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Systematic review

Efficacy and safety of tocilizumab in COVID-19 patients: a living systematic review and meta-analysis

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ABSTRACT

Objectives: Cytokine release syndrome with elevated interleukin-6 (IL-6) levels is associated with multiorgan damage and death in severe coronavirus disease 2019 (COVID-19). Our objective was to perform a living systematic review of the literature concerning the efficacy and toxicity of the IL-6 receptor antagonist tocilizumab in COVID-19 patients.
Methods: Data sources were Ovid MEDLINE(R) and Epub Ahead of Print, In-Process & Other Non-Indexed Citations and Daily, Ovid Embase, Ovid Cochrane Central Register of Controlled Trials, Ovid Cochrane Database of Systematic Reviews, Web of Science, Scopus up, preprint servers and Google up to October 8, 2020. Study eligibility criteria were randomized controlled trials (RCTs) and observational studies at low or moderate risk of bias. Participants were hospitalized COVID-19 patients. Interventions included tocilizumab versus placebo or standard of care. We pooled crude risk ratios (RRs) of RCTs and adjusted RRs from cohorts, separately. We evaluated inconsistency between studies with I^2. We assessed the certainty of evidence using the GRADE approach.
Results: Of 1156 citations, 24 studies were eligible (five RCTs and 19 cohorts). Five RCTs at low risk of bias, with 1325 patients, examined the effect of tocilizumab on short-term mortality; pooled RR was 1.09 (95% CI 0.80—1.49, $I^2 = 0\%$). Four RCTs with 771 patients examined the effect of tocilizumab on risk of mechanical ventilation; pooled RR was 0.71 (95%CI 0.52—0.96, $I^2 = 0\%$), with a corresponding number needed to treat of 17 (95%CI 9—100). Among 18 cohorts at moderate risk of bias with 9850 patients, the pooled adjusted RR for mortality was 0.58 (95%CI 0.51—0.66, $I^2 = 2.5\%$). This association was observed over all degrees of COVID-19 severity. Data from the RCTs did not show a higher risk of infections or adverse events with tocilizumab: pooled RR 0.63 (95%CI 0.38—1.06, five RCTs) and 0.83 (95%CI 0.55—1.24, five RCTs), respectively.
Conclusions: Cumulative moderate-certainty evidence shows that tocilizumab reduces the risk of mechanical ventilation in hospitalized COVID-19 patients. While RCTs showed that tocilizumab did not reduce short-term mortality, low-certainty evidence from cohort studies suggests an association between tocilizumab and lower mortality. We did not observe a higher risk of infections or adverse events

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with tocilizumab use. This review will continuously evaluate the role of tocilizumab in COVID-19 treatment. **Imad M. Tleyjeh, Clin Microbiol Infect 2021;27:215**

Introduction

Since its emergence in December 2019, the severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) has infected millions of people across the globe and claimed hundreds of thousands of human lives, as well as negatively impacting the economy of numerous countries. Although the majority of SAR-CoV-2-infected patients who develop coronavirus disease 2019 (COVID-19) manifest only mild symptoms, about 14% of patients develop severe symptoms and 5% develop critical disease defined by respiratory failure, shock and/or multiorgan failure [1].

The innate and adaptive immune responses react appropriately in the majority of infected people and lead to control of the infection with no significant damage to the host tissues. Macrophages are activated by damage-associated molecular patterns (DAMPs) from damaged cells (such as heat-shock proteins and hyaluronan fragments) and liberated pathogen-associated molecular patterns (PAMPS) such as viral RNA. These molecules activate toll-like receptors (TLRs) and NLRP3 inflammasome. Cytosolic DNA also triggers cGAS-STING and RIG-I-MAVS pathways. These responses lead to activation of antiviral immune responses (such as INF–I and –III response and production of other cytokines) that result in the amplification of the innate response and activation of adaptive immunity, leading to viral clearance and tissue repair [2,3].

Patients with severe COVID-19 disease manifest immune system dysregulation, which is believed to be triggered by a particular mode of programmed cell death called pyroptosis. This form of cell death induces several proinflammatory cytokines and chemokines—such as IL-1β, IL-2, IL-6, tumour necrosis factor α (TNF-α), and monocyte chemoattractant protein 1 (MCP1)—and lymphopenia with attrition of both CD4+ and CD8+ T cells and natural killer T cells [3–5]. IL-6 and IL-1® production promote neutrophil and cytotoxic T cell recruitment to the affected tissues, both of which contribute to tissue damage resulting in acute lung injury through production of oxygen free radicals and inflammatory mediators such as leukotrienes [2]. Indeed, elevated blood levels of IL-6 have been shown to correlate with COVID-19 disease severity and SARS-CoV-2 RNA blood levels in COVID-19 patients, and is also associated with a worse prognosis [6].

It is hypothesized that the elevation of cytokines in COVID-19 diseases is similar to other cytokine release syndromes (CRSs) observed with diseases such as haemophagocytic lymphohistiocytosis (HLH), macrophage activation syndrome (MAS) and chimeric antigen receptor T-cell therapy (CAR-T), and that this cytokine storm is responsible for the multiorgan damage observed in patients with severe COVID-19 [2]. A number of immunomodulatory therapies targeting these cytokines have recently garnered interest and have been tested in COVID-19 [7–15]. Among these, IL-6 receptor blockade with the humanized monoclonal antibody tocilizumab is used routinely as a disease-modifying agent in the treatment of rheumatoid arthritis [16], and has been shown to be effective in the treatment of CRS associated with CAR-T therapy [17]. These observations formed the basis for targeting IL-6 as a therapeutic approach for severe COVID-19 disease [18,19].

Several studies addressing the role of tocilizumab were subsequently published with variable results. Two recent systematic reviews [20,21] pooled crude unadjusted data from cohort studies and concluded that tocilizumab is associated with better outcomes in COVID-19. However, their conclusions are flawed by the confounding effect inherent in crude data from observational data. Moreover, the two reviews did not address the adverse events and did not evaluate the certainty of cumulative evidence. Therefore, we sought to perform a living systematic review of randomized trials and observational studies addressing the efficacy and safety of tocilizumab in the treatment of COVID-19 patients.

Methods

We followed the Preferred Reporting Items for Systematic Reviews and Meta-analyses (PRISMA) guidelines for reporting systematic reviews [22]. Our review is a living systematic review. A living systematic review is a cumulative synthesis that is updated regularly as new evidence becomes available [23].

Inclusion criteria

We included in separate analyses (a) RCTs or (b) cohort or case–control studies (at low or moderate risk of bias) reporting on the adjusted effect estimates (derived for example from conventional regression models or propensity score matching or analysis) of the association between tocilizumab use in COVID-19 patients and one of the following a-priori outcomes: in-hospital mortality, need for mechanical ventilation, need for ICU admission, composite outcomes if reported, adverse events (data from RCTs only), and infections (data from RCTs only).

Data sources and search strategies

A comprehensive search of several databases from 2019 to 8th October 2020, excluding animal studies, was conducted. The databases included Ovid MEDLINE(R) and Epub Ahead of Print, In-Process & Other Non-Indexed Citations and Daily, Ovid Embase, Ovid Cochrane Central Register of Controlled Trials, Ovid Cochrane Database of Systematic Reviews, Web of Science, and Scopus. The search strategy is available in the Appendix (Supplementary Material). We also searched for unpublished manuscripts using the medRxiv services and Researchsquare.com, Google, and the references of eligible studies and review articles.

Data extraction

Eight reviewers (IT, HT, MA, MA, MD, YA, TK and ZAK) in groups of two independently identified eligible studies and extracted the data into a prespecified data collection form in duplicate. Any discrepancies were resolved by two senior reviewers (IT and TK).

Living systematic review

In this living systematic review, two independent investigators (IT and ZK) receive an updated literature search file every 3 months and continuously include relevant newly published or unpublished studies as per above inclusion criteria. The relevant meta-analyses will be continuously updated, and if new evidence becomes available (judged by the author group in coordination with the Journal Editor in Chief), the results will be submitted for publication at regular intervals.

Quality assessment

Five reviewers (IT, HT, MA, TK and ZK) independently and in duplicate assessed the risk of bias for each study using the RoB 2 of the Cochrane risk-of-bias tool for randomized trials and the ROBINS-I ('Risk Of Bias In Non-randomized Studies of Interventions') for observational studies [24]. We also assessed all included cohort studies for risk of survivor bias (or immortal time bias). We considered the following analytical approaches as acceptable tools to account for survivor bias [25−27]: (a) tocilizumab use as a time-dependent variable in the regression analysis, (b) landmark analysis, (c) structural nested accelerated failure time model, and (d) marginal structure models. Reviewers judged each criterion for risk of bias and resolved any disagreements with a senior reviewer (IT). Finally, two reviewers (HT and IT) assessed the certainty of evidence for each of our outcomes using the GRADE (Grading of Recommendations Assessment, Development, and Evaluation) approach [28].

Statistical analysis

We evaluated heterogeneity between studies using the I^2 statistic [29]. We pooled crude data from RCTs using the Mantel−Haenszel methods using fixed effect model [30]. Due to substantial heterogeneity in cohort studies, we pooled the adjusted effect estimates of the included studies using the DerSimonian−Laird random-effects model and constructed corresponding forest plots [31]. Prior to pooling, the ORs were converted to RRs using the method of Zhang and Yu [32]. For the main analyses, we included only the studies at low or moderate risk of bias.

We conducted a priori determined subgroup analyses to assess the impact of COVID-19 disease severity on response to tocilizumab therapy. We also conducted meta-regression using a priori chosen study level baseline characteristics of patients' populations to explore causes of heterogeneity of treatment effects across studies. These variables included characteristics of study population (age, sex, comorbidities), dosing regimen and number of tocilizumab doses, adjustment for survivor bias, and levels of ferritin, interleukin 6 (IL-6), C-reactive protein (CRP) and lactate dehydrogenase (LDH). Other considered variables were not included because of missing data. Finally, we constructed contour-enhanced funnel plots and performed an Egger precision-weighted linear regression test as a statistical test of funnel plot asymmetry and publication bias [33]. All analyses of cohort studies were conducted using Stata version 16 statistical software (StataCorp, College Station, Texas, USA) and those of RCTs using Review Manager, Version 5.4, The Cochrane Collaboration.

Results

Efficacy

Of 1156 citations, 24 studies (five RCTs and 19 cohorts) (Supplementary Material Tables S1 and S2) [7,34−36,38−58] with 1325 patients (RCTs) and 10 021 patients (cohorts), including single-centre and multicentre studies from different countries, were included in our systematic review. Two studies [7,59] reported on the same cohort, and we included data from the report that focused on tocilizumab use [7]. To date, five RCTs (Supplementary Material Table S1) have been completed but only four have been published [47,54,56,57]. Preliminary data from another RCT (EMPACTA) [58] was identified on Google news as a press release and cross-identified on clinicaltrials.gov to identify its objectives and details (NCT04372186). Fig. 1 shows the result of our search strategy (PRISMA flow diagram). Supplementary Material Tables S1 and S2 (Tables 1 and 2) illustrate the general characteristics of the included studies. All studies reported on patients hospitalized with COVID-19 with varying degrees of disease severity.

Four RCTs were at low risk of bias as per ROB 2 scale (Fig. 2A). We could not assess the risk of bias in the EMPACTA trial even though it was a double-blind placebo-controlled trial. The quality of the observational studies was assessed using the ROBINS-I tool (Supplementary Material Fig. S1). Among included cohort studies, survivor bias was addressed in the analysis in four studies only [36,38,39,55] (Supplementary Material Table S2).

Randomized controlled trials

Five RCTs at low risk of bias, with 1325 patients, examined the effect of tocilizumab on short-term mortality; pooled risk ratio (RR) was 1.09 (95%CI 0.80−1.49, $I^2 = 0\%$) (Fig. 2A). Four RCTs with 771 patients examined the effect of tocilizumab on risk of mechanical ventilation; pooled RR was 0.71 (95%CI 0.52−0.96, $I^2 = 0\%$) (Fig. 2B), with a corresponding number needed to treat of 17 (95%CI 9−100). Five RCTs at low risk of bias, with 1325 patients, examined the effect of tocilizumab on a composite of poor outcome; pooled RR was 0.71 (95%CI 0.56−0.89, $I^2 = 0\%$) (Fig. 2C). The definition of this composite outcome in each trial is summarized in Supplementary Material Table S1.

Data from the RCTs did not show higher risk of infections (Fig. 3A) or adverse events (Fig. 3B) with tocilizumab; pooled RR 0.63 (95%CI 0.38−1.06, five RCTs) and 0.83 (95%CI 0.55−1.24, five RCTs), respectively.

Cohort studies

Data from 18 out of the 19 identified cohorts were used for this analysis. The study by Garcia et al. [60] did not report on mortality as an individual endpoint and therefore was not included in the quantitative analysis. Among 18 cohorts at moderate risk of bias, with 9850 patients, the pooled adjusted RR for mortality was 0.58 (95%CI 0.51−0.66, $I^2 = 2.5\%$) (Supplementary Material Fig. S2). This association was observed across all stages of disease severity (Fig. 4). Multivariate meta-regression analyses of the variables listed in the methods section did not identify any study level variable associated with RR of mortality. Contour-enhanced funnel plot and Egger's test for small-study effects (p 0.120) did not show evidence of publication bias (Supplementary Material Fig. S3). Only three studies reported on other outcomes (Supplementary Material Figs S4 and S5).

All included cohort studies were at moderate risk of bias (Supplementary Material Fig. S1). Survivor bias was addressed in the analysis in four studies [36,38,39,55] only (Supplementary Material Table S2). To study the potential effect of survivor bias on the observed results, we performed a post-hoc analysis where we assumed that cohort studies which did not adjust for this bias as per above approaches reported over-estimated effect estimates. Based on data from a COVID-19 study [61] and another influenza study [62], relative risk for mortality increased by 40−60% when treatment (such as steroids or oseltamivir) was considered as a time-dependent variable in Cox regression analysis [61]. We therefore multiplied the RR or HR of studies that did not adjust for survivor bias and their corresponding 95%CIs by 1.6 and pooled the new adjusted effect estimates. The corrected pooled adjusted RR for mortality from the 18 cohorts was 0.77 (95%CI 0.63−0.95, $I^2 = 41\%$) (Supplementary Material Fig. S6).
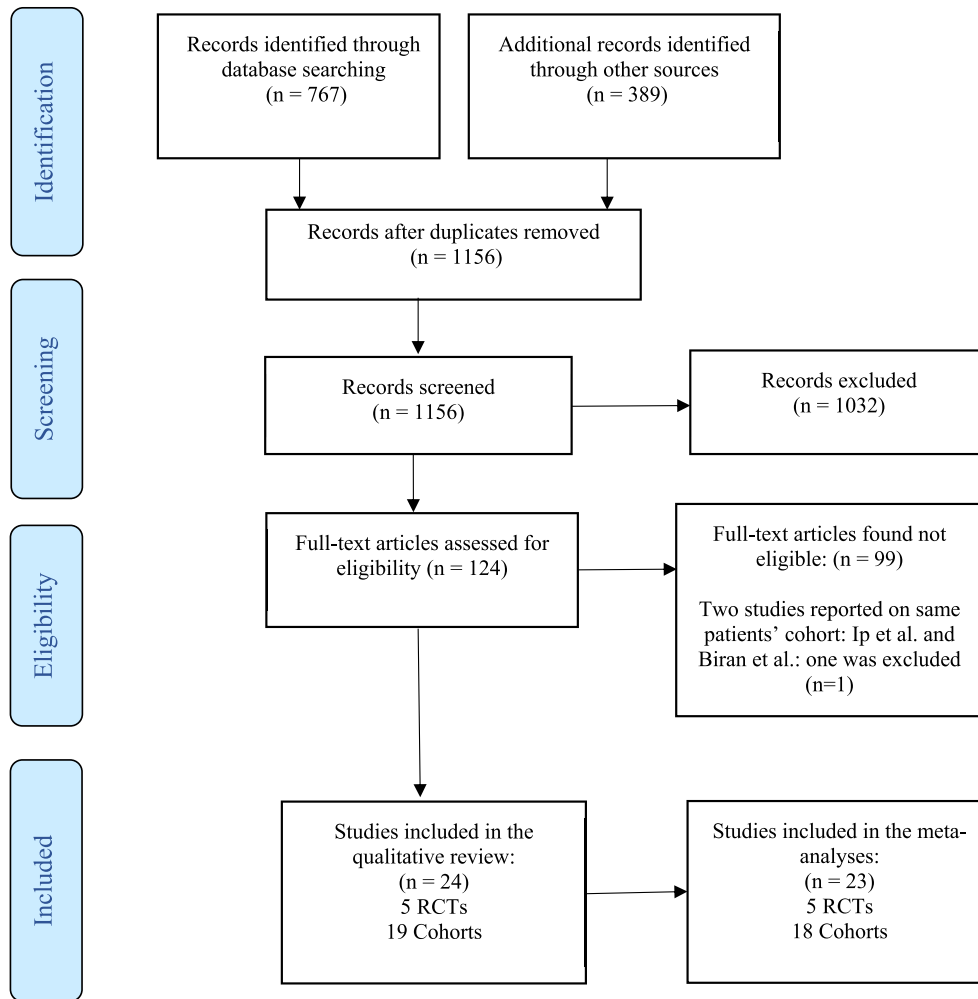
Fig. 1. Flow diagram of the assessment of studies identified in the systematic review.

GRADE of evidence

To facilitate interpretation and grading of the results, we calculated the absolute effect for mortality and other outcomes for both RCTs and cohort studies. Data from RCTs, at low risk of bias, showed that in hospitalized COVID-19 patients tocilizumab reduces the risk of mechanical ventilation with a corresponding number needed to treat of 17 (95%CI 9—100). Because of the imprecision of the 95%CI for the number needed to treat, this evidence was downgraded to moderate-certainty evidence. Although RCTs did not show that tocilizumab has an effect on mortality, the pooled RR of 1.09 (95%CI 0.80—1.49) was imprecise, with a wide 95%CI, suggesting that more studies may be needed for a definitive answer. In the five identified RCTs, the risk of mortality in the control group was 57/553 (10.3%). The sample size required for an RCT to detect an RR of 0.73 for mortality with tocilizumab (with 80% power and α 0.05) is 4506 patients (2253 in each arm). The total number of patients in the five RCTs is 772 patients in the tocilizumab group and 553 patients in the control group.

For the cohort studies, we used the baseline risk for mortality (27.3%) from the International Severe Acute Respiratory and Emerging Infection COVID-19 database [63]. The absolute risk difference in mortality was −11.5% (95%CI −13.4% to −9.3%) with a number needed to treat to prevent one death of 9 (95%CI 7−11). The overall quality of evidence (classified as low in observational studies) remained low given the moderate risk of study bias, low

risk of publication bias, direct evidence, low inconsistency, and precise estimate.

Discussion

Main findings: efficacy

In this living systematic review and meta-analysis that included five RCTs and 18 cohorts as of October 2020, cumulative moderate certainty evidence shows that tocilizumab reduces the risk of mechanical ventilation in hospitalized COVID-19 patients. While RCTs showed that tocilizumab did not reduce short-term mortality, low-certainty evidence from cohort studies suggests an association between tocilizumab and lower mortality.

Although the effect of tocilizumab on mortality from RCTs are discordant with the results of observational studies, there are many possible explanations beyond study design and residual confounding [7,64]. These include different patient populations, inflammatory status, and timing of administration and dosing of tocilizumab. Moreover, the RCTs did not consider mortality as an individual primary endpoint, and therefore they were not powered enough to detect difference in mortality between tocilizumab and control groups as suggested by the wide 95%CI of their pooled RR 1.09 (95%CI 0.80−1.49). The sample size required for an RCT to detect an RR of 0.73 for mortality (similar RR to the cohort studies adjusted for survivor bias) is 4506 patients (2253 in each arm). The
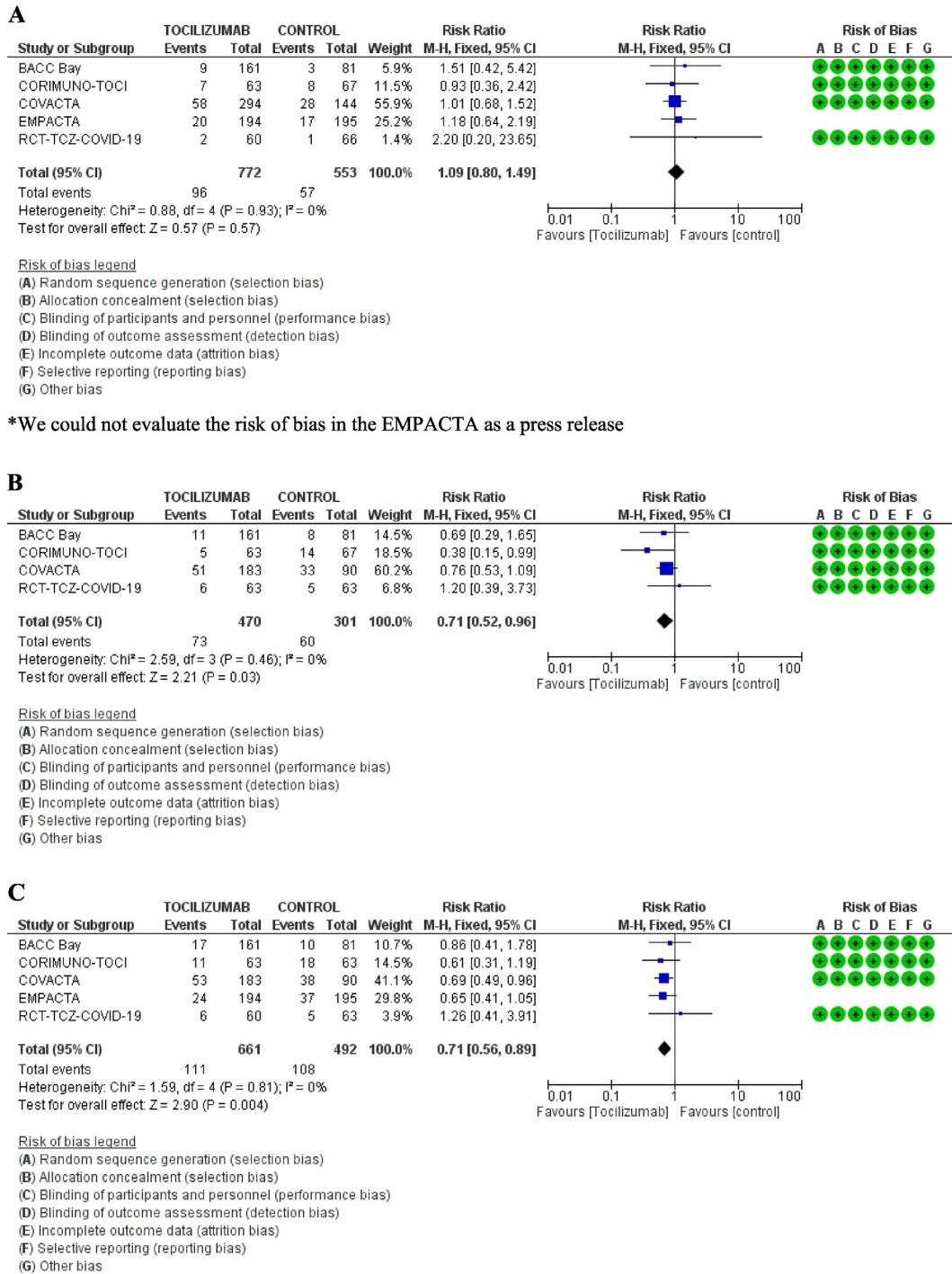
A

Study or Subgroup	TOCILIZUMAB Events	Total	CONTROL Events	Total	Weight	Risk Ratio M-H, Fixed, 95% CI
BACC Bay	9	161	3	81	5.9%	1.51 [0.42, 5.42]
CORIMUNO-TOCI	7	63	8	67	11.5%	0.93 [0.36, 2.42]
COVACTA	58	294	28	144	55.9%	1.01 [0.68, 1.52]
EMPACTA	20	194	17	195	25.2%	1.18 [0.64, 2.19]
RCT-TCZ-COVID-19	2	60	1	66	1.4%	2.20 [0.20, 23.65]
Total (95% CI)		**772**		**553**	**100.0%**	**1.09 [0.80, 1.49]**
Total events	96		57			

Heterogeneity: Chi² = 0.88, df = 4 (P = 0.93); I² = 0%
Test for overall effect: Z = 0.57 (P = 0.57)

Favours [Tocilizumab]   Favours [control]

Risk of bias legend
(A) Random sequence generation (selection bias)
(B) Allocation concealment (selection bias)
(C) Blinding of participants and personnel (performance bias)
(D) Blinding of outcome assessment (detection bias)
(E) Incomplete outcome data (attrition bias)
(F) Selective reporting (reporting bias)
(G) Other bias

*We could not evaluate the risk of bias in the EMPACTA as a press release

B

Study or Subgroup	TOCILIZUMAB Events	Total	CONTROL Events	Total	Weight	Risk Ratio M-H, Fixed, 95% CI
BACC Bay	11	161	8	81	14.5%	0.69 [0.29, 1.65]
CORIMUNO-TOCI	5	63	14	67	18.5%	0.38 [0.15, 0.99]
COVACTA	51	183	33	90	60.2%	0.76 [0.53, 1.09]
RCT-TCZ-COVID-19	6	63	5	63	6.8%	1.20 [0.39, 3.73]
Total (95% CI)		**470**		**301**	**100.0%**	**0.71 [0.52, 0.96]**
Total events	73		60			

Heterogeneity: Chi² = 2.59, df = 3 (P = 0.46); I² = 0%
Test for overall effect: Z = 2.21 (P = 0.03)

Favours [Tocilizumab]   Favours [control]

Risk of bias legend
(A) Random sequence generation (selection bias)
(B) Allocation concealment (selection bias)
(C) Blinding of participants and personnel (performance bias)
(D) Blinding of outcome assessment (detection bias)
(E) Incomplete outcome data (attrition bias)
(F) Selective reporting (reporting bias)
(G) Other bias

C

Study or Subgroup	TOCILIZUMAB Events	Total	CONTROL Events	Total	Weight	Risk Ratio M-H, Fixed, 95% CI
BACC Bay	17	161	10	81	10.7%	0.86 [0.41, 1.78]
CORIMUNO-TOCI	11	63	18	63	14.5%	0.61 [0.31, 1.19]
COVACTA	53	183	38	90	41.1%	0.69 [0.49, 0.96]
EMPACTA	24	194	37	195	29.8%	0.65 [0.41, 1.05]
RCT-TCZ-COVID-19	6	60	5	63	3.9%	1.26 [0.41, 3.91]
Total (95% CI)		**661**		**492**	**100.0%**	**0.71 [0.56, 0.89]**
Total events	111		108			

Heterogeneity: Chi² = 1.59, df = 4 (P = 0.81); I² = 0%
Test for overall effect: Z = 2.90 (P = 0.004)

Favours [Tocilizumab]   Favours [control]

Risk of bias legend
(A) Random sequence generation (selection bias)
(B) Allocation concealment (selection bias)
(C) Blinding of participants and personnel (performance bias)
(D) Blinding of outcome assessment (detection bias)
(E) Incomplete outcome data (attrition bias)
(F) Selective reporting (reporting bias)
(G) Other bias

*Footnote: definitions of composite outcomes for each trial are listed in table 1

Fig. 2. A: Forest plot for the effect of tocilizumab on 28-30 days mortality in randomized controlled trials with corresponding risk of bias*. **B:** Forest plot for the effect of tocilizumab on risk for mechanical ventilation in randomized controlled trials with corresponding risk of bias. **C:** Forest plot for the effect of tocilizumab on 28-30 days composite outcome in randomized controlled trials with corresponding risk of bias*.

total number of patients in the five RCTs is 772 patients in the tocilizumab group and 553 patients in the control group. Finally, empirical studies have shown that pooled estimates from meta-analyses of observational studies yield similar estimates to those pooled from RCTs [65–68].

Main findings: safety

Although IL-6 inhibition dampens the host immune response and may theoretically increase the risk of infections, we did not observe a higher risk of infections or adverse events with

Table 1
Characteristics of completed randomized controlled trials of tocilizumab for patients with coronavirus disease 2019 (COVID-19)

RCT	Design	Number of patients	Country, centres	Inclusion criteria	Tocilizumab	Primary outcome	Composite outcome used in meta-analysis	Completed versus stopped early
RCT-TCZ-COVID-19 NCT04346355	Open label	60 TCZ versus 66 Controls	Italy, 24 centres	COVID-19 pneumonia documented by radiological imaging, PaO2/FIO2 between 200 and 300 mmHg, and an inflammatory phenotype defined by fever and elevated CRP	8 mg/kg up to a maximum of 800 mg, followed by a second dose after 12 hours	Composite outcome: entry into the ICU with MV, death from all causes, or clinical aggravation documented by the finding of a PaO2/FIO2 ratio <150 mmHg, whichever came first	Death or continuous need for hospitalization at day 30	Stopped early for futility
CORIMUNO-19 NCT04331808	Open label	64 TCZ versus 67 controls	France, 9 centres	COVID-19 and moderate or severe pneumonia requiring at least 3 L/min of oxygen but without ventilation or admission to ICU	8 mg/kg on day 1 and on day 3 if clinically indicated	Scores >5 on the World Health Organization 10-point Clinical Progression Scale (WHO-CPS) on day 4 and survival without need of ventilation (including non-invasive ventilation) at day 14	Death or continuous need for hospitalization at day 28	Completed
BACC Bay Tocilizumab Trial NCT04356937	Double-blind, placebo-controlled trial	161 TCZ versus 81 controls	USA, 7 centres	(SARS-CoV-2) infection, hyperinflammatory states, and at least two of the following signs: fever (body temperature >38°C), pulmonary infiltrates, or the need for supplemental oxygen in order to maintain an oxygen saturation >92%	Single dose of tocilizumab 8 mg/kg	Intubation or death	Mechanical ventilation or death at day 28	Completed
COVACTA NCT04320615	Double-blind, placebo-controlled trial	294 TCZ versus 144 controls	Nine countries (Canada, Denmark, France, Germany, Italy, The Netherlands, Spain, United Kingdom, United States), 67 centres	Patients ≥18 years with severe COVID-19 pneumonia confirmed by PCR test in any body fluid and evidenced by bilateral chest infiltrates. Blood oxygen saturation ≤93% or partial pressure of oxygen/fraction of inspired oxygen <300 mm/Hg	8 mg/kg infusion, maximum 800 mg second infusion could be administered 8–24 hours after the first	Clinical status on a 7-category ordinal scale at day 28 (1, discharged/ready for discharge; 7, death)	Death, withdrawal during hospitalization, transfer to ICU, or requirement for invasive mechanical ventilation within 28 days of baseline	Completed
EMPACTA NCT04372186	Double-blind, placebo-controlled trial	194 TCZ versus 195 controls	Six countries (Brazil, Kenya, Mexico, Peru, South Africa, US), 69 centres	COVID-19 pneumonia confirmed by PCR of any specimen and radiographic imaging SpO2 <94% while on ambient air	8 mg/kg × 1, Possible second dose	Death or MV by day 28	Death or MV by day 28	Completed

NIV, non-invasive ventilation, PaO$_2$/FIO$_2$, ratio of partial pressure of arterial oxygen to fraction of inspired oxygen; HFNC, high-flow nasal cannula; MV, mechanical ventilation respiratory support; DNR, do not resuscitate; ARDS, acute respiratory distress syndrome; CRP, C-reactive protein; PaO$_2$, arterial partial pressure of oxygen; FiO$_2$, inspired fraction of oxygen; SpO$_2$, oxygen pulse oximetry; ICU, intensive care unit; WHO, World Health Organization; PCR, polymerase chain reaction; TCZ, tocilizumab.

Table 2
Characteristics of included cohort studies

Study/ Publication type	Country	No. of pts	Study type	Cohort selection	Treatment criteria	Exclusion criteria	Primary outcome	Analytical method	Variables adjusted for	Immortal time bias adjustment
Biran et al.[a] (same cohort as Ip et al.)	United States	630	Multicentre retrospective cohort	Patients admitted to ICU without prior exposure to tocilizumab	ARDS on MV, or worsening oxygenation with high oxygen requirements (80 −100%) on high-flow nasal cannula or 15 L non-rebreather mask Symptoms had to be present for 7 days	Pregnant, enrolled in an RCT, death within 24 h	30-day mortality	Multivariate Cox regression with propensity score	Comorbidities, gender, age, race Steroids: no (equal in both groups)	No
Colaneri et al.[a]	Italy	42	Single-center retrospective cohort	Hospitalized adult patients with a confirmed COVID-19 pneumonia	TCZ was administered if: CRP > 5 mg/dl, PCTI < 0.5 ng/mL, PF ratio <300	ALT >500 U/L	ICU admission and 7-day in-hospital mortality rate.	Propensity matched logistic regression	Age, sex, LDH, neutrophils Steroids: (100%) received	No
Eimer[b] et al.	Sweden	44	Single-center retrospective cohort	Patients admitted to ICU for severe ARDS	Rising O2 at least 5L/ min, ≥ 7 days from symptom onset, CRP >100 mg/L or ferritin >500 ug/L and no contraindication to tocilizumab	Patients with COVID-19 admitted to ICU for primary diagnosis other than ARDS	30-day all-cause mortality after ICU admission	Cox proportion-hazard model	Age, diabetes, hypertension, obesity, d-dimer, interlukin-6, troponin T and PaO2/FiO2 ratio	No
Garcia[a] et al.	Spain	171	Single-center Retrospective Cohort	COVID-19 patients with pneumonia who did not require ICU transfer during the first 24h of admission	Patients with pneumonia, progressive respiratory failure CRP ≥8 mg/dL or ferritin ≥800 ng/mL or lymphocyte count <800	NR	In-hospital mortality or ICU admission	Multivariate logistic regression analysis with propensity scoring	Age, HTN, DM, heart disease, respiratory disease, lymphoma Steroids: No	Inadequate: patients not requiring ICU admission within 24 hours
Gokhale[b] et al.	India	269	Single-center Retrospective Cohort	Severe COVID-19 pneumonia with persistent hypoxia	Persistent hypoxia, bilateral pulmonary infiltrates and raised CRP, LDH and ferritin	Altered sensorium, terminal malignancy, EF < 20%	Survival	Multivariate Cox regression analysis	Age, oxygen saturation, creatinine, tocilizumab and invasive ventilation	No
Guaraldi[a] et al.	Italy	544	Multi-center Retrospective Cohort	Patients with severe* COVID-19 infection	SaO2< 93% and a PaO2/ FiO2 ratio <300 mm Hg in room air or a >30% decrease in their PaO2/ FiO2 ratio in the past 24 h during hospitalization.	Coexisting infection, PaO2/FiO2 > 300, steroid use, neutrophils <500 platelet< 50,000 High risk for bowel perforation	Composite of mortality or invasive mechanical ventilation	Multivariate Cox regression analysis	Sex, age, recruiting center, duration of symptoms, and baseline SOFA score Steroids: yes	Yes
Hill[b] el al.	United States	88	Multi-center retrospective cohort	Patients admitted to hospital and requiring supplemental oxygen	Persistent fever with impending or current respiratory failure, hemodynamic instability, IL-6 > 5 times normal.	Sepsis, transaminases >5 times normal, ANC <500 cells/mm³ or platelets <50 cells/mm³	Clinical improvement i.e. two-point reduction in severity, discharge and 28 day mortality	Cox proportion-hazard model	Age, sex, ethnicity, BMI, diabetes, cardiovascular disease, hospital, code status, oxygen support category	No
Holt[b] et al.	United States	62	Single-center case-control study	Patients admitted to hospital meeting inclusion criteria	oxygen ≥ 4L and; IL-6 > 40 pg/mL, CRP >10 mg/ dL, D- dimer >1 mcg/ mL FEU, ferritin >1000 ng/mL, or LDH >350 units	NR	Effect of tocilizumab on mortality	Cox proportion-hazard model	Age, chronic hypoxia, nursing home, IL6 > 580, ferritin >1631, ICU admission, tocilizumab, hypoxia on admission, solid	No

Table 2 (*continued*)

Study/ Publication type	Country	No. of pts	Study type	Cohort selection	Treatment criteria	Exclusion criteria	Primary outcome	Analytical method	Variables adjusted for	Immortal time bias adjustment
									tumor, diabetes, Caucasian and altered mental status	
Martinez-Sanz[b] et al.	Spain	1229	Multi-center Retrospective Cohort	COVID-19 patients.	Treatment criteria not reported	Death or transfer within 24 hours	In-hospital? Mortality	Multivariate Cox regression with Propensity score	Age, gender, comorbidities, lab values Steroids: NA	Yes: Marginal structure model
Mikulska[b] et al.	Italy	196	Single-center Retrospective Cohort	Patients with severe* or critical** COVID-19 infection	Treatment criteria was not reported	Death intubation or discharge before day 3 of admission	Intubation mechanical ventilation or mortality	Overlap-weighted Cox proportional hazard regression model	Age, gender, presence of comorbidities, week treatment began, NIV, and labs Steroids: NA, Tcz compared with steroids	Yes: landmark analysis
Narain[a] et al.	United States	3098	Multi-center Retrospective Cohort	Patients with cytokine storm defined as ferritin >700ng/mL or CRP >30mg/dL or LDH >300U/L	Treatment criteria not reported	Immunomodulatory drugs used prior to the diagnosis of cytokine storm	In-Hospital mortality	Multivariate Cox proportional hazard regression model	Age, gender, race, comorbidities, lab data, insurance status Steroids: NA, patients receiving steroids were in their own group	No
Ramaswamy[b] et al.	United States	86	Multi-center Case-Control	Patients who died during hospitalization were cases, while patients discharged alive were controls. The exposure was treatment with tocilizumab	Treatment criteria was not reported	NR	In-hospital Mortality	IPSW Cox regression adjusted for competing risk of mortality	Age, race, gender, Elixhauser comorbidity score, MEWS score Steroids: included in IPW analysis	No
Roomi[b] et al.	United States	176	Single-center Retrospective Cohort	Hospitalized adult patients with a confirmed COVID-19 infection	Treatment criteria was not reported	NR	In-hospital mortality, ICU admission, mechanical ventilation	Multivariate logistic regression	Baseline comorbidities and medication use	No
Rossi[b] et al.	France	246	Single-center Case Control	Patients with severe* COVID-19 pneumonia	Treatment criteria was not reported	Patients on IMV or admitted to the ICU	Composite of mortality and mechanical ventilation	IPSW Cox regression	Age, engagement status, systolic blood pressure SpO2/FiO2 ratio Steroids: Equal between groups after matching	Inadequate: time between admission and inclusion was adjusted for in propensity-score matching
Rossotti[a] et al.	Italy	222	Single-center Retrospective Cohort	Patients with severe [c]COVID-19 infection	CT scan showing severe bilateral pneumonia; CRP >1 mg/dL, IL-6 >40 pg/mL, D-dimer >1.5 mcg/mL, or ferritin >500 ng/mL	ALT value > 5 x ULN; neutrophil cell count <500 cell/mmc; platelet count <50,000 cell/mmc	In-hospital Mortality	Cox regression models	NR Steroids: NA	No
Roumier[b] et al.	France	59	Single-center Retrospective Cohort	NR	COVID-19 patients with requiring >6L O$_2$, elevated CRP levels	NR	Mortality, Mechanical ventilation, and ICU admission	IPTW matched logistic regression	Age, gender and disease severity Steroids: No	No

Study	Country	N	Study design	Inclusion criteria	Outcome/exclusion	Outcome		Statistical model	Adjustment variables	Adequacy of adjustment
Somers[a] et al.	United States	154	Single-center retrospective Cohort	Critical[d] COVID-19 patients requiring invasive mechanical ventilation	Death within 48 hours of intubation, intubation for conditions unrelated to COVID-19 or enrolled into an RCT for sarilumab	In-hospital mortality		IPTW Cox regression model	Age, gender, race, ferritin, LDH, AST Steroids: No (equal between groups)	Inadequate: patients who died within 48 hours of intubation were excluded
Tsai[b] et al.	United States	132	Single-center consecutive Cohort	Hospitalized patients who meet treatment criteria	Ferritin >300 ug/mL and SpO2 < 94% requiring supplemental oxygen or mechanical ventilation	In-hospital mortality	NR	Propensity matched logistic regression	Age, sex, body mass index, lactic acid, ferritin, LDH, procalcitonin, creatinine, hypertension and comorbidity score	No

ALT: Alanine aminotransferase; ARDS: acute respiratory distress syndrome; CRP: C-reactive protein; DM: Diabetes mellitus; HTN: Hypertension; NR: Not reported; NA: Not applicable; IMV: Invasive mechanical ventilation; IPTW: Inverse probability treatment weighted; IPW: Inverse probability weighted; MV: Mechanical ventilation; UNL: upper normal limit; TCZ: Tocilizumab.
[a] Peer-reviewed.
[b] Pre-print.
[c] Severe COVID-19 defined by one of the following 1) respiratory rate ≥30 breaths/min, 2) peripheral capillary oxygen saturation (SpO2) ≤ 93% while breathing room air, 3) PaO2/FiO2 ≤ 300 mmHg.
[d] Critical patients requiring mechanical ventilation.

tocilizumab use in the RCTs. Moreover, many of the cohort studies included in our review assessed patients receiving tocilizumab in the ICU, of whom many were on mechanical ventilation. The high absolute incidence of secondary bacterial infections in the ICU [69–71] that was also observed in the cohort studies is probably related to critical illness and ICU admission as opposed to the use of tocilizumab. Tocilizumab use in CRS following CAR-T therapy was not associated with increased infections [72].

Mechanisms

IL-6 has pleotropic effects and plays important roles in host defence against invading organisms and tissue repair in acute environmental conditions such as trauma and burns [73,74]. It is produced by immune cells and several other types of cells in response to TLR activation and in response to other proinflammatory cytokines such as TNFα and IL-1®, and its production is also upregulated by coagulation factors like factor VIIa and thrombin [73]. IL-6 plays a pivotal role in acute-phase reaction and rapidly induces acute-phase proteins, including CRP, antitrypsin, serum amyloid protein A (SAA), fibrinogen, ferritin, hepcidin and others [73,74]. Some of these proteins are involved in host defence; CRP works as an opsonin through its binding to bacterial phosphorylcholine and activates the complement pathway, while antitrypsin inactivates proteases released by pathogens and damaged cells, and hepcidin possesses antimicrobial activities [73]. Moreover, IL-6 induces B-cell differentiation and antibody production, and induces cytotoxic-T-cell differentiation from CD8 T cells. It also promotes the induction of C3 complement and C5a receptor [73,74]. IL-6 also increases the production of intercellular adhesion molecule-1 (ICAM-1), MCP-1 and IL-8, which play roles in the migration of inflammatory cells to sites of inflammation and tissue damage [73].

All of these effects are very important in host defence against invading pathogens. However, persistent and dysregulated production of IL-6 may have detrimental effects, and IL-6 has been identified as an important mediator in different autoimmune and chronic inflammatory diseases such as rheumatoid arthritis, systemic lupus erythematosus (SLE), and others [73,74]. Additionally, the production of IL-6 has been shown to be significantly increased in conditions associated with CRS and cytokine storm, such as CAR-T therapy, MAS, HLH syndromes and viral infections associated with cytokine storm [73,74]. IL-6 plays a crucial role in the pathogenesis of CRS associated with these conditions. IL-6 increases vascular permeability leading to interstitial oedema, increased tissue pressure and damage through activation of the complement system, induction of vascular endothelial growth factor (VEGF) and histamine release from mast cells. It also activates the coagulation system through increased production of factor VIIa, thrombin and tissue factor, and induction of megakaryocyte maturation and platelet production. These procoagulation effects can lead to disseminated intravascular coagulation (DIC). Furthermore, IL-6 has direct myocardial depressing effects [73]. Therefore, these effects of IL-6 could explain the manifestations of COVID-19-associated cytokine storm which include hypoxaemia, hypotension, intravascular thrombosis, myocardial dysfunction and multiorgan failure. Several lines of evidence support this contention. For example, IL-6 injection in patients with breast and lung cancer have been shown to produce fever and influenza-like symptoms [75]. It has also been shown that injecting metastatic renal cancer patients with IL-6 induces coagulation activation as evidenced by increased thrombin–antithrombin III complexes and increased plasma levels of prothrombin activation fragment [76]. Additionally, daily subcutaneous IL-6 injections of 1–10 μg/kg/day for 7 days in patients receiving chemotherapy resulted in elevation of several acute-phase proteins, with the fastest response observed with CRP and
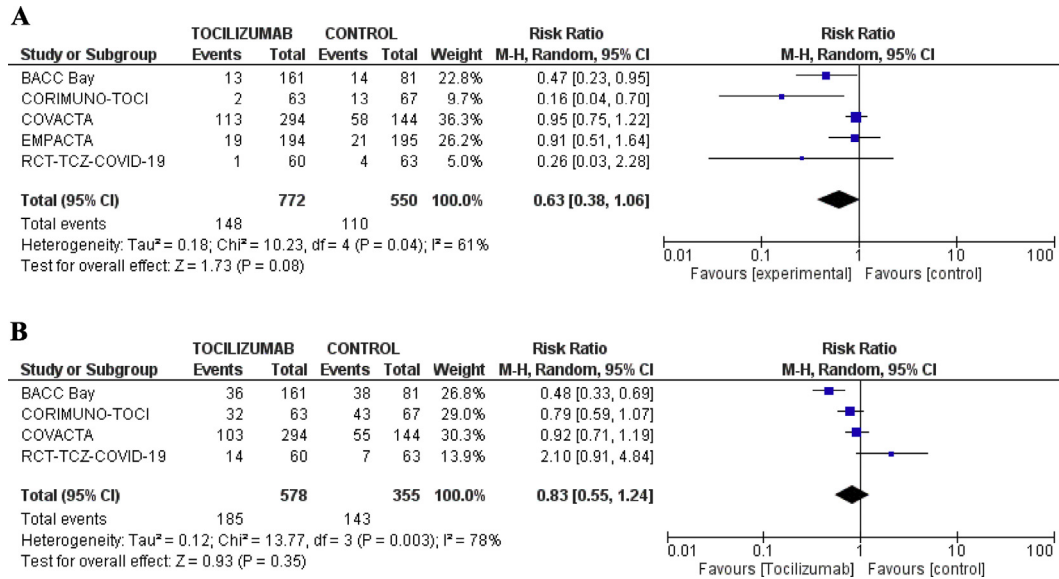
Fig. 3. A: Forest plot for relative risk of infections with tocilizumab vs. control in randomized controlled trials. **B:** Forest plot for relative risk of adverse events with tocilizumab vs. control in randomized controlled trials.

serum amyloid protein A [77]. On the other hand, treatment of patient with CAR-T therapy induced cytokine storm with a single injection of the humanized anti-IL-6 monoclonal antibody, and

tocilizumab led to a rapid resolution of the manifestations of this cytokine storm [17]. Further, in MAS, which is a well-known complication of systemic juvenile idiopathic arthritis (sJIA), it has
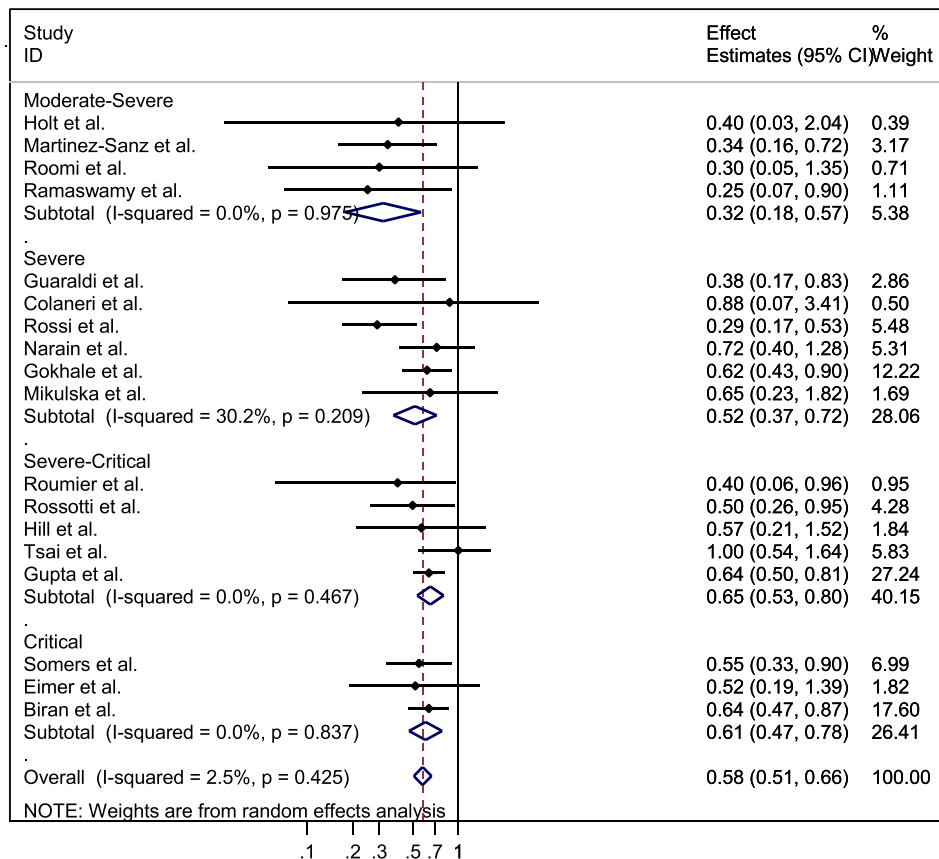
*Footnote: critical group: patients admitted to intensive care unit (ICU). Severe group: patients requiring respiratory support outside ICU. Moderate group: patients who did not require any respiratory support.

Fig. 4. Forest plot of the association between tocilizumab use and short-term mortality in COVID-19 patients from cohorts at moderate risk of bias: stratified by disease severity*.

been demonstrated that activation of haemophagocytic macrophages is linked to decreased functioning of natural killer cells with reduced expression of perforin and granzyme B, and it is believed that IL-6 plays a role in this process as treatment of patients with sJIA with tocilizumab results in normalization of reduced perforin and granzyme B [73]. The findings of our meta-analysis further support the role of IL-6 in CRS and establishes the potential therapeutic benefits of tocilizumab in CRS syndromes in general, and more specifically in severe COVID-19 disease.

Phenotypes/markers

Although IL-6 level determination is not performed routinely in most hospitals, acute-phase proteins can serve as surrogate markers for elevated IL-6 in COVID-19 disease, especially CRP and ferritin, as their assays are widely available. Therefore, CRP and ferritin can be used as markers of elevated IL-6 to help in selecting candidate COVID-19 patients for tocilizumab therapy. Indeed, in a multicentre study that included 1229 COVID-19 patients from Spain, it was found that tocilizumab reduced the risk of mortality only in patients with baseline CRP levels >150 mg/L (aHR 0.34, 95%CI 0.16—0.72, p 0.005) [38]. This indicates that patients with low CRP likely had low IL-6 levels and hence did not respond favourably to tocilizumab. Tocilizumab was also shown to significantly reduce CRP and ferritin among COVID-19 disease patients [78]. D-dimer—which is another biomarker that is usually elevated in patients with COVID-19 disease and which may indicate presence of intravascular thrombosis—was also found to predict outcomes to tocilizumab [78]. These studies indicate that not all COVID-19 patients respond equally to tocilizumab, which is likely reflecting the heterogeneous nature of the disease and the possibility that it encompasses different sub-phenotypes. Post-mortem studies lend support to this hypothesis as autopsy series from Italy and the USA showed that some COVID-19 patients exhibit extensive pulmonary inflammation and alveolar damage while others manifest severe vascular endothelial damage and intravascular thrombosis [79,80]. In addition, in the RECOVERY trial, only patients with underlying hypoxaemia had improved survival with dexamethasone therapy, reflecting the possible presence of different sub-phenotypes with differential responses to immuno-modulatory therapies. A similar phenomenon has been observed in patients with sepsis. Re-analysis of the phase III trial of anti-IL-1® antibody (anakinra) therapy in sepsis patients revealed that only patients who had features of MAS (5.6% of patients) had markedly reduced mortality in response to anakinra [81]. Further studies are needed to identify COVID-19 patients who would respond favourably to immunomodulatory therapies.

Strengths and limitations

Our meta-analysis has several strengths. We included published and unpublished randomized trials, employed rigorous methodologies, and excluded unadjusted crude effect estimates from cohort studies. Our inclusion of real-world data from good-quality observational studies and randomized trials allowed us to explore sources of heterogeneity and to compare the results of the RCTs with those of cohort studies. Although observational studies are prone to different biases—including confounding by indication, survivor bias and residual confounding—empirical studies have shown that pooled estimates from meta-analyses of observational studies yield similar estimates to those pooled from RCTs [65—68].

Conclusion

Cumulative moderate-certainty evidence shows that tocilizumab reduces the risk of mechanical ventilation in hospitalized COVID-19 patients. While RCTs showed that tocilizumab did not reduce short-term mortality, low-certainty evidence from cohort studies suggests an association between tocilizumab and lower mortality. We did not observe a higher risk of infections or adverse events with tocilizumab use. This review will continuously evaluate the role of tocilizumab in COVID-19 treatment.

Author contributions

ZK, MD, MR and HT contributed equally to this work. IT: conception and design of the study. IT, ZK, MD, HT, MA, YA, MA, RT, LH and TK: acquisition of the data. IT and MR: analysis of the data. IT, ZK, HT, MR and TK: interpretation of data. IT, ZK, LH and TK: drafting of the article. All authors: critical revision of the article for important intellectual content and final approval of the version to be submitted.

Transparency declaration

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.cmi.2020.10.036.

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