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ORIGINAL RESEARCH / *ÖZGÜN ARAŞTIRMA*

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Secondary Infection and Co-infection in COVID-19 Patients Receiving Tocilizumab

Tocilizumab Alan COVİD-19 Hastalarında Sekonder Enfeksiyon ve Ko-enfeksiyon

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ABSTRACT *Objective:* Tocilizumab (TCZ) is a recombinant humanized anti-interleukin-6 receptor monoclonal antibody that is beneficial in critically ill coronavirus disease-2019 (COVID-19) patients. However, the clinical efficacy and safety of immunosuppressants (including TCZ, sarilumab and anakinra) in COVID-19 patients are not yet known. These treatments may predispose patients to infection. The aim of this study was to find any connection between the use of TCZ and increased secondary bacterial infections.
Materials and Methods: In this study, we conducted retrospective analyses of secondary bacterial infections in COVID-19 patients in the intensive care unit (ICU). This study included patients with laboratory-confirmed COVID-19 infection or clinically and radiologically confirmed COVID-19 infections who were admitted to the university hospital adult ICUs between March 2020 and January 2022. Demographic data, recent exposure and travel history, clinical symptoms or signs, laboratory findings, and comorbidities were recorded. Microbial cultures from tracheal aspirates, blood, and urine were obtained at admission and throughout the hospital stay. The patients who received TCZ treatment noted and analyzed for seconder infections. Blood cultures were taken at least 48 hours after the first dose of TCZ.
Results: We found that 80 patients (%37) had positive culture samples at admission, and most of these cases were admitted to the ICU from various hospital wards. The analyzed data showed that the TCZ group had a higher incidence of positive culture samples (75% vs. 35%, p=0.0001). The results showed that culture of TCZ taken patients had more incidence with methicillin resistance *Staphylococcus aureus*, *Klebsiella* spp., and *Acinetobacter* spp. (p=0.0001). Infection and mortality rates were much higher than those in the usual care group.
Conclusion: Secondary infections and sepsis are major risk factors for mortality. The pathogens detected were drug resistant and had a lower chance of treatment. The benefit of TCZ treatment was lost in these patients because of secondary infections. Future studies are needed to help determine the risks of TCZ treatments.
Keywords: Seconder infection, COVID-19, tocilizumab

ÖZ *Amaç:* Tocilizumab (TCZ), kritik durumdaki koronavirüs hastalığı-2019 (COVİD-19) hastalarında fayda sağlayan, rekombinant bir anti-interlökin-6 reseptörü monoklonal antikordur. Bununla birlikte, COVİD-19 hastalarında immünosüpresan tedavilerin (TCZ, sarilumab ve anakinra dahil) klinik etkinliği ve güvenliği henüz bilinmemektedir. Bu tedaviler hastaları enfeksiyona yatkın hale getirebilir. Bu çalışmanın amacı, TCZ kullanımı ile artmış sekonder bakteriyel enfeksiyonlar arasında herhangi bir bağlantı bulmaktır.
Gereç ve Yöntem: Bu çalışmada yoğun bakım ünitesindeki (YBÜ) COVİD-19 hastalarında sekonder bakteriyel enfeksiyonların retrospektif analizlerini yaptık. Bu çalışmaya Mart 2020 ile Ocak 2022 tarihleri arasında üniversite hastanesinin yetişkin YBÜ'lerine kabul edilen laboratuvarca doğrulanmış COVİD-19 enfeksiyonu veya klinik ve radyolojik olarak doğrulanmış COVİD-19 enfeksiyonu olan hastalar dahil edilmiştir. Demografik veriler, yakın zamandaki maruziyet ve seyahat öyküsü, klinik semptomlar veya bulgular, laboratuvar bulguları ve eşlik eden hastalıklar kaydedildi. Trakeal aspiratlardan, kan ve idrardan mikrobiyal kültürler, hastaneye yatışta ve hastanede kaldıkları süre boyunca alındı. TCZ tedavisi alan hastalar sekonder enfeksiyonları not etmiş ve analiz etmişlerdir. Kan kültürleri ilk TCZ dozundan en az 48 saat sonra alınmıştır.

Bulgular: Seksen hastada (%37) başvuru sırasında kültür örneğinin pozitif olduğunu ve bu olguların çoğunun çeşitli hastane servislerinden YBÜ'ye kabul edildiğini saptadık. Analiz edilen veriler, TCZ grubunun pozitif kültür örnekleri insidansının daha yüksek olduğunu gösterdi (%75'e karşı %35, p=0,0001). Sonuçlar, TCZ kültürü alan hastalarda metisilin direnci insidansının daha yüksek olduğunu göstermiştir. *Staphylococcus aureus*, *Klebsiella* spp. ve *Acinetobacter* spp. (p=0,0001). Enfeksiyon oranı ve ölüm oranı normal bakım grubundan çok daha yüksekti.
Sonuç: Sekonder enfeksiyonlar ve sepsis mortalite için önemli bir risktir. Tespit edilen patojenler ilaca dirençliydi ve tedavi şansı daha düşüktü. Bu hastalarda sekonder enfeksiyonlar nedeniyle TCZ tedavisinin yararı kaybolmuştur. TCZ tedavilerinin risklerini belirlemeye yardımcı olmak için gelecekteki çalışmalara ihtiyaç vardır.
Anahtar Kelimeler: Sekonder enfeksiyon, COVİD-19, tocilizumab

Introduction

Most of the coronavirus disease-2019 (COVID-19) patients are asymptomatic or have symptoms that don't need hospitalization. However, there are patients who develop a respiratory failure requiring oxygen support and hospital care. Many of them need intensive care unit (ICU) admission and ventilator support (1). In these patients, COVID has a progressive clinical characteristic. The disease usually begins as an upper respiratory tract infection. Following days, patients have rapid deterioration and increased oxygen support. This results acute respiratory distress syndrome (ARDS), multi-organ failure and death (2).

The pathogenesis of COVID-19 is thought a dysregulated inflammatory response causing clinical manifestations in patients (3). This systemic response includes massive releasing of cytokines such as interleukin (IL)-1, IL-6 (4). This process causes alveolar damage and microvascular thrombosis (5). Treatment of COVID-19 focuses on stopping hyperinflamation response using corticosteroids and immune suppressive agents.

Tocilizumab (TCZ) is a recombinant humanized anti-IL-6 receptor monoclonal antibody that inhibits the binding of IL-6 to both membrane and soluble IL-6 receptors, blocking IL-6 signaling and reducing inflammation. The drug is used in rheumatoid arthritis, juvenile inflammatory arthritis and refractory giant cell arteritis (6). TCZ is also approved for systemic inflammatory response caused by the massive release of proinflammatory cytokines (7,8). TCZ was tested in many COVID-19 cases due to these characteristics and shown that many laboratory parameters improved such as C-reactive protein, lactate dehydrogenase, ferritin and total leukocyte count. TCZ usage in severe COVID-19 patients causes less complications, decreased duration of hospitalization, decreased needs for ICU admission (9).

Secondary infections are common in viral respiratory diseases. There are studies that shows secondary bacterial infection (SBI) is seen 5-15% of patients with COVID-19. According to reports, 50% of COVID-19 deaths had history of SBIs. SBIs have a higher risk of mortality (10). Using immune suppressive treatment makes patients proned to SBI. In most cases, benefit of avoiding pulmonary fibrosis due to COVID infection more beneficial then avoiding SBI.

In this study, we conducted a retrospective analysis of SBIs in COVID-19 patients at ICU. The aim of this study is to find any connection between usage of TCZ and increased SBI in these patients. This connection may lead better clinical follow-up and making health providers aware of SBI risk.

Materials and Methods

The permission for this retrospective study had taken from Non-invasive Clinical Research Ethics Committee of Pamukkale University (no: E-60116787-020-14359, date: 02.02.2021).

This research involved individuals who were admitted to the adult ICUs at the university hospital between March 2020 and January 2022, with confirmed cases of COVID-19 either through laboratory tests or clinical and radiological examinations. COVID-19 diagnosis relied on either a positive outcome from a reverse-transcriptase–polymerase-chain reaction test or antibody Rapid Test using samples collected from nasopharyngeal swabs or endotracheal aspirates. Patient information was retrieved from electronic records stored in the hospital's computer system.

Data Collection

We gathered information on demographics, recent exposure, travel history, clinical symptoms, laboratory results, and existing health conditions. Additionally, we calculated and documented scores for acute physiology and chronic health evaluation-II and sequential organ failure assessment. Parameters related to invasive mechanical ventilation were also noted. Radiological evaluations, including chest X-rays or computed tomography scans, were conducted upon admission and as necessary. We recorded arterial partial

pressure of oxygen (PaO_2), PaO_2/FiO_2 ratio, and occurrences of ARDS. Sepsis and septic shock were defined and treated according to established guidelines and recommendations from the Turkey Ministry of Health for managing COVID-19 patients (11,12). Microbial cultures from tracheal aspirates, blood, and urine were obtained at admission and throughout the hospitalization period. The patients evaluated with infection diseases departments and rheumatology departments for TCZ treatment. The patients who took TCZ treatment had noted and analyzed for seconder infections. The blood cultures had taken at least 48 hours after first dose of TCZ. The patients discharge status (dead, alive), and length of stay in the ICU were also recorded.

Statistical Analysis

All statistical analyses were performed using SPSS 25.0 (IBM SPSS Statistics 25 software (Armonk, NY: IBM Corp.). Continuous variables were defined by the mean ± standard deviation and categorical variables were defined by number and percent. Difference between categorical variables were analyzed with chi-square analysis. Statistical significance was determined as $p<0.05$.

Results

Two hundred and sixteen patients admitted to ICU with laboratory confirmed COVID-19 infection between March 2020 and January 2022.

66.7% of the patients were male. Mean age was 65.93±14.45 years. 49.1% of the patients admitted to ICU from emergency service and the others were from COVID-19 wards and other wards. One or more comorbidities was found in 192 patients. Twenty-four patients had no comorbidity. Three hundred thirty eight comorbidities had detected in these patients. Hypertension was the most common comorbidity in these patients (44.4%). Diabetes mellitus (38%) and oncological diseases (17.6%) followed hypertension (Table 1). The infection parameters at admission are shown at Table 1.

Sixty patients were intubated at beginning of admission (27.7%). Forty patients were intubated in course of admission. One hundred and sixteen patients were followed with high-flow nasal oxygen (HFNO) and non-invasive mechanic ventilation (NIMV) (53.7%). HFNO and NIMV administered alternately. Sixteen patient who received TCZ were intubated which only one of them survived and discharged.

At the admission, empiric antibiotics were started by infection diseases department according to laboratory and clinical findings. Thirty-three patients didn't receive any empiric treatment. Tigecycline, piperacillin-tazobactam and ceftriaxone were the most chosen options in treatment. The microbial samples of patients had taken at admission. Eighty patients had positive culture. 94% of these cultures were blood samples. Most common pathogens were coagulase negative *staphylococcus*, methicillin resistant *Staphylococcus aureus* and *Enterococcus* spp. After positive culture samples, empiric treatment had changed according to antimicrobial resistance testing in 28 patients (13%). The microbiological findings and treatments at admission is shown at Table 2.

Patients with macrophage activation syndrome who have no or little response to glucocorticoids had treated with TCZ (humanized monoclonal antibody against the IL-6 receptor). The dosage used for TCZ is 8 mg/kg (patients ≥30 kg) or 12 mg/kg (patients <30 kg) as a single dose (maximum: 800 mg/dose). TCZ treatment was decided with the cooperation of infection diseases department and rheumatology department. Twenty four of 216 patients has taken TCZ treatment with approvement of Turkish Health Ministry.

Table 1. Demographics and clinical characteristics of the patients

Age (mean ± SD)	65.93±14.45
Sex (M/F)	144 (66.7%)/72 (33.3%)
SOFA score (mean ± SD)	2.6±1.32
Admission service (emergency/other wards)	106 (49.1%)/110 (50.9%)
Length of ICU stay (day) (mean ± SD)	11.17±9.77
Exitus	94 (43.5%)
Comorbidities	
Hypertension	96 (44.4%)
Diabetes mellitus	82 (38%)
Oncological diseases	38 (17.6%)
Cardiac failure	20 (9.3%)
Coronary artery disease	19 (8.8%)
Hematological disease	13 (6%)
Laboratory findings at admission	
Procalcitonin (mean ± SD)	2.9±10.51
C-reactive protein (mean ± SD)	123.83±86
Ferritin (mean ± SD)	1023.33±1335.09

SD: Standard deviation, ICU: intensive care unit, SOFA: sequential organ failure assessment

Eighteen of 24 patients who taken TCZ treatment, had worsened clinical conditions and increased level of infection markers. Culture samples were taken. Methicillin resistance *S. aureus* (n=11) *Klebsiella* spp. (n=6) and *Acinetobacter* spp. (n=6) were most common in these patients.

Almost all of the patients needed to repeat cultures due to clinical and laboratory worsening. Fifty seven of patients who didn't receive anti-cytokine treatment had positive result in their cultures. The pathogens were methicillin resistance *S. aureus* (n=28) *Klebsiella* spp. (n=10) and *Acinetobacter* spp. (n=10), *Enterococcus* spp. (n=8), *Pseudomonas* spp. (n=6), *Candida* spp. (n=3).

The difference of clinical and microbiological characteristics is shown at Table 3. The results have compared between patients who received TCZ and who didn't. There was no statistical difference in cultures at admission. There was an increased positive rating in cultures that were utilized after clinical worsening (p=0.0001). The results showed that culture of TCZ taken patients had more incidence with methicillin resistance *S. aureus*, *Klebsiella* spp. and *Acinetobacter* spp. (p=0.0001). Death was more common in TCZ group. There was no difference in admission service.

Table 2. Microbiological findings at admission and antimicrobial treatment characteristics

Positive culture samples (positive/negative) (total n=216)	80 (37%)/136 (63%)
Pathogens at culture positive patients	
Coagulase negative *Staphylococcus*	35 (43.8%)
Methicillin resistant *Staphylococcus aureus*	33 (41.3%)
Enterococcus spp.	7 (8.8%)
Klebsiella spp.	3 (3.8%)
Corynebacterium spp.	3 (3.8%)
Candida spp.	2 (2.5%)
Acinetobacter spp.	1 (1.3%)
Pseudomonas spp.	1 (1.3%)
Other pathogens	5 (6.3%)
Positive sample location	
Blood	78 (97.5%)
Tracheal aspiration	4 (5%)
Urine	1 (1.3%)
Empiric treatment at admission	
Tigecycline	66 (12%)
Piperacillin-tazobactam	50 (9.1%)
Ceftriaxone	42 (7.6%)
Meropenem	17 (3.1%)
Teicoplanin	14 (2.5%)
No antibiotics	33 (6%)

Discussion

After COVID-10 outbreak, many immunocompromised patients were admitted to ICUs. There has been an increased need of ICUs. Many of these patients had SBIs and ICU specialists fought with sepsis and co-infection beside COVID-19. There are many studies, reviews and case report about secondary infections in COVID. The mechanism of increased SBI thought to be the failure of the adaptive immune reaction toward viral infection against bacterial infection (13).

In one study, researchers utilized the data of 1,495 cases and 6.8% of these cases had secondary bloodstream infections. The pathogens in these cases were mostly Gram-negative bacteria such as *Acinetobacter baumanii* (35.8%) and *Klebsiella pneumoniae* (%30.8) (10). In a study, Zhang et al. (14) analyzed 148,221 patients with severe acute respiratory syndrome coronavirus-2 pneumonia were admitted to Zhongnan Hospital, Wuhan, China. 25.8% (57/221) patients had co-infections, 29.8% (17/57) of these cases were co-infected with bacteria (14).

In our study, secondary infection rate was higher like these studies. Eighty patients (37%) had positive culture sample at admission and most of these cases was admitted to ICU from various hospital wards. The reason for high rate of positive cultures at admission is thought to be long duration of hospital admission. Most patients had come to ICU after being in infection wards for days. Most of the positive cultures had the pathogens such as coagulase negative *Staphylococcus*, methicillin resistant *S. aureus* and *Enterococcus* spp. Most of our patients had worsened clinically (fever, decreased consciousness) and had cultures repeated. Seventy five of these patients (35%) had SBI with positive cultures. These results are consistent with other studies.

There are limited studies about secondary infections in patients who take anti-cytokine, anti-inflammatory treatment. It is known that these treatments cause predisposition with secondary infections. TCZ is most used immunomodulatory treatment in our ICU. These patients evaluated about infections, immunosuppressive conditions, tuberculosis, human immunodeficiency virus. After this evaluation, TCZ admitted.

Table 3. Comparison of clinical and microbiological characteristics				
		Tocilizumab group	**Non-tocilizumab group**	**p-value**
Admission service	Emergency	12 (50%)	94 (48.96%)	0.923
	Wards	12 (50%)	98 (51.04%)	
Outcome	Discharge	9 (37.5%)	113 (58.85%)	0.047*
	Death	15 (62.5%)	79 (41.15%)	
Cultures	Negative	6 (25%)	153 (79.69%)	0.0001*
	Positive	18 (75%)	39 (20.31%)	
Coagulase negative *Staphylococcus*	Negative	209 (96.76%)	187 (97.4%)	0.176
	Positive	7 (3.24%)	5 (2.6%)	
Methicillin resistance *S. aureus*	Negative	13 (54.17%)	175 (91.15%)	0.0001*
	Positive	11 (45.83%)	17 (8.85%)	
Corynebacterium	Negative	22 (91.67%)	187 (97.4%)	0.176
	Positive	2 (8.33%)	5 (2.6%)	
***Klebsiella* spp.**	Negative	18 (75%)	188 (97.92%)	0.0001*
	Positive	6 (25%)	4 (2.08%)	
***Acinetobacter* spp.**	Negative	18 (75%)	188 (97.92%)	0.0001*
	Positive	6 (25%)	4 (2.08%)	
***Enterococcus* spp.**	Negative	20 (83.33%)	188 (97.92%)	0.006*
	Positive	4 (16.67%)	4 (2.08%)	
***Pseudomonas* spp.**	Negative	22 (91.67%)	188 (97.92%)	0.135
	Positive	2 (8.33%)	4 (2.08%)	
***Candida* spp.**	Negative	23 (95.83%)	190 (98.96%)	0.299
	Positive	1 (4.17%)	2 (1.04%)	
*p<0.05 statistically significant; chi-square test				

The analyzed data showed that TCZ group has a higher incidence of positive culture samples (75% vs. 35%, p=0.0001). Methicillin resistance *S. aureus*, *Klebsiella* spp. and *Acinetobacter* spp. had increased incidence in TCZ group's cultures against usual care group's (respectively, 45-8%, 25-2% and 24-2%; p=0.0001 for each).

Giacobbe et al. (15), studied secondary bloodstream infections among critically ill patients with COVID-19. They found the cumulative risk of SBI was 25% after 15 days and 50% after 30 days of ICU stay. The study also showed that TCZ was associated with an increased risk of secondary infection (p=0.003) (15). Our data is consistent with these ratios.

RECOVERY study showed that the patients receiving TCZ has higher chance for discharge at 28 day of admission and lower rates for mortality and MV needs. RECOVERY study didn't analyze infection situation in patients (6).

In one study, receiving TCZ was associated with a higher risk of secondary bacterial (48.1% vs. 28.1%; p=0.029 infections and higher mortality (35.2% vs. 19.3%; p=0.020) (16). Our mortality rate is higher in TCZ group consistent with higher positive culture rates (62% vs. 41%, p=0.004). Our results are similar with this study.

In our study, infection rate and mortality rate were much higher than usual care group. Secondary infections and sepsis are a major risk for mortality. The pathogens detected were drug-resistance and had a lower chance of treatment. The benefit of TCZ treatment lost in these patients because of secondary infections.

Another reason for higher mortality is thought to need of MV. Our data showed that 66% of the patients who received TCZ were intubated. Only one of them survived and discharged while other patients were lost. MV is a major risk for both infection and mortality. This is also a controversial point. TCZ prones to infection but TCZ is given to patients

who are in severe condition like need of MV. Further studies are needed in more specific groups on this subject.

In an ongoing study, the mortality rate in TCZ-treated patients was 24.1%. There was an association between mortality and seniority, the need for mechanic support, the presence of critical COVID-19 and severe lung parenchymal disease. The same study found that invasive MV support, immunosuppression and extended lung injury may increase the risk for SBIs (17).

This study has several limitations: small sample size, retrospective nature (selection and information biases) and single-center nature (contamination and flora of the same ICU).

Conclusion

The incidence rate of SBI is higher in critically ill patients in COVID-19. TCZ is a promising treatment for COVID patients who has overactive immune system due to cytokines. In response to this, TCZ can create predisposition to infection which causes sepsis and mortality. These risks cast a suspicion of benefit in TCZ treatment. These findings should be confirmed with a larger randomized clinical trial with longer follow-up. Future studies are needed to help determine about risks of TCZ treatments.

Ethics

Ethics Committee Approval: The permission for this retrospective study had taken from Non-invasive Clinical Research Ethics Committee of Pamukkale University (no: E-60116787-020-14359, date: 02.02.2021).

Informed Consent: Retrospective study.

Authorship Contributions

Concept: Ç.E., H.S., Design: Ç.E., H.S., Data Collection and Process: Ç.E., M.K., B.Ş., Analysis or Interpretation: Ç.E., M.K., H.S., Literature Search: Ç.E., M.K., Writing: Ç.E.

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