Journal of Microbiology, Immunology and Infection 57 (2024) 38—47

Available online at www.sciencedirect.com

ScienceDirect

journal homepage: www.e-jmii.com

Original Article

The association between tocilizumab and the secondary bloodstream infection maybe nonsignificant in hospitalized patients with SARS-CoV-2 infection: A cohort study

De-En Lu [a,b], Tsong-Yih Ou [c,d], Jyun-Wei Kang [e], Jie Ywi Ong [a,b], I-Ju Chen [f], Chih-Hsin Lee [b,g,h], Ming-Chia Lee [d,e,i,*]

[a] *Division of Nephrology, Department of Internal Medicine, Wan Fang Hospital, Taipei Medical University, Taipei, Taiwan*
[b] *Pulmonary Research Center, Wan Fang Hospital, Taipei Medical University, Taipei, Taiwan*
[c] *Division of Infectious Diseases, Department of Internal Medicine, Wan Fang Hospital, Taipei Medical University, Taipei, Taiwan*
[d] *Department of Nursing, Cardinal Tien College of Healthcare and Management, Taipei, Taiwan*
[e] *Department of Pharmacy, New Taipei City Hospital, New Taipei City, Taiwan*
[f] *Division of Pulmonary Medicine, Department of Internal Medicine, New Taipei City Hospital, New Taipei City, Taiwan*
[g] *Division of Pulmonary Medicine, Department of Internal Medicine, Wan Fang Hospital, Taipei Medical University, Taipei, Taiwan*
[h] *Division of Pulmonary Medicine, Department of Internal Medicine, School of Medicine, College of Medicine, Taipei Medical University, Taipei, Taiwan*
[i] *School of Pharmacy, College of Pharmacy, Taipei Medical University, Taipei, Taiwan*

Received 13 September 2022; received in revised form 25 October 2023; accepted 29 October 2023
Available online 7 November 2023

KEYWORDS
SARS-CoV-2 infection;
Secondary bloodstream infections;
Tocilizumab

Abstract *Background:* Immunomodulatory agents, such as tocilizumab (TCZ), exert promising effects against SARS-CoV-2 infection. However, growing evidence indicates that using TCZ may carry higher risks of secondary bloodstream infection (sBSI). This study determined whether TCZ is associated with an increased risk of sBSI.
Methods: We retrospectively collected the demographic and clinical data of hospitalized patients with SARS-CoV-2 infection from two Taiwanese hospitals. The time-to-incident sBSI in the TCZ users and nonusers was compared using the log-rank test. A multivariate Cox proportional hazards model was performed to identify independent risk factors for sBSI.

* Corresponding author. Department of Pharmacy, New Taipei City Hospital, No. 3, Sec. 1, New Taipei Blvd., Sanchong Dist., New Taipei City, Taiwan.
E-mail addresses: 108100@w.tmu.edu.tw (D.-E. Lu), 93023@w.tmu.edu.tw (T.-Y. Ou), jwkang0825@gmail.com (J.-W. Kang), 109225@w.tmu.edu.tw (J.Y. Ong), laby0510@gmail.com (I.-J. Chen), 103001@w.tmu.edu.tw (C.-H. Lee), ymkbaz60@gmail.com (M.-C. Lee).

https://doi.org/10.1016/j.jmii.2023.10.011

Results: Between May 1 and August 31, 2021, among 453 patients enrolled, 12 (2.65 %) developed sBSI. These patients were in hospital for longer duration (44.2 ± 31.4 vs. 17.6 ± 14.3 days, $p = 0.014$). Despite sBSI being more prevalent among the TCZ users (7.1 % vs. 1.6 %, $p = 0.005$), Kaplan—Meier survival analysis and multivariate Cox proportional hazards model both revealed no significant difference in risks of sBSI between the TCZ users and nonusers [adjusted HR (aHR) = 1.32 (95 % confidence interval (CI) = 0.29—6.05), $p = 0.724$]. Female sex [aHR = 7.00 (95 % CI = 1.45—33.92), $p = 0.016$], heavy drinking [aHR = 5.39 (95 % CI = 1.01—28.89), $p = 0.049$], and mechanical ventilation [aHR = 5.65 (95 % CI = 1.67—19.30), $p = 0.006$] were independently associated with a higher sBSI risk.
Conclusion: This real-world evidence indicates that in hospitalized patients with SARS-CoV-2 infection, TCZ does not significantly increase the risk of sBSI.

Introduction

The coronavirus disease of 2019 (COVID-19), caused by zoonotic severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2), remains an international health crisis and continues to have a devastating effect. Until September 2023, 770 million COVID-19 cases had been detected, and the World Health Organization reported 6.95 million confirmed deaths.[1] Most patients with SARS-CoV-2 infection have only mild symptoms. However, 14 % and 5 % of patients develop severe symptoms and critical disease, respectively.[2] Vaccines against SARS-CoV-2 effectively reduce the risks of hospitalization and critical illness, but the protection they afford declines over time.[3,4] Although the risk of severe disease is lower for Omicron infection than for infection with other variants,[5] the overall number of deaths remains high because the Omicron variant spreads more extensively than its ancestor variants.

The cytokine storm, also known as cytokine release syndrome (CRS), is associated with a high disease severity of SARS-CoV-2 infection.[6] Because spike proteins on the surface of the virion bind to cellular angiotensin-converting enzyme 2 receptors, the immediate immune response involving proinflammatory cytokines, such as endogenous interleukin 1 (IL-1), IL-4, IL-6, and interferon-gamma (IFN-R),[7,8] causes extensive collateral damage in the lung parenchyma (Fig. 1). Xing et al. demonstrated that IL-6 plays a crucial role in controlling the extent of tissue inflammatory response.[9] An increased IL-6 level was associated with poor clinical outcomes in patients with SARS-CoV-2 infection.[10] Severe acute respiratory distress syndrome is the predominant cause of mortality in patients with SARS-CoV-2 infection. The mortality rate of patients with SARS-CoV-2 infection and ARDS has ranged from 12 % to 78 %.[11,12]

Tocilizumab (TCZ), a monoclonal antibody targeting the IL-6 receptor to block the downstream signal transduction pathway of IL-6, was confirmed to be effective and have a favorable safety profile when used to treat patients with rheumatoid arthritis.[13] To our knowledge, excessive IL-6 production is associated with thrombosis, neutrophil recruitment, platelet aggregation, and vascular hyperpermeability, subsequently contributing to alveolar damage.[14—17] Because IL-6 receptor antagonists play a pivotal role in limiting CRS, TCZ has improved the survival of critically ill patients with SARS-CoV-2 infection receiving organ support therapy.[18] Current guidelines endorse the addition of TCZ to standard care for hospitalized patients with SARS-CoV-2 infection and elevated markers of systemic inflammation.[19]

Secondary bloodstream infection (sBSI) is a dangerous complication in hospitalized patients with SARS-CoV-2 infection and leads to longer hospitalization and a higher in-hospital mortality risk.[20] For hospitalized patients with SARS-CoV-2 infection, the prevalence of sBSI has been reported to vary from 3.7 % to 34.1 %.[20,21] IL-6 is a crucial mediator of neutrophil trafficking, hematopoiesis activation, and lymphocyte differentiation in response to offending pathogens. IL-6 protects neutrophils from apoptosis in individuals with bacterial infection and plays a fundamental role in competent host defense.[22,23] However, IL-6 blocker raises a concern of vulnerability to secondary infection,[24] although such risk was not discovered in several randomized trials.[25,26] Patients with active infection other than SARS-CoV-2 infection have typically been excluded from participating in trials investigating IL-6. Guaraldi et al. and Khatri et al. have indicated that TCZ use is associated with higher risks of new infection and sBSI.[21,24] Herein, we report our real-world experience of using TCZ in a cohort of hospitalized patients with SARS-CoV-2 infection to provide evidence regarding the possibility of an association between TCZ use and sBSI incidence.

Methods

TCZ use in Taiwan

The Guidelines for Clinical Management of SARS-CoV-2 Infection published by the Taiwan Centers for Disease Control[27] suggest the use of immunomodulators, including TCZ and corticosteroids, to ameliorate systemic inflammation in patients with moderate to severe stage infection, defined as the presence of pneumonia patches on chest radiographs or oxyhemoglobin desaturation ($SpO_2 \leq 94$ %) requiring supplemental oxygen or mechanical ventilation. In addition, TCZ could be used when C-reactive protein of patients are more than 7.5 mg/dL.[27] The recommended dose of TCZ is a single intravenous infusion of 8 mg/kg body weight (not exceeding 800 mg). The dose of dexamethasone suggested by the guideline was 6 mg daily for 10 days.

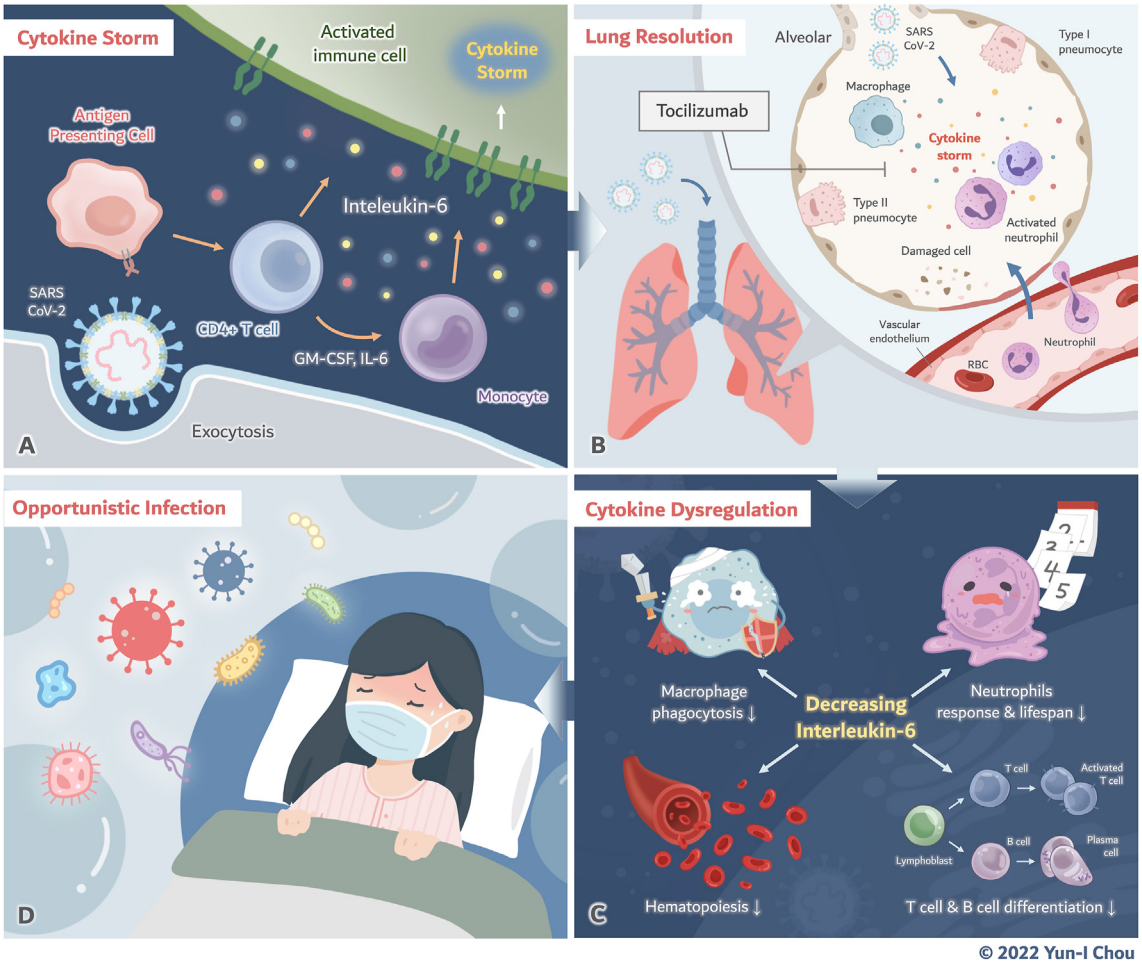

Figure 1. Therapeutic efficacy of tocilizumab in SARS-CoV-2 infection and its possible immunopathogenesis of secondary infection. **A.** SARS-CoV-2 triggers an uncontrolled release of proinflammatory cytokines. IL-6, in particular, delays apoptosis of neutrophils and promotes leukocyte recruitment to induce overactivation of immune cells. **B.** Impaired clearance of activated neutrophils results in damage to alveolar units. Blockage of IL-6 prevents the aggregation of neutrophils in the pulmonary capillary endothelium, modulating the self-magnifying inflammatory response. **C.** Treatment of CRS with TCZ, a monoclonal antibody that inhibits IL-6 receptors, accelerates the apoptosis of neutrophils and inhibits macrophage phagocytosis, hematopoiesis, and lymphocyte differentiation. **D.** The long-lasting immunosuppressive effect of TCZ may predispose patients to opportunistic infections. (Abbreviations: GM-CSF, granulocyte-macrophage colony-stimulating factor; IL-6, interleukin-6; SARS CoV-2, severe acute respiratory syndrome coronavirus 2).

Study design

This multicenter cohort study investigated the association between TCZ use and bacterial or fungal sBSI in hospitalized patients with SARS-CoV-2 infection. The investigation period was from May 1, 2021, to August 31, 2021. All patients were followed until the incident sBSI, discharge, or death. Eligible patients were those aged 20 years or older, hospitalized, and with SARS-CoV-2 infection that was confirmed by a positive SARS-CoV-2 polymerase chain reaction test via a nasopharyngeal swab sampled before admission.

The TCZ group consisted of patients with SARS-CoV-2 infection who were administered TCZ, whereas the non-TCZ group consisted of patients with SARS-CoV-2 infection who were not administered TCZ. The Institutional Review Boards of New Taipei City Hospital (Protocol No: 111004-E) and Taipei Medical University (Protocol No: N202109021) approved this study and a waiver of informed consent. We reviewed the electronic medical records (EMRs) of patients admitted with SARS-CoV-2 infection at these two hospitals. Using a standardized data collection tool, we extracted demographic, clinical, laboratory, microbiological, and outcome data from the EMRs during the time of admission. Heavy drinking was defined as the consumption of more than 4 alcoholic drinks on any day or more than 14 per week for men and more than 3 alcoholic drinks on any day or more than 7 per week for women.[28] Central venous catheter placement was recorded. For patients with sBSIs, only the central venous catheters being placed prior to the occurrence of sBSIs were recorded. Comorbidities—hypertension, type 2 diabetes mellitus, hyperlipidemia, coronary artery disease, chronic kidney disease, chronic viral hepatitis infection, respiratory diseases, and malignancy—were

recorded. The respiratory diseases considered were chronic obstructive pulmonary disease, asthma, and diffuse parenchymal lung diseases.

Definition of the outcome

The outcome of interest was the time to incident sBSI, defined as bacterial or fungal infection identified on blood cultures after TCZ administration and at least 48 h after hospital admission. The half-life of TCZ in adults was 11—13 days.[29] We only consider the sBSI that occurred within 8 weeks after TCZ use as a valid event. If the patients have no incident sBSI, the days of follow-up would be until discharge or mortality. The microorganisms were identified using matrix-assisted laser desorption ionization time-of-flight mass spectrometry. The antimicrobial susceptibility test was performed using commercial Phoenix automated machine kits (Becton, Dickinson and Company, Franklin Lakes, New Jersey, United states) in accordance with performance standards recommended by the Clinical and Laboratory Standards Institute. For blood cultures, multiple blood samples were collected from different veins. Blood cultures were considered to be contaminated if a coagulase-negative *Staphylococcus* species was present in only one out of two blood cultures. The microbiology laboratories of Wan Fang Hospital and New Taipei City Hospital have both been accredited by the Taiwan Accreditation Foundation.

Statistical analysis

The following descriptive statistics were used to describe the clinical characteristics of patients with SARS-CoV-2 infection: the mean and standard deviation, median and interquartile range (IQR) for continuous variables, and proportions for categorical variables. Group comparisons were performed using the Student *t* test for normally distributed continuous variables and the Mann—Whitney U test for nonnormally distributed continuous variables. Differences in proportions were compared using the chi-squared or Fisher's exact test. Kaplan—Meier curves plotting the time to incident sBSI in the TCZ and non-TCZ groups were compared using the log-rank test. A Cox proportional hazards model in a backward stepwise algorithm incorporating demographic characteristics, comorbidities, respiratory support, use of a central venous catheter, and medication use was employed to calculate adjusted hazard ratios (aHRs) and 95 % confidence intervals (CIs) for describing associations with sBSI risk. We also performed two different discrete survival models, i.e., the conditional logistic regression and the exact logistic regression. Considering the outcome events were relatively rare in this cohort, discrete survival model was conducted. The medication use included dexamethasone, remdesivir, tocilizumab, monoclonal antibody, colchicine, NRICM101, and fluvoxamine. Subgroup analysis stratified by the dexamethasone use was performed. We used *p-values* ≥ 0.2 as the removal criterion in our automatic selection of variables. We removed variables with *p-values* > 0.1 in determining the final model. All tests of significance were two-tailed. The α level was set at 0.05. Analyses were performed using the R program Version 4.1. (The R Foundation, Indianapolis, Indiana, United States).

Results

Between May 1 and August 31, 2021, a total of 464 patients with SARS-CoV-2 infection were hospitalized at two hospitals: 184 (39.7 %) in Wang Fang Hospital and 280 (60.3 %) in New Taipei City Hospital. After excluding 11 patients who were younger than 20 years, we analyzed the data of 453 patients (Fig. 2). Eighty-five patients (18.8 %) were administered TCZ. The baseline characteristics of the TCZ users and nonusers are listed in Table 1. The incidence of sBSI was higher among the TCZ users than the nonusers (7.1 % vs. 1.6 %, $p = 0.005$). The mean and median follow-up durations were 17.7 versus 14.4 days ($p = 0.054$) and 15 versus 11 days ($p = 0.002$) for the TCZ users and non-users, respectively. Compared the TCZ nonusers, the TCZ users were significantly older (age $\geq$65 years old; $p < 0.001$) and a greater proportion of them were men (65.9 % vs. 51.4 %, $p = 0.015$). The patients receiving TCZ were more likely to be active smokers (21.2 % vs. 11.7 %, $p = 0.021$). The prevalence of the following comorbidities was higher in the TCZ group: hypertension (67.1 % vs. 42.7 %, $p < 0.001$), diabetes mellitus (55.3 % vs. 23.1 %, $p < 0.001$), hyperlipidemia (28.2 % vs. 15.2 %, $p = 0.005$), coronary artery disease (25.9 % vs. 15.8 %, $p = 0.027$), chronic kidney disease (24.7 % vs. 10.6 %, $p = 0.001$) and chronic viral hepatitis (27.1 % vs. 8.4 %, $p < 0.001$).

The patients with SARS-CoV-2 infection frequently reported cough, dyspnea, fever, and anorexia as presenting symptoms. The rates of dyspnea (52.9 % vs. 33.2 %, $p = 0.001$), fever (76.5 % vs. 51.9 %, $p < 0.001$), and muscle weakness (43.5 % vs. 28.3 %, $p = 0.006$) were higher in the TCZ group. The white blood cell count (8.0 $\pm$ 5.1 vs. 6.2 $\pm$ 2.7 $\times$ $10^3/\mu L$, $p = 0.002$), C-reactive protein level (9.2 $\pm$ 10.1 vs. 5.9 $\pm$ 6.6 mg/dL, $p = 0.04$), D-dimer level (3.5 $\pm$ 5.7 vs. 1.7 $\pm$ 4.0 μg/mL, $p = 0.043$), and erythrocyte sedimentation rate (38.3 $\pm$ 26.3 vs. 27.2 $\pm$ 24.3 mm/h, $p < 0.001$) were higher in the TCZ group than in the non-TCZ group, whereas the albumin level was lower in the TCZ group. The TCZ users were more likely to require invasive mechanical ventilator support (38.8 % vs. 5.4 %, $p < 0.001$) and a central venous catheter (36.5 % vs. 5.4 %, $p < 0.001$). Higher proportions of the TCZ users were treated with dexamethasone (97.6 % vs. 41.3 %, $p < 0.001$) and remdesivir (75.3 % vs. 24.2 %, $p < 0.001$).

Twelve patients (2.65 %) developed sBSI at a median of 14 (IQR = 11.3—20.0) days after admission, i.e., 19 (IQR = 12.0—27.0) days after SARS-CoV-2 PCR sampling. Among the 12 patients with sBSI, 6 (50 %) required mechanical ventilation and central venous catheterization. The patients with sBSI were hospitalized for longer duration (44.2 $\pm$ 31.4 vs. 17.6 $\pm$ 14.3 days, $p = 0.014$). Six patients had multiple episodes of positive blood cultures, and several strains were isolated simultaneously in six sBSI episodes. Among the 28 isolates, 14 (50.0 %), 7 (25.0 %), and 7 (25.0 %) were gram-negative bacteria (GNB), gram-positive cocci (GPC), and *Candida* species, respectively (Fig. 3). The most prevalent pathogens were *Elizabethkingia meningosepticum* (five isolates), *Enterococcus faecium* (three

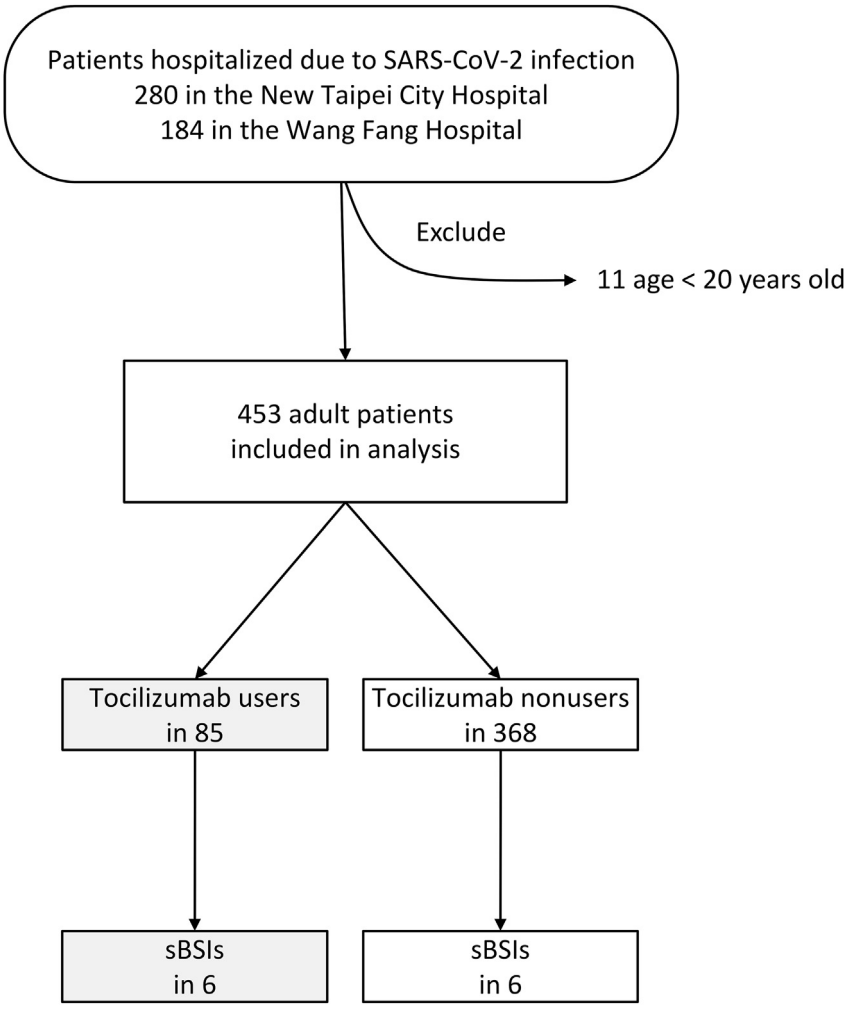

Figure 2. Flowchart for selection of patients with SARS-CoV-2 infection. (Abbreviations: sBSI, secondary bloodstream infection).

isolates), and *Klebsiella pneumoniae* (three isolates). Of the seven patients with isolates containing GPC, four (57.1 %) required a central venous catheter.

The Kaplan–Meier analysis revealed a nonsignificant difference in the risk of sBSI between the TCZ users and nonusers ($p = 0.052$ by log-rank test; Fig. 4). Both univariate and multivariate Cox proportional hazards models showed a nonsignificant association between TCZ use and sBSI risk (Table 2), with the HR (95 % CI) being 2.93 (0.94–9.14, $p = 0.064$) and the aHR (95 % CI) being 1.32 (0.29–6.05, $p = 0.724$). In the univariate Cox proportional hazards model, hyperlipidemia [HR = 3.29 (95 % CI = 1.04–10.40), $p = 0.042$], chronic kidney disease [HR = 3.24 (95 % CI = 1.02–10.30), $p = 0.046$], mechanical ventilation [HR = 4.96 (95 % CI = 1.56–15.76), p = 0.007] and central venous catheter use [HR = 3.68 (95 % CI = 1.13–11.97), $p = 0.030$] were associated with a higher risk of sBSI. In the multivariate Cox proportional hazards model, female sex [aHR = 7.00 (95 % CI = 1.45–33.92), $p = 0.016$], heavy drinking [aHR = 5.39 (95 % CI = 1.01–28.89), $p = 0.049$], and mechanical ventilation [aHR = 5.65 (95 % CI = 1.67–19.30), $p = 0.006$] were independently associated with a higher risk of sBSI. When TCZ use was retained in the multivariate model along with female sex, heavy drinking, and mechanical ventilation, we found that TCZ use and heavy drinking were both non-significant variables. The results of discrete survival models were similar and shown in the supplemental file (Table S1). In addition, among dexamethasone users, female sex [aHR = 14.63 (95 % CI = 1.58–135.63), $p = 0.018$], heavy drinking [aHR = 13.96 (95 % CI = 1.45–134.48), $p = 0.023$] were independently associated with a higher risk of sBSI in the multivariate Cox proportional hazards model.

Discussion

Although only 2.65 % of the patients analyzed in the present study developed sBSI, this complication was associated with prolonged hospitalization. Despite the higher prevalence of sBSI during hospitalization in the TCZ group, the survival analysis demonstrated that TCZ use was not related to the risk of sBSI. The independent predictors of sBSI were female sex, heavy drinking, and mechanical ventilation. The prevalence of sBSI among the TCZ users was higher than that among the non-TCZ users because TCZ tended to be prescribed to older patients with more severe initial clinical

Table 1 Clinical characteristics of hospitalized patients with SARS-CoV-2 infection, with stratification by tocilizumab use.

	Tocilizumab		*p-value*
	Users (N = 85)	Non-users (N = 368)	
Age, years			<0.001
< 65, n (%)	34 (40.0)	234 (63.6)	
≥ 65, n (%)	51 (60.0)	134 (36.4)	
Male sex, n (%)	56 (65.9)	189 (51.4)	0.015
Body mass index kg/m^2			0.098
< 18.5, n (%)	1 (1.2)	14 (3.8)	
18.5–24, n (%)	27 (31.8)	151 (41.0)	
≥ 24, n (%)	57 (67.1)	203 (55.2)	
Smoking status, n (%)	18 (21.2)	43 (11.7)	0.021
Heavy drinking, n (%)	12 (14.1)	28 (7.6)	0.057
Comorbidities			
Hypertension, n (%)	57 (67.1)	157 (42.7)	<0.001
Diabetes mellitus, n (%)	47 (55.3)	85 (23.1)	<0.001
Hyperlipidemia, n (%)	24 (28.2)	56 (15.2)	0.005
Coronary artery disease, n (%)	22 (25.9)	58 (15.8)	0.027
Chronic kidney disease, n (%)	21 (24.7)	39 (10.6)	0.001
Chronic viral hepatitis, n (%)	23 (27.1)	31 (8.4)	<0.001
Respiratory diseases, n (%)	7 (8.2)	17 (4.6)	0.180
Malignancy, n (%)	7 (8.2)	22 (6.0)	0.444
Maintenance hemodialysis, n (%)	4 (4.7)	6 (1.6)	0.098
Hospitalization course			
Length of hospitalization			
Mean ± SD, days	17.7 ± 13.1	14.4 ± 14.4	0.054
Median (IQR), days	15 (2)	11 (8)	0.002
Intensive care unit admission, n (%)	26 (30.6)	31 (8.4)	<0.001
Central line, n (%)	31 (36.5)	20 (5.4)	<0.001
Respiratory support, n (%)	79 (92.9)	199 (54.1)	<0.001
Nasal cannula, n (%)	63 (74.1)	182 (49.5)	<0.001
Simple mask, n (%)	51 (60.0)	51 (13.9)	<0.001
Nonrebreathing mask, n (%)	34 (40.0)	45 (12.2)	<0.001
HFNC, n (%)	16 (18.8)	21 (5.7)	<0.001
Mechanical ventilation, n (%)	33 (38.8)	20 (5.4)	<0.001
Medication use			
Dexamethasone, n (%)	83 (97.6)	152 (41.3)	<0.001
Remdesivir, n (%)	64 (75.3)	89 (24.2)	<0.001
Monoclonal antibody, n (%)	2 (2.4)	24 (6.5)	0.195
Casirivimab + Imdevimab	0 (0)	9 (2.4 %)	0.219
Bamlanivimab + Etesevimab	2 (2.4)	15 (4.1 %)	0.751
Colchicine, n (%)	13 (15.3)	31 (8.4)	0.054
NRICM101, n (%)	11 (12.9)	22 (6.0)	>0.999
Fluvoxamine, n (%)	6 (7.1)	12 (3.3)	0.106
Laboratory data			
Albumin, mean ± SD, g/L	3.3 ± 0.5	3.7 ± 0.7	<0.001
WBC count, mean ± SD, x $10^3/\mu L$	8.0 ± 5.1	6.2 ± 2.7	0.002
Platelet, mean ± SD, $10^3/\mu L$	200.6 ± 89.8	208.8 ± 87.5	0.496
D-dimer, mean ± SD, μg/mL	3.5 ± 5.7	1.7 ± 4.0	0.043
CRP, mean ± SD, mg/dL	9.2 ± 10.1	5.9 ± 6.6	0.011
Ferritin, mean ± SD, μg/L	1600 ± 1779	902 ± 2742	0.062
LDH, mean ± SD, U/L	372 ± 170	287 ± 833	0.361
CPK, mean ± SD, U/L	234.9 ± 336.5	180.8 ± 380.9	0.238
Fibrinogen, mean ± SD, mg/dL	454.8 ± 203.3	449.0 ± 181.0	0.867
Procalcitonin, mean ± SD, ng/mL	1.7 ± 6.3	1.6 ± 14.9	0.929
ESR, mean ± SD, mm/h	38.3 ± 26.3	27.2 ± 24.3	<0.001
Symptoms			
Cough, n (%)	64 (75.3)	243 (66.0)	0.1
Fever, n (%)	65 (76.5)	191 (51.9)	<0.001

(*continued on next page*)

Table 1 (*continued*)

	Tocilizumab		*p-value*
	Users (N = 85)	Non-users (N = 368)	
Dyspnea, n (%)	45 (52.9)	122 (33.2)	0.001
Muscle weakness, n (%)	37 (43.5)	104 (28.3)	0.006
Anorexia, n (%)	29 (34.1)	102 (27.7)	0.241
sBSI, n (%)	6 (7.1)	6 (1.6)	0.005
Days from SARS-CoV-2 test to the outcome, mean ± SD	23.7 ± 12.9	19.9 ± 12.2	0.01
Days from after admission to the outcome, mean ± SD	17.7 ± 13.1	14.4 ± 14.42	0.054
Mortality, n (%)	28 (32.9)	31 (8.4)	<0.001

Abbreviations: CPK, creatinine phosphokinase; CRP, C-reactive protein; ESR, erythrocyte sedimentation rate; HFNC, high-flow nasal cannula; LDH, lactate dehydrogenase; sBSI, secondary bloodstream infections; SD, standard deviation; WBC, white blood cell.

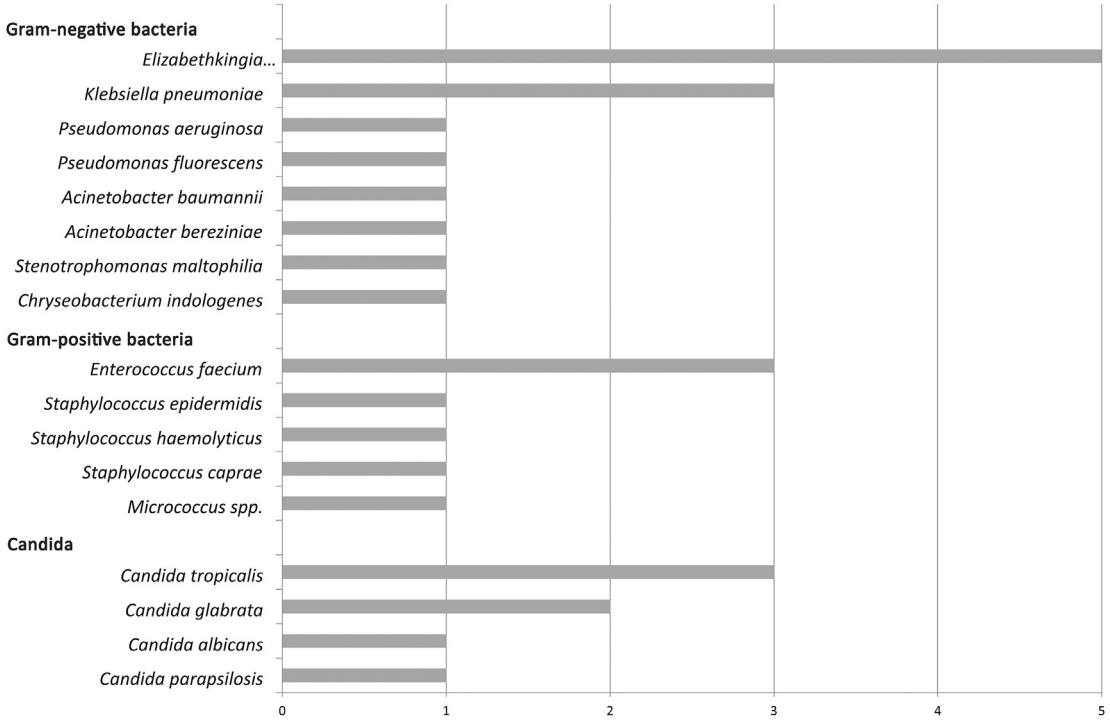

Figure 3. Identification of organisms from all blood cultures in hospitalized patients with SARS-CoV-2 infection and sBSI.

manifestations and who were more vulnerable to secondary bacterial infection.

TCZ, a humanized monoclonal antibody, competitively blocks the interaction of IL-6 with its receptor. IL-6 regulates the immune response, specifically the proliferation and differentiation of T cells and terminal differentiation of B cells. Selective inhibition of the host immune system may be a double-edged sword in the treatment of COVID-19-associated CRS. CRS depletes the host immune reserve, causes mucociliary dysfunction, and breaks the mucosal integrity of the lower respiratory and gastrointestinal tracts, predisposing patients to secondary bacterial and fungal infections.[30,31] By contrast, IL-6 is crucial to the protection of neutrophils from apoptosis in bacterial infection. Blockade of the downstream IL-6 pathway may render the host immune system prone to secondary bacterial infection[22,23]; Inconsistent results were obtained in one meta-analysis study.[32] Similar to the present study, in an observational study, Guaraldi et al. determined a higher rate of new infection among 179 patients with SARS-CoV-2 infection who were treated with TCZ compared with that among 365 patients treated with standard care only: 13 % and 4 %, respectively ($p < 0.0001$).[24] Such a difference was not observed in two randomized trials.[25,26] Several studies have also reported no association between TCZ use and secondary infection in patients with SARS-CoV-2 infection.[20,33–37] In a cohort study including 144 patients with SARS-CoV-2 infection who were admitted to an emergency intensive care unit, Kuwahara et al. demonstrated an association of TCZ treatment with bacteremia [odds ratio = 1.01 (95 % CI = 0.37–3.02), $p = 0.99$] by performing multivariate logistic regression.[37] Moore et al. indicated that TCZ use was not associated with health-care-associated infection [aHR = 0.85 (95 % CI = 0.29–2.52), $p = 0.780$) compared with nonuse.[36] Those results are consistent with our findings.

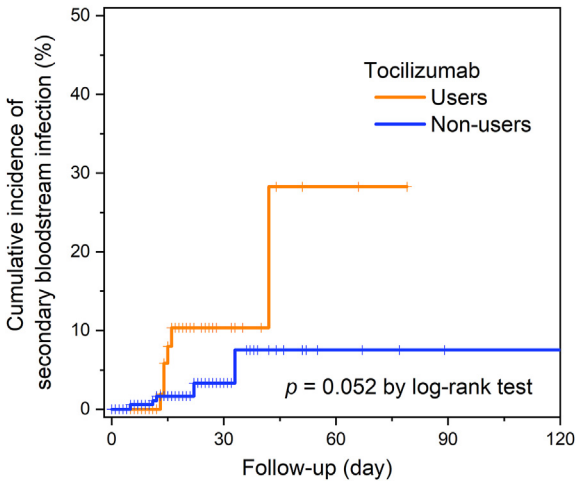

Figure 4. Kaplan–Meier curves depicting time to sBSI in the TCZ and non-TCZ groups.

The development of secondary infection in patients with severe SARS-CoV-2 infection can be attributed to several factors. CRS can induce immune anergy and epithelial disruption of the lower respiratory and gastrointestinal tracts, which facilitate the entry of bacteria and *Candida* into the bloodstream. Endothelial cell damage leads to vasculature leakage, causing bacterial translocation and failure of the intestinal barrier.[38] Administration of broad-spectrum antibiotics in a patient with clinical presentation of severe sepsis results in less diversity among microorganisms. It shifts the equilibrium of microbial flora, causing the expansion of multi-drug-resistant strains and *Candida* species.[39] Thus, in secondary infection, glucose fermentative enteric GNB or *Candida* species, commonly found as normal flora in the gastrointestinal tract, were cultured as pathogens of secondary bacterial infections in our study. Mechanical ventilation for the supportive care of critically ill patients also breaches the natural anatomical barrier between the oropharyngeal cavity and sterile lower respiratory tract, predisposing the patients to secondary bacterial infection. In our report, 11 out of 14 GNB identified were glucose-nonfermenting bacteria that are commonly present in the medical care environment and causative pathogens for nosocomial infection in health-care-associated or ventilator-associated pneumonia. Bhatt et al. reported an association between sBSI and invasive mechanical ventilation, which is consistent with our findings.[20] Regarding SARS-CoV-2 infection, a newly emerging and highly transmissible viral pulmonary disease, infections must be controlled by utilizing personal protective equipment to protect health-care workers. However, this protective equipment may interfere with the convenience, fluency, and integrity of medical care, increasing the risk of contamination during procedures that require aseptic

Table 2 Predictors of sBSI in the univariate and multivariate Cox proportional hazards model.

Analysis	Univariate analysis		Multivariate analysis 1		Multivariate analysis 2	
	HR (95 % CIs)	*p*	Adjusted HR (95 % CIs)	*p*	Adjusted HR (95 % CIs)	*p*
Tocilizumab use	2.93 (0.94–9.14)	0.064			1.32 (0.29–6.05)	0.724
Age, years		0.430				
< 65	Reference					
≥ 65	3.60 (0.43–29.90)					
Female sex	2.82 (0.83–9.60)	0.096	7.00 (1.45–33.92)	0.016	7.06 (1.45–34.44)	0.016
Smoking status	1.24 (0.27–5.69)	0.778				
Heavy drinking	3.24 (0.88–11.98)	0.078	5.39 (1.01–28.89)	0.049	5.09 (0.92–28.29)	0.063
Hypertension	2.52 (0.68–9.34)	0.168				
Diabetes mellitus	2.36 (0.76–7.35)	0.138				
Hyperlipidemia	3.29 (1.04–10.40)	0.042				
Coronary artery disease	0.99 (0.27–3.68)	0.984				
Chronic kidney disease	3.24 (1.02–10.30)	0.046				
Chronic viral hepatitis	1.58 (0.42–5.91)	0.494				
Mechanical ventilation	4.96 (1.56–15.76)	0.007	5.65 (1.67–19.30)	0.006	4.82 (1.06–22.02)	0.042
Intensive care unit admission	2.10 (0.62–7.11)	0.232				
Central line	3.68 (1.13–11.97)	0.030				
Remdesivir	1.34 (0.43–4.18)	0.612				
NRICM101	1.90 (0.42–8.72)	0.408				
Dexamethasone	3.82 (0.84–17.45)	0.084				
Colchicine	0.93 (0.12–7.19)	0.941				
Fluvoxamine	3.37 (0.43–26.65)	0.250				
Monoclonal antibody	0.97 (0.12–7.62)	0.977				

Abbreviations: CI, confidence interval; HR, hazard ratio.
Multivariate analysis 1 was the final result after backward stepwise analysis.
Multivariate analysis 2 included only tocilizumab use, female sex, heavy drinking, and mechanical ventilation.
The sample size of patients who had underlying respiratory disease and malignancy, and were undergoing hemodialysis was so small that accurate estimate of the odds ratios are not practical.

manipulation. A high proportion of cases of commensal skin flora, such as *Candida* species and GPC belonging to the Micrococcaceae family, could be due to the use of a central venous catheter.

This study found that the female patients with SARS-CoV-2 infection were more likely to develop sBSI than were the male patients. The sexual dimorphism in bacterial infection has been described as a susceptibility to gastrointestinal and respiratory bacterial infections in men and vulnerability to genitourinary tract bacterial infections in women.[40] Limited evidence has been obtained to support sex differences in the risk of bacterial infection among patients with SARS-CoV-2 infection. Cohen et al. reported that female patients had a lower risk of healthcare-associated bloodstream infection [odds ratio = 0.82 (95 % CI = 0.74—0.91)] in a large academic medical center in New York.[41] Studies have previously indicated no significant difference in the risk of sBSI between men and women.[20,21] Our results can provide a new direction for research concerning the complex interactions of genetic and hormonal differences with the host immune system and the subsequent risk of bacterial infection in hospitalized patients with SARS-CoV-2 infection. Heavy drinking could contribute to a patient being immunocompromised and having increased susceptibility to infection.[42] Our results confirm the association between heavy drinking and sBSI in hospitalized patients with SARS-CoV-2 infection.

This study has some limitations. First, confounding factors are unavoidable in a retrospective cohort study. However, adjustment was performed by performing a multivariable Cox proportional hazards model. Second, variables that were unbalanced between the two groups could have resulted in confounding by indications; however, a multivariable Cox proportional hazards model was performed. Third, the study enrolled an Asian cohort, which could limit its extrapolation. Finally, larger studies are needed to confirm this result due to a small sample size and a limited number of patients developing sBSI in the present study.

Conclusion

The hospital stay of patients with SARS-CoV-2 infection is significantly increased if they develop sBSI. The risk of secondary infection is complex and multifactorial. In practice, TCZ is prescribed to older patients with multiple comorbidities and severe clinical conditions. TCZ treatment does not increase the risk of sBSI in this vulnerable population. The causative microbes identified in this study were mainly healthcare-associated pathogens, especially those found in the airway, on the skin, in the intestines, and in intensive care unit environments. The present microbiology data provide clinicians with a direction for antibiotic selection. Additional studies are warranted to optimize infection control and environmental cleaning strategies during the SARS-CoV-2 infection pandemic era.

Funding

This study was not funded by any institutions.

Author contributions

D.E.L. and T.Y.O. wrote the first draft of the manuscript. D.E.L., J.W.K., and I.J.C. reviewed and collected the data on patients. T.Y.O. and M.C.L. performed the statistical analysis. M.C.L. and C. H. L. critically revised the manuscript. All authors contributed to the final version of the manuscript.

Declaration of competing interest

All authors declare that no competing interests exist.

Acknowledgments

This manuscript was edited by Wallace Academic Editing.

References

1. *WHO coronavirus (COVID-19) dashboard* [cited 2023 4 September]; Available from: https://covid19.who.int/; 2023.
2. Wiersinga WJ, Rhodes A, Cheng AC, Peacock, SJ, Prescott HC. Pathophysiology, transmission, diagnosis, and treatment of coronavirus disease 2019 (COVID-19): a review. *JAMA* 2020; **324**:782—93.
3. Britton A, Fleming-Dutra KE, Shang N, Smith ZR, Dorji T, Derado G, et al. Association of COVID-19 vaccination with symptomatic SARS-CoV-2 infection by time since vaccination and delta variant predominance. *JAMA* 2022;**327**:1032—41.
4. Rosenberg ES, Holtgrave DR, Dorabawila V, Conroy M, Greene D, Lutterloh E, et al. New COVID-19 cases and hospitalizations among adults, by vaccination status - New York, may 3-july 25, 2021. *MMWR Morb Mortal Wkly Rep* 2021;**70**:1150—5.
5. Maslo C, Friedland R, Toubkin M, Laubscher A, Akaloo T, Kama B. Characteristics and outcomes of hospitalized patients in South Africa during the COVID-19 Omicron wave compared with previous waves. *JAMA* 2022;**327**:583—4.
6. Mehta P, Mcauley DF, Brown M, Sanchez E, Tattersall RS, Manson JJ. COVID-19: consider cytokine storm syndromes and immunosuppression. *Lancet* 2020;**395**:1033—4.
7. Huang C, Wang Y, Li X, Ren L, Zhao J, Hu Y, et al. Clinical features of patients infected with 2019 novel coronavirus in Wuhan, China. *Lancet* 2020;**395**:497—506.
8. Fara A, Mitrev Z, Rosalia RA, Assas BM. Cytokine storm and COVID-19: a chronicle of pro-inflammatory cytokines. *Open Bio* 2020;**10**:200160.
9. Xing Z, Gauldie J, Cox G, Baumann H, Jordana M, Lei XF, et al. IL-6 is an antiinflammatory cytokine required for controlling local or systemic acute inflammatory responses. *J Clin Invest* 1998;**101**:311—20.
10. Chen X, Zhao B, Qu Y, Chen Y, Xiong J, Feng Y, et al. Detectable serum severe acute respiratory syndrome coronavirus 2 viral load (RNAemia) is closely correlated with drastically elevated interleukin 6 level in critically ill patients with coronavirus disease 2019. *Clin Infect Dis* 2020;**71**:1937—42.
11. Sjoding MW, Admon AJ, Saha AK, Kay SG, Brown CA, Co I, et al. Comparing clinical features and outcomes in mechanically ventilated patients with COVID-19 and acute respiratory distress syndrome. *Ann Am Thorac Soc* 2021;**18**:1876—85.
12. Grasselli G, Greco M, Zanella A, Albano G, Antonelli M, Bellani G, et al. Risk factors associated with mortality among patients with COVID-19 in intensive care units in lombardy, Italy. *JAMA Intern Med* 2020;**180**:1345—55.

13. Haraoui B, Casado G, Czirják L, Taylor A, Bernasconi C, Reiss W, et al. Patterns of tocilizumab use, effectiveness and safety in patients with rheumatoid arthritis: core data results from a set of multinational observational studies. *Clin Exp Rheumatol* 2017;**35**:899—906.
14. Mcgonagle D, Sharif K, O'regan A, Bridgewood C. The role of cytokines including interleukin-6 in COVID-19 induced pneumonia and macrophage activation syndrome-like disease. *Autoimmun Rev* 2020;**19**:102537.
15. Rose-John S, Winthrop, K, Calabrese L. The role of IL-6 in host defence against infections: immunobiology and clinical implications. *Nat Rev Rheumatol* 2017;**13**:399—409.
16. Pelaia C, Tinello C, Vatrella A, De Sarro G, Pelaia G. Lung under attack by COVID-19-induced cytokine storm: pathogenic mechanisms and therapeutic implications. *Ther Adv Respir Dis* 2020;**14**:1753466620933508.
17. Narazaki M, Kishimoto T. The two-faced cytokine IL-6 in host defense and diseases. *Int J Mol Sci* 2018;**19**:3528.
18. Gordon AC, Mouncey PR, Al-Beidh F, Rowan KM, Nichol AD, Arabi YM, et al. Interleukin-6 receptor antagonists in critically ill patients with covid-19. *N Engl J Med* 2021;**384**:1491—502.
19. *Infectious diseases society of America guidelines on the treatment and management of patients with COVID-19*. https://www.idsociety.org/practice-guideline/covid-19-guideline-treatment-and-management/. [Accessed 24 May 2021].
20. Bhatt PJ, Shiau S, Brunetti L, Xie Y, Solanki K, Khalid S, et al. Risk factors and outcomes of hospitalized patients with severe coronavirus disease 2019 (COVID-19) and secondary bloodstream infections: a multicenter case-control study. *Clin Infect Dis* 2021;**72**:e995—1003.
21. Khatri A, Malhotra P, Izard S, Kim A, Oppenheim M, Gautam-Goyal P, et al. Hospital-acquired bloodstream infections in patients hospitalized with severe acute respiratory syndrome coronavirus 2 infection (coronavirus disease 2019): association with immunosuppressive therapies. *Open Forum Infect Dis* 2021;**8**:ofab339.
22. Fielding CA, Mcloughlin RM, Mcleod L, Colmont CS, Najdovska M, Grail D, et al. IL-6 regulates neutrophil trafficking during acute inflammation via STAT3. *J Immunol* 2008;**181**:2189—95.
23. Asensi V, Valle E, Meana A, Fierer J, Celada A, Alvarez V, et al. In vivo interleukin-6 protects neutrophils from apoptosis in osteomyelitis. *Infect Immun* 2004;**72**:3823—8.
24. Guaraldi G, Meschiari M, Cozzi-Lepri A, Milic J, Tonelli R, Menozzi M, et al. Tocilizumab in patients with severe COVID-19: a retrospective cohort study. *Lancet Rheumatol* 2020;**2**:e474—84.
25. Hermine O, Mariette X, Tharaux PL, Resche-Rigon M, Porcher R, Ravaud P. Effect of tocilizumab vs usual care in adults hospitalized with COVID-19 and moderate or severe pneumonia: a randomized clinical trial. *JAMA Intern Med* 2021;**181**:32—40.
26. Stone JH, Frigault MJ, Serling-Boyd NJ, Fernandes AD, Harvey L, Foulkes AS, et al. Efficacy of tocilizumab in patients hospitalized with covid-19. *N Engl J Med* 2020;**383**:2333—44.
27. *Interim guidelines for clinical management of SARS-CoV-2, 24th edition* [cited 2023 4 September]; Available from: https://www.cdc.gov.tw/File/Get/Tkmk1mDdnEWdDyjMplfrSQ.
28. *Drinking levels defined* [cited 2022 28 June]; Available from: https://www.niaaa.nih.gov/alcohol-health/overview-alcohol-consumption/moderate-binge-drinking; 2022.
29. *Actemra (tocilizumab) [prescribing information]*. South San Francisco, CA: Genentech Inc; 2022.
30. Langford BJ, So M, Raybardhan S, Leung V, Westwood D, Macfadden DR, et al. Bacterial co-infection and secondary infection in patients with COVID-19: a living rapid review and meta-analysis. *Clin Microbiol Infect* 2020;**26**:1622—9.
31. Shah VK, Firmal P, Alam A, Ganguly D, Chattopadhyay S. Overview of immune response during SARS-CoV-2 infection: lessons from the past. *Front Immunol* 2020;**11**:1949.
32. Tleyjeh IM, Kashour Z, Damlaj M, Riaz M, Tlayjeh H, Altannir M, et al. Efficacy and safety of tocilizumab in COVID-19 patients: a living systematic review and meta-analysis. *Clin Microbiol Infect* 2021;**27**:215—27.
33. Ignatius EH, Wang K, Karaba A, Robinson M, Avery RK, Blair P, et al. Tocilizumab for the treatment of COVID-19 among hospitalized patients: a matched retrospective cohort analysis. *Open Forum Infect Dis* 2021;**8**:ofaa598.
34. Omrani AS, Koleri J, Ben Abid F, Daghfel J, Odaippurath T, Peediyakkal MZ, et al. Clinical characteristics and risk factors for COVID-19-associated Candidemia. *Med Mycol* 2021;**59**:1262—6.
35. Taramasso L, Magnasco L, Portunato F, Briano F, Vena A, Giacobbe DR, et al. Clinical presentation of secondary infectious complications in COVID-19 patients in intensive care unit treated with tocilizumab or standard of care. *Eur J Intern Med* 2021;**94**:39—44.
36. Moore JL, Stroever SJ, Rondain PE, Scatena RN. Incidence of secondary bacterial infections following utilization of tocilizumab for the treatment of COVID-19 - a matched retrospective cohort study. *J Global Infect Dis* 2021;**13**:67—71.
37. Kuwahara M, Kamigaito M, Nitta S, Hasegawa K, Murakami H, Kobayashi T, et al. Effect of tocilizumab treatment on patients with coronavirus disease 2019 and bacteremia: a retrospective cohort study. *Infect Dis Ther* 2022;**11**:533—41.
38. Cardinale V, Capurso G, Ianiro G, Gasbarrini A, Arcidiacono PG, Alvaro D. Intestinal permeability changes with bacterial translocation as key events modulating systemic host immune response to SARS-CoV-2: a working hypothesis. *Dig Liver Dis* 2020;**52**:1383—9.
39. Ong JY, Wang CH, Tsai YS, Chen FL, Lee CH, Ou TY. Nosocomial septicemia in COVID-19 nosocomial K. pneumoniae, A. baumannii, and Elizabethkingia meningoseptica septicemia in a patient of COVID-19. *J Infect* 2022;**85**:90—122.
40. Vázquez-Martínez ER, García-Gómez E, Camacho-Arroyo I, González-Pedrajo B. Sexual dimorphism in bacterial infections. *Biol Sex Differ* 2018;**9**:27.
41. Cohen B, Choi YJ, Hyman S, Furuya EY, Neidell M, Larson E. Gender differences in risk of bloodstream and surgical site infections. *J Gen Intern Med* 2013;**28**:1318—25.
42. Girard DE, Kumar KL, McAfee JH. Hematologic effects of acute and chronic alcohol abuse. *Hematol Oncol Clin N Am* 1987;**1**:321—34.

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.jmii.2023.10.011.