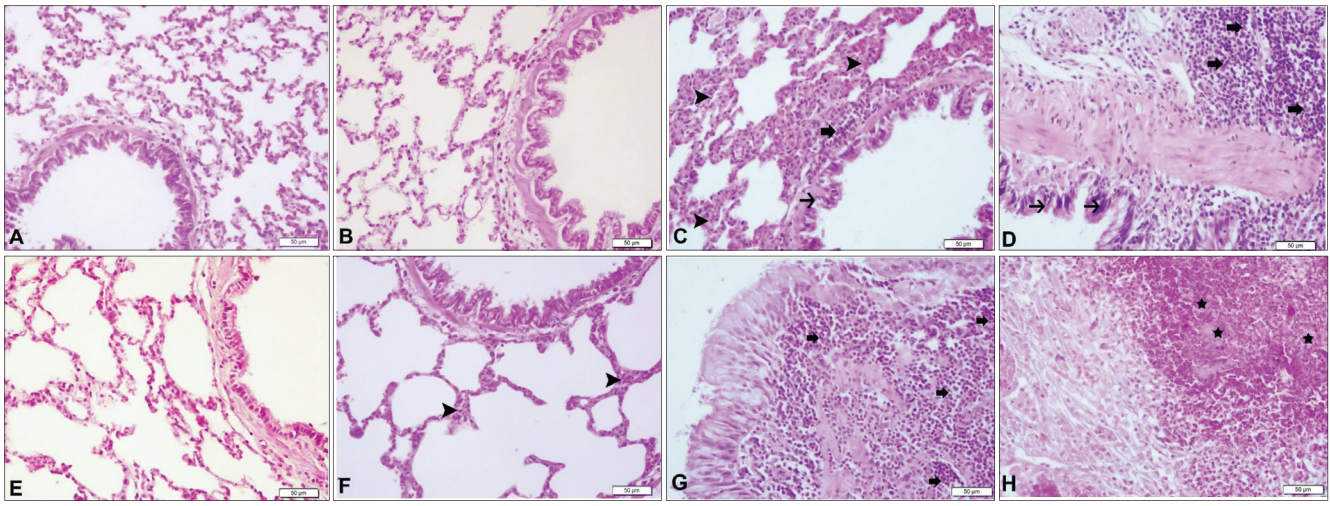

Fig 4. Lung Tissue H&E staining. Healthy group **(A)**, Immunosuppressed group **(B)**, Healthy rats given *A. baumannii* bacteria *(C)*, Immunosuppressed rats given *A. baumannii* bacteria **(D)**, Healthy rats given tocilizumab **(E)**, Immunosuppressed rats given tocilizumab **(F)**, Healthy rats given *A. baumannii* bacteria and tocilizumab **(G)**, Immunosuppressed rats given tocilizumab and *A. baumannii* bacteria **(H)**. Interstitial pneumonia (arrowheads), degeneration of bronchial epithelia *(thin arrows)*, peribronchiolar cell infiltration *(thick arrows)*, granuloma *(star)*, H&E, Bar: 50 µm. The Kruskal-Wallis test, one of the non-parametric tests, was used for the analysis of the differences between the groups for the data obtained semi-quantitatively in the histopathological examination, and the Mann-Whitney U Statistical analysis test was used for the comparison of paired groups. SPSS 13.0 package program was used for all statistical analyses

group given tocilizumab (IM+TCZ) compared to the healthy group given tocilizumab (H+TCZ) (P<0.05). NLRP3 mRNA expressions decreased in the immunosuppression group given tocilizumab and bacteria together (IM+BAC+TCZ) compared to the healthy group given tocilizumab and bacteria together (H+BAC+TCZ) (P<0.05). NLRP3 mRNA expressions considerably increased in the healthy group given tocilizumab and bacteria together (H+BAC+TCZ), difference (P<0.05) was detected when compared to group H.

It was determined that the group of healthy rats given tocilizumab (H+TCZ) had a normal histological appearance of the bronchi-bronchioles and alveoli *(Fig. 4-E)*. The group of immunosuppressed rats given tocilizumab (IM+TCZ) had a very mild interstitial pneumonia *(Fig. 4-F)*. When the

healthy group given tocilizumab and bacteria together (H+BAC+TCZ) were evaluated it was found that the lungs had interstitial pneumonia, lymphoid hyperplasia, degeneration of the bronchial-bronchiol epithelium, mild granuloma, and hyperemia in the vessels *(Fig. 4-G)*. Statistically significant difference ($P<0.05$) was detected when compared to healthy group (H). When the immunosuppressed group given tocilizumab and bacteria together (IM+BAC+TCZ) were evaluated it was found that the lungs had a very severe interstitial pneumonia, moderate granuloma, dense bacterial clusters in the middle of the granulomas, severe degeneration of the bronchiole epithelium *(Fig. 4-H)*. Statistically significant difference ($P<0.05$) was detected when compared to healthy group (H).

The histopathological results of the groups are summarized in *Table 4*. The presence of Interstitial Pneumonia, Peribronchiolar Cell Infiltration and Granuloma in the lung tissues are given in *Table 1*, *Table 2* and *Table 3*, respectively.

Discussion

In this study, we investigated the effect of IL-6 receptor antagonist on the lungs and systemic inflammatory response in healthy and immunosuppressed rats in which pneumonia was induced by *A. baumannii*. When we examined our results, we found that NF-*κB*, NLRP3 and IL-6 mRNA expressions significantly decreased in rats that were given steroids (group 2, 4, 6, 8) compared to the healthy rats (group 1, 3, 5, 7). NF-*κB*, NLRP3 and IL-6 mRNA expressions significantly increased in the groups in which *A. baumannii* pneumonia was induced without immunosuppression while this increase was significantly reduced in the immunosuppressed rats infected with *A. baumannii*. Our results indicate that treatment with IL-6 receptor antagonists caused a significant decrease in systemic inflammatory response in rats with *A. baumannii* pneumonia in the presence of steroid immunosuppression compared to the rats with *A. baumannii* pneumonia without steroid immunosuppression. Considering only the histopathological analyses, the results were reserved. The use of IL-6 receptor antagonist in *A. baumannii* infection in immunosuppressed rats caused significant lung damage compared to the rats that were not given the drug.

Lung injury due to *A. baumannii* infection is a clinical condition that causes serious morbidity and mortality. Pneumonia infections caused by *Acinetobacter* have been reported to increase the mortality rate to 42% in immunocompromised patients treated in the intensive care unit [32]. The increased release of immune modulatory mediators is required to stop this process and achieve recovery. It is important to understand the basics of host-bacterial interactions, especially the host immune response, for the development of effective treatments against *A. baumannii*, which has recently become the leading cause of pneumonia [33]. Different innate immune cells such as monocytes, macrophages, dendritic cells and natural killer cells have been identified as important factors in the body's defense against *A. baumannii*, and among them, neutrophils represent an important immune cell indispensable for the control of infection [33]. With the activation of alveolar macrophages in *Acinetobacter* infections, the production of mediators such as IL-6, tumor necrosis factor alpha (TNF-α) and NF-*κB* increase and play the main protective role for the host against the causative agent [34]. IL-6 is released from infected or lesioned cells and recognized by the pathogen recognition receptors of immune cells. These receptors consist of toll-like receptors, retinoic acid-inducible gene-1-like receptors, nucleotide-binding oligomerization domain-like receptors, DNA receptors, and NOD-like receptors [35]. In addition, IL-6 released during infection plays a supporting role for the immune system by regulating NF-κB synthesis and increasing TNF-α, and IL-1 β mRNA transcription [36]. Steroids with a very strong anti-inflammatory effect can stop the cytokine storm [19]. However, the use of steroids can be considered to lead to immunosuppression through NF-κB and AP-1 and by causing neutrophil phagocytic dysfunction. This can increase the risk of nosocomial infections. However, studies in the literature report conflicting results. It has been determined that the down-regulation of inflammation due to glucocorticoid therapy reduces the growth factors of bacteria, which decreases the risk of nosocomial infection [37]. In an *in vitro* study, glucocorticoid use downregulated inflammation, and LPS-activated monocytes were shown to reduce the gene transcription of TNF-α, IL-1β, and IL-6 [38]. Similar results have been obtained from clinical studies [39]. Studies have shown that inflammation caused by infections has a bidirectional effect. It is stated that cytokines that increase with inflammation can also be a growth factor for bacteria [37]. In addition to treatment with many steroids in immunosuppressed rat models, among the most commonly used methods are those involving the use of hydrocortisone [40]. In our study, the immunosuppression model was successfully created with the hydrocortisone administration. The use of hydrocortisone reduced NLRP3 expression and IL-6 and NF-κB levels, with the expected lung damage remaining lower.

In this study, we showed that especially in *A. baumannii* infections, the reduction of immunosuppression; i.e., increased cytokine storm could reduce the prevalence of infection and the damage it can cause. However, this was not the only focus of our study. In fact, this work was an experimental demonstration of problems that exist in many intensive care patients and always present a difficult time for the clinician. The balance between pro-inflammatory and anti-inflammatory mediators regulates the inflammatory process, which includes adhesion, chemotaxis, phagocytosis of invading bacteria, bacteria killing, and phagocytosis of debris from injured tissue. However, if this proinflammatory and anti-inflammatory mediator

balance is not disturbed, homeostasis can be restored by eliminating bacterial invasion. Otherwise, it progresses to sepsis, shock, and multiorgan insufficiency due to the direct effect of the microorganism, as well as the effect of proinflammatory cytokines [31]. Therefore, a well-planned anti-cytokine therapy and anti-inflammatory therapy is very important. The question was what we would clinically face if an IL-6 receptor antagonist was required in a patient developed *A. baumannii* pneumonia when on steroids. We demonstrated, both at molecular and histopathological levels, that the use of steroids could prevent the development of damage due to *A. baumannii* pneumonia, contrary to expectations.

NOD-like group inflammasomes are complexes of the natural immune response and they are multiproteins released at the time of tissue damage, inflammation, and infection. It has recently been stated that inflammasomes which are complexes located in the cytoplasm of multiple proteins, are responsible for the maturation of pro-inflammatory cytokines such as IL-1β and IL-18, and the initiation of pyroptosis, a highly inflammatory form of programmed cell death [30]. Inflammasomes sense either microbial stimuli or danger and send protective signals to the host. This signaling and regulation are achieved through proinflammatory cytokine release or proptosis induction. NLRP3 is one of the strongest inflammatory signal proteins among inflammasomes. It is one of the most important response elements in inflammatory response to acute respiratory distress syndrome, sepsis, and many bacterial, parasitic, viral, and fungal infections [41]. In individuals without immune system disorders, these cytokines are regulated, while in immunosuppressive cases, the host becomes susceptible to infection with the lack or absence of these cytokines. Thus, immunomodulatory therapies are seen as a strategy in the combat against *A. baumannii* infections in patients with a weak immune system [42]. IL-6, being rapidly produced through pathogen-linked molecular patterns or molecules that recognize damage-dependent molecular patterns, triggers innate immune responses in a manner that is controlled by transcriptional and posttranscriptional mechanisms; however, the irregular continued release of IL-6 has pathological effects leading to chronic inflammation and autoimmunity [43].

Damage-related inflammatory response is specific to infection, and IL-6 release increases in severe injuries and sepsis. Although IL-6 plays a role in protecting the host as a proinflammatory response, serious complications and even death can be seen as a result of its continuous and irregular release. TCZ is a humanized anti-human antibody of the immunoglobulin G1 class that prevents IL-6 from binding to IL-6R. It has been shown that the use of IL1 and IL6 antagonists provide significant benefits in diseases presenting hyperinflammation, such as SARS-CoV [23]. Although TCZ was previously only indicated for use in autoimmune diseases, the FDA extended its use to cytokine release syndrome in 2017 [24]. In a clinical study, it was shown that TCZ was used in 38% of intensive care unit-acquired bloodstream infections. This shows that this drug, which can play an active role in cytokine storm, can also contribute to many infections [44]. However, in the same study, it was emphasized that this increased risk could further increase with steroid use. There is no information about clinical progression in these patients. Therefore, the current study was the first to provide an insight into the potential results of *A. baumannii* reproduction in patients using both steroids and TCZ. Our results showed that *A. baumannii* pneumonia induced in rats that were given steroids could lead to a worse state with TCZ use. With these results, we showed that more attention should be paid to the immune status and risk of infection in the transition to TCZ use in patients using steroids. Our findings also indicated that while the use of TCZ with steroids suppressed cytokine production very strongly, this substantially reduced cytokine level led to the inability to stop *A. baumannii* infection and exacerbated lung damage.

In this study, the use of TCZ in the presence of *A. baumannii* infection significantly decreased inflammatory cytokines in rats immunosuppressed with steroids. However, despite the decreasing amounts of cytokines, *A. baumannii* was shown to increase lung damage due to infection. This suggests that in patients with a current or recent use of steroids, tocilizumab can increase organ damage due to opportunistic infection. Therefore, more care should be taken in patients using tocilizumab together with steroids.

Availability of Data and Materials

The datasets during and/or analyzed during the current study available from the corresponding author (D. Çelebi) on reasonable request.

Funding Support

There is no funding source.

Conflict of Interest

The authors report no conflicts of interest. The authors alone are responsible for the content and writing of paper.

Acknowledgements

The author sincerely acknowledges the Atatürk University for for the opportunities it provides.

Author Contributions

DC, ZH and OC conceived and supervised the study. DC, ZH and IC carried out animal experiments. KTC and IC made laboratory measurements. NA, IH and HK collected and analyzed data. SY applied the histopathological examination

of the study. All authors contributed to the critical revision of the manuscript and have read and approved the final version.

References

1. Baumann P, Doudoroff M, Stanier RY: A study of the *Moraxella* group II. Oxidative-negative species (genus *Acinetobacter*). *J Bacteriol,* 95 (5): 1520-1541, 1968. DOI: 10.1128/jb.95.5.1520-1541.1968

2. Bouvet PJM, Grimont PAD: Taxonomy of the genus *Acinetobacter* with the recognition of *Acinetobacter baumannii* sp. *nov., Acinetobacter haemolyticus* sp. *nov., Acinetobacter johnsonii* sp. *nov.,* and *Acinetobacter junii* sp. *nov.* and emended descriptions of *Acinetobacter calcoaceticus* and *Acinetobacter lwoffii. Int J Syst Evol,* 36 (2): 228-240, 1986. DOI: 10.1099/00207713-36-2-228

3. Dijkshoorn L, Nemec A, Seifert H: An increasing threat in hospitals: Multidrug-resistant *Acinetobacter baumannii. Nat Rev Microbiol,* 5 (12): 939-951, 2007. DOI: 10.1038/nrmicro1789

4. Munoz-Price LS, Weinstein RA: *Acinetobacter* infection. *N Engl J Med,* 358 (12): 1271-1281, 2008. DOI: 10.1056/NEJMra070741

5. Wong D, Nielsen TB, Bonomo RA, Pantapalangkoor P, Luna B, Spellberg B: Clinical and pathophysiological overview of *Acinetobacter* infections: A century of challenges. *Clin Microbiol Rev,* 30 (1): 409-447, 2017. DOI: 10.1128/CMR.00058-16

6. Chung DR, Song JH, Kim SH, Thamlikitkul V, Huang SG, Wang H, So TMK, Yasin RMD, Hsueh PR, Carlos CC, Hsu LY, Buntaran L, Lalitha MK, Kim MJ, Choi LY, Kim SII, Ko KS, Kang CI, Peck KR, Asian Network for Surveillance of Resistant Pathogens Study Group: High prevalence of multidrug-resistant nonfermenters in hospital-acquired pneumonia in Asia. *Am J Respir Crit Care Med,* 184 (12): 1409-1417, 2011. DOI: 10.1164/rccm.201102-0349OC

7. Tognim MCB, Andrade SS, Silbert S, Gales AC, Jones RN, Sader HS: Resistance trends of *Acinetobacter* spp. in Latin America and characterization of international dissemination of multi-drug resistant strains: Five-year report of the SENTRY Antimicrobial Surveillance Program. *Int J Infect Dis,* 8 (5): 284-291, 2004. DOI: 10.1016/j.ijid.2003.11.009

8. Van Dessel H, Dijkshoorn L, van der Reijden T, Bakker N, Paauw A, van den Broek P, Verhoef J, Brisse S: Identification of a new geographically widespread multiresistant *Acinetobacter baumannii* clone from European hospitals. *Res Microbiol,* 155 (2): 105-112, 2004. DOI: 10.1016/ j.resmic.2003.10.003

9. Gaddy JA, Arivett BA, McConnell MJ, López-Rojas R, Pachón J, Actis LA: Role of acinetobactin-mediated iron acquisition functions in the interaction of *Acinetobacter baumannii* strain ATCC 19606T with human lung epithelial cells, *Galleria mellonella* caterpillars, and mice. *Infect Immun,* 80 (3): 1015-1024, 2012. DOI: 10.1128/IAI.06279-11

10. Howard A, O'Donoghue M, Feeney A, Sleator RD: *Acinetobacter baumannii:* An emerging opportunistic pathogen. *Virulence,* 3 (3): 243-250, 2012. DOI: 10.4161/viru.19700

11. McConnell MJ, Actis L, Pachón J: *Acinetobacter baumannii:* Human infections, factors contributing to pathogenesis and animal models. *FEMS Microbiol Rev,* 37 (2): 130-155, 2013. DOI: 10.1111/j.1574-6976.2012.00344.x

12. Mortensen BL, Skaar EP: The contribution of nutrient metal acquisition and metabolism to *Acinetobacter baumannii* survival within the host. *Front Cell Infect Microbiol,* 3:95, 2013. DOI: 10.3389/fcimb.2013.00095

13. Mortensen BL, Skaar EP: Host-microbe interactions that shape the pathogenesis of *Acinetobacter baumannii* infection. *Cell Microbiol,* 14 (9): 1336-1344, 2012. DOI: 10.1111/j.1462-5822.2012.01817.x

14. Seed KD: Battling phages: How bacteria defend against viral attack. *PLoS Pathog,* 11 (6): e1004847, 2015. DOI: 10.1371/journal.ppat.1004847

15. Weber BS, Hennon SW, Wright MS, Scott NE, de Berardinis V, Foster LJ, Ayala JA, Adams MD, Feldman MF: Genetic dissection of the type VI secretion system in *Acinetobacter* and identification of a novel peptidoglycan hydrolase, TagX, required for its biogenesis. *MBio,* 7 (5): e01253-16, 2016. DOI: 10.1128/mBio.01253-16

16. Sousa C, Botelho J, Silva L, Grosso F, Nemec A, Lopes J, Peixe L: MALDI-TOF MS and chemometric based identification of the *Acinetobacter calcoaceticus-Acinetobacter baumannii* complex species. *Int J Med Microbiol,* 304 (5-6): 669-677, 2014. DOI: 10.1016/j.ijmm.2014.04.014

17. Vickers NJ: Animal communication: When I'm calling you, will you answer too? *Curr Biol,* 27 (14): R713-R715, 2017. DOI: 10.1016/j.cub.2017.05.064

18. Cerqueira GM, Peleg AY: Insights into *Acinetobacter baumannii* pathogenicity. *IUBMB Life,* 63 (12): 1055-1060, 2011. DOI: 10.1002/iub.533

19. Park JH, Lee HK: Re-analysis of single cell transcriptome reveals that the NR3C1-CXCL8-neutrophil axis determines the severity of COVID-19. *Front Immunol,* 11: 2145, 2020. DOI: 10.3389/fimmu.2020.02145

20. Meduri GU, Annane D, Confalonieri M, Chrousos GP, Rochwerg B, Busby A, Ruaro B, Meibohm B: Pharmacological principles guiding prolonged glucocorticoid treatment in ARDS. *Intensive Care Med*, 46 (12): 2284-2296, 2020. DOI: 10.1007/s00134-020-06289-8

21. García-Patiño MG, García-Contreras R, Licona-Limón P: The immune response against *Acinetobacter baumannii,* an emerging pathogen in nosocomial infections. *Front Immunol,* 8:441, 2017. DOI: 10.3389/fimmu.2017.00441

22. Chen W: Host innate immune responses to *Acinetobacter baumannii* infection. *Front Cell Infect Microbiol,* 10:486, 2020. DOI: 10.3389/fcimb.2020.00486

23. Rokni M, Hamblin MR, Rezaei N: Cytokines and COVID-19: friends or foes? *Hum Vaccin Immunother,* 16 (10): 2363-2365, 2020. DOI: 10.1080/21645515.2020.1799669

24. Cortegiani A, Ippolito M, Greco M, Granone V, Protti A, Gregoretti C, Giarratano A, Einav S, Cecconi M: Rationale and evidence on the use of tocilizumab in COVID-19: A systematic review. *Pulmonology,* 27 (1): 52-66, 2021. DOI: 10.1016/j.pulmoe.2020.07.003

25. Ramzy D, Tumiati LC, Tepperman E, Sheshgiri R, Jackman J, Badiwala M, Rao V: Dual immunosuppression enhances vasomotor injury: Interactive effect between endothelin-1 and nitric oxide bioavailability. *J Thorac Cardiovasc Surg,* 135 (4): 938-944, 2008. DOI: 10.1016/j.jtcvs.2007.09.075

26. Chen KL, Lv ZY, Yang HW, Liu Y, Long FW, Zhou B, Sun XF, Peng ZH, Zhou ZG, Li Y: Effects of tocilizumab on experimental severe acute pancreatitis and associated acute lung injury. *Crit Care Med,* 44 (8):e664-e677, 2016. DOI: 10.1097/CCM.0000000000001639

27. Wang Y, Zhang X, Feng X, Liu X, Deng L, Liang ZA: Expression of toll-like receptor 4 in lungs of immune-suppressed rat with *Acinetobacter baumannii* infection. *Exp Ther Med,* 12 (4): 2599-2605, 2016. DOI: 10.3892/etm.2016.3624

28. Keskin H, Keskin F, Tavaci T, Halici H, Yuksel TN, Ozkaraca M, Bilen A, Halici Z: Neuroprotective effect of roflumilast under cerebral ischaemia/reperfusion injury in juvenile rats through NLRP-mediated inflammatory response inhibition. *Clin Exp Pharmacol Physiol*, 48 (8): 1103-1110, 2021. DOI: 10.1111/1440-1681.13493

29. Livak KJ, Schmittgen TD: Analysis of relative gene expression data using real-time quantitative PCR and the 2-ΔΔCT method. *Methods,* 25 (4): 402-408, 2001. DOI: 10.1006/meth.2001.1262

30. Bilen A, Calik I, Yayla M, Dincer B, Tavaci T, Cinar I, Bilen H, Cadirci E, Halici Z, Mercantepe F: Does daily fasting shielding kidney on hyperglycemia-related inflammatory cytokine via TNF-α, NLRP3, TGF-β1 and VCAM-1 mRNA expression. *Int J Biol Macromol*, 190, 911-918, 2021. DOI: 10.1016/j.ijbiomac.2021.08.216

31. Keskin H, Tavaci T, Halici H, Yuksel TN, Ozkaraca M, Bilen A, Kose D, Mendil AS, Halici Z: Early administration of milrinone ameliorates lung and kidney injury during sepsis in juvenile rats. *Pediatr Int*, 2021 *(Article in Press).* DOI: 10.1111/ped.14917

32. Almomani BA, McCullough A, Gharaibeh R, Samrah S, Mahasneh F: Incidence and predictors of 14-day mortality in multidrug-resistant *Acinetobacter baumannii* in ventilator-associated pneumonia. *J Infect Dev Ctries,* 9 (12): 1323-1330, 2015. DOI: 10.3855/jidc.6812

33. Joly-Guillou ML, Wolff M, Pocidalo JJ, Walker F, Carbon C: Use of a new mouse model of *Acinetobacter baumannii* pneumonia to evaluate the postantibiotic effect of imipenem. *Antimicrob Agents Chemother,* 41 (2): 345-351, 1997. DOI: 10.1128/AAC.41.2.345

34. Qiu H, KuoLee R, Harris G, Chen W: Role of NADPH phagocyte oxidase in host defense against acute respiratory *Acinetobacter baumannii* infection in mice. *Infect Immun,* 77 (3): 1015-1021, 2009. DOI: 10.1128/IAI.01029-08

35. Kumar H, Kawai T, Akira S: Pathogen recognition by the innate immune system. *Int Rev Immunol,* 30 (1): 16-34, 2011. DOI: 10.3109/08830185.2010.529976

36. Kimura A, Naka T, Nakahama T, Chinen I, Masuda K, Nohara K, Fujii-Kuriyama Y, Kishimoto T: Aryl hydrocarbon receptor in combination with Stat1 regulates LPS-induced inflammatory responses. *J Exp Med,* 206 (9): 2027-2035, 2009. DOI: 10.1084/jem.20090560

37. Meduri GU: Clinical review: A paradigm shift: The bidirectional effect of inflammation on bacterial growth. Clinical implications for patients with acute respiratory distress syndrome. *Crit Care,* 6 (1): 24-29, 2002. DOI: 10.1186/cc1450

38. Meduri GU, Kanangat S, Bronze M, Patterson DR, Meduri CU, Pak C, Tolley EA, Schaberg DR: Effects of methylprednisolone on intracellular bacterial growth. *Clin Diagn Lab Immunol,* 8 (6): 1156-1163, 2001. DOI: 10.1128/CDLI.8.6.1156-1163.2001

39. Tongyoo S, Permpikul C, Mongkolpun W, Vattanavanit V, Udompanturak S, Kocak M, Meduri GU: Hydrocortisone treatment in early sepsis-associated acute respiratory distress syndrome: Results of a randomized controlled trial. *Crit Care,* 20:329, 2016. DOI: 10.1186/s13054-016-1511-2

40. Sugui JA, Pardo J, Chang YC, Zarember KA, Nardone G, Galvez EM, Müllbacher A, Gallin JI, Simon MM, Kwon-Chung KJ: Gliotoxin is a virulence factor of *Aspergillus fumigatus:* gliP deletion attenuates virulence in mice immunosuppressed with hydrocortisone. *Eukaryot Cell,* 6 (9): 1562-1569, 2007. DOI: 10.1128/EC.00141-07

41. Wu CL, Lee YL, Chang KM, Chang GC, King SL, Chiang CD, Niederman MS: Bronchoalveolar interleukin-1β: A marker of bacterial burden in mechanically ventilated patients with community-acquired pneumonia. *Crit Care Med,* 31 (3): 812-817, 2003. DOI: 10.1097/01.CCM.0000054865.47068.58

42. Bergogne-Berezin E, Towner K: *Acinetobacter* spp. as nosocomial pathogens: Microbiological, clinical, and epidemiological features. *Clin Microbiol Rev,* 9 (2): 148-165, 1996. DOI: 10.1128/CMR.9.2.148

43. Kang S, Tanaka T, Kishimoto T: Therapeutic uses of anti-interleukin-6 receptor antibody. *Int Immunol,* 27 (1): 21-29, 2015. DOI: 10.1093/intimm/dxu081

44. Giacobbe DR, Battaglini D, Ball L, Brunetti I, Bruzzone B, Codda G, Crea F, De Maria A, Dentone C, Di Biagio A, Icardi G, Magnasco L, Marchese A, Mikulska M, Orsi A, Patroniti N, robba C, Signori A, Taramasso L, Vena A, Pelosi P, Bassetti M: Bloodstream infections in critically ill patients with COVID-19. *Eur J Clin Invest,* 50 (10): e13319, 2020. DOI: 10.1111/eci.13319