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ΔΙΠΛΩΜΑΤΙΚΗ ΕΡΓΑΣΙΑ

Ασφάλεια και αποτελεσματικότητα των ανταγωνιστών ιντερλευκίνης-1 στη νόσο COVID-19, με έμφαση στη νοσηρότητα και τη θνητότητα: Συστηματική ανασκόπηση και μετά-ανάλυση

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Safety and efficacy of interleukin-1 antagonists in coronavirus disease-2019, with emphasis on morbidity and mortality: a systematic review and meta-analysis.

Athina K. Dimosiari

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ABSTRACT (Greek)

Εισαγωγή-Σκοπός: Η νόσος COVID-19 παραμένει ένα παγκόσμιο πρόβλημα δημόσιας υγιεινής που σχετίζεται με σημαντική θνητότητα και θνησιμότητα. Παρά την πρόοδο στην κατανόηση της παθοφυσιολογίας της νόσου, δεν υπάρχει εγκεκριμένη στοχευμένη θεραπεία μέχρι και σήμερα. Η ιντερλευκίνη IL-1 έχει καταδειχθεί βασικός κρίκος στην αλυσίδα των γεγονότων που ενεργοποιούν την καταιγίδα κυτταροκινών και οδηγούν στην ανάπτυξη των κύριων επιπλοκών της νόσου. Σε αυτή τη γνώση βασίστηκε και η δοκιμή της αναστολής της IL-1, ως θεραπευτική προσέγγιση για την τροποποίηση της πορείας ασθενών με σοβαρή νόσο COVID-19. Σκοπός της παρούσας συστηματικής ανασκόπησης και επικαιροποιημένης μετά-ανάλυσης τυχαιοποιημένων κλινικών δοκιμών, είναι η αξιολόγηση της αποτελεσματικότητας και της ασφάλειας των αναστολέων της ιντερλευκίνης IL-1 σε νοσηλευόμενους ασθενείς με νόσο COVID-19.

Μέθοδος: Πραγματοποιήθηκε αναζήτηση στις βάσεις δεδομένων PubMed, Cochrane Library, clinicaltrials.gov, European Union (EU) Clinical Trials Register και medrxiv.gov έως τις 1 Απριλίου 2022 για τυχαιοποιημένες κλινικές δοκιμές σε νοσηλευόμενους ασθενείς, που έλαβαν είτε κάποιον αναστολέα IL-1 (anakinra ή canakinumab) είτε placebo, και εκτιμήθηκαν σημεία ασφάλειας και αποτελεσματικότητας.

Αποτελέσματα: Χρησιμοποιήθηκαν στοιχεία από 7 κλινικές δοκιμές με συνολικό αριθμό 2120 ασθενών. Η αναστολή της IL-1 δεν συνεισέφερε στατιστικά σημαντικό όφελος στην σχετιζόμενη με τη νόσο θνητότητα [risk ratio (RR) = 0.93, 95% CI; 0.70 – 1.22, I2 = 28%, p = 0.22], στον κίνδυνο εφαρμογής μηχανικού αερισμού (RR = 1.05, 95% CI; 0.77 – 1.42, I2 = 41%, p = 0.13) και στον κίνδυνο εφαρμογής μη μηχανικού αερισμού (RR = 1.03, 95% CI; 0.65 – 1.62, I2 = 0%, p = 0.9). Στην ανάλυση υποομάδων δεν παρατηρήθηκε διαφορά μεταξύ anakinra και canakinumab. Τέλος, κανένας από τους δύο παράγοντες δεν ανέδειξε στατικά σημαντική αύξησή σε κύρια σοβαρά ανεπιθύμητα συμβάματα.

Συμπέρασμα: Στην παρούσα μετά-ανάλυση δεν καταδεικνύεται θεραπευτικό όφελος από τη χρήση αναστολέων IL-1 σε νοσηλευόμενους ασθενείς με νόσο COVID-19, όταν προστίθενται στη συνήθη θεραπευτική, παρότι είναι ασφαλής η χορήγησή τους. Τα σύγχρονα δεδομένα δεν υποστηρίζουν τη χορήγησή τους σε ασθενείς με σοβαρή νόσο COVID-19.

Λέξεις Κλειδιά

COVID-19, σοβαρή νόσος, θνητότητα, ιντερλευκίνη IL-1, anakinra, canakinumab

ABSTRACT

Introduction: Coronavirus disease-2019 (COVID-19) remains a global public health problem, associated with significant morbidity and mortality. Despite the progress in the understanding of its pathophysiology, there is no approved, targeted treatment option so far. Interleukin-1 (IL-1) appears to be crucial in the cytokine storm mediating major complications of the disease. Therefore, it appears reasonable that blockage of IL-1 could modify disease course in patients with severe COVID-19. Herein we sought to perform an updated meta-analysis of randomized controlled trials (RCTs), assessing the safety and efficacy of IL-1 blockers in hospitalized patients with COVID-19.

Methods: We searched PubMed, Cochrane Library, clinicaltrials.gov, European Union (EU) Clinical Trials Register and medrxiv.gov databases from inception to 1st April 2022 for RCTs enrolling hospitalized adult subjects with COVID-19, assigned either to an IL-1 antagonist (either anakinra or canakinumab) or control (placebo or active comparator). We assessed a number of safety and efficacy outcomes.

Results: We pooled data from 7 trials in a total of 2120 enrolled subjects. IL-1 blockage did not confer any significant benefit on COVID-19 related mortality [risk ratio (RR) = 0.93, 95% CI; 0.70 – 1.22, I2 = 28%, p = 0.22], on risk for invasive mechanical ventilation (RR = 1.05, 95% CI; 0.77 – 1.42, I2 = 41%, p = 0.13) and on risk for non-invasive mechanical ventilation (RR = 1.03, 95% CI; 0.65 – 1.62, I2 = 0%, p = 0.9). No subgroup difference between anakinra and canakinumab was shown. Neither anakinra nor canakinumab were associated with a significant increase in the risk for serious adverse events.

Conclusion: We failed to document any treatment benefit with IL-1 blockers in hospitalized patients with COVID-19, as added to standard of care, despite being a safe treatment option. Current evidence does not support their administration in patients with severe COVID-19.

Keywords

COVID-19, severe disease, mortality, interleukin-1, anakinra, canakinumab

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INTRODUCTION

Coronaviruses belong to the family of Coronaviridae within the order Nidovirales and were first identified in 1960. They broadly affect vertebrates and are associated with upper and lower respiratory tract infections. Of note, rhinoviruses and coronaviruses account for more than 50% of cases of upper respiratory tract infections worldwide, the so called "common cold" [1-2].

In 2003, severe acute respiratory syndrome coronavirus (SARS-CoV) emerged as the pathogen implicated into the pathogenesis of a highly contagious atypical pneumonia pattern, which resulted in an epidemic that affected 8422 subjects worldwide, leading 11% of them to death. Implementation of public health measures, due to the absence of effective treatment options or appropriate vaccines, led to the delay and final to the limitation in the spread of the disease [3].

In 2012, in the Middle East region, another outbreak of lower respiratory tract infections emerged. This was finally attributed to the Middle East respiratory syndrome (MERS) coronavirus, which led to 170 confirmed cases, with a extremely high fatality rate, since 72 patients of those infected eventually died. Identification of MERS coronavirus resulted in a significant improvement in the understanding of the pathophysiology of infections caused by coronaviruses [4-5].

However, in late December 2019, in Wuhan, Huabei Province, China, a novel coronavirus (2019-nCoV) emerged, later named after SARS-CoV-2. Affected patients suffered from atypical pneumonia, with the majority of cases initially linked to the Huanan Seafood Wholesale Market. Mean incubation period was calculated to be 5.2 days, while the epidemic doubled in size every 7.4 days [6]. Epidemic rapidly spread outside China and the World Health Organization (WHO) declared the coronavirus outbreak to be a public health emergency of international concern on 31 January, 2020. The WHO finally characterized the disease as a "pandemic" on March 11, 2020, since WHO authorities stated that they are "deeply concerned both by the alarming levels of spread and severity and by the alarming levels of inaction" [7-8]. Until now, SARS-CoV-2 has affected patients in almost every country across the world.

As of May 25, 2022, almost 527 million subjects have been infected worldwide, with more than 6 million documented deaths [coronavirus disease-2019 (COVID-19) Dashboard by the Center for Systems Science and Engineering at Johns Hopkins University]. In Greece, at the same time point, almost 3.5 million cases have been confirmed, with almost 30000 recorded deaths, attributed to SARS-CoV-2.

A significant improvement in the understanding of the pathogenesis of COVID-19 has been noted during the last 2 years, however the disease burden remains high [9]. Several treatment options and therapeutic strategies have been utilized so far, while the development of vaccines highly effective against the development of severe disease has been widely adopted, despite initial considerations.

Severe COVID-19 is characterized by systemic hyper-inflammation, cytokine storm and rapid progression to respiratory failure and acute respiratory distress syndrome (ARDS). Major inflammatory cytokines, such as interleukin (IL)-6, IL-8, tumor necrosis factor (TNF)-α and IL-1, have been shown to be predictors of disease severity and mortality, therefore, it was relatively early proposed that they should represent both prognostic biomarkers, but also treatment targets in COVID-19 [9].

Therefore, there has been a vivid and ongoing discussion regarding the role of antirheumatic drugs targeting various stages of the inflammatory cascade in COVID-19, including drug classes against specific cytokines or their receptors, in the treatment of COVID-19 and the prevention of surrogate outcomes. Concerning the role of IL-1 blockers, former meta-analyses of observational studies resulted in enthusiasm about the promising role of anakinra, for the reduction of COVID-19 related mortality, while data concerning the role of canakinumab, a monoclonal antibody targeting IL-1β, was limited and scarce [10].

Thus, we sought to perform an updated systematic review and meta-analysis of relevant randomized controlled trials, in order to evaluate the safety and efficacy of both IL-1 blockers, anakinra and canakinumab, in patients with severe COVID-19, providing high-level evidence on their place in the treatment algorithm of COVID-19.

SYSTEMIC REVIEW

Epidemiology

The emergence of Coronavirus Disease-19 (COVID-19) in China at the end of 2019 has quickly developed in a worldwide health crisis, causing the World Health Organization (WHO) to declare it a pandemic on March 11th, 2020, as it transmitted with speed and in a scale not witnessed since the Spanish influenza back in the 1918-19 [7]. A novel coronavirus, initially named 2019-nCoV, was identified after isolation as the etiology of the disease and was quickly redesignated as severe acute respiratory syndrome coronavirus-2 (SARS-CoV-2) by the International Committee for Taxonomy of Viruses, due to genetic relation to the coronavirus responsible for the SARS outbreak of 2003. The suspected first case was reported on 8 December 2019 in the town of Wuhan, Hubei Province, China, while on 31 December, WHO was notified of an unidentified pneumonia outbreak in the region [6].

Coronaviruses belong to the Coronaviridae family, with the subgroups of alpha (a-CoV) and beta (β-CoV) being infectious to mammals in contrast to the remaining subgroups of gamma (γ-CoV), and delta (δ-CoV) coronaviruses. Both SARS-CoV and SARS-CoV-2 are members of the B lineage of β-CoV (further 4 different lineages A,B,C,D), with more than 200 viral sequences known [5]. Although coronaviruses are rather diverse when infecting animals in forms of respiratory, gastrointestinal, and central nervous system diseases, they can also cause respiratory infections in humans of whichever severity, as in 2002 with the severe acute respiratory syndrome coronavirus (SARS-CoV) and in 2013 with the Middle East respiratory syndrome coronavirus (MERS-CoV). Those two outbreaks of coronavirus both fatally infected humans via respiratory tract [1,2].

The SARS-CoV-2, the third zoonotic human coronavirus of the century is a single, positive-strand ribonucleic acid (RNA) virus, with a stronger ability to be transmitted from human to human compared to SARS-CoV and a higher adaption capacity through mutating and host tropism modification, thus forming a long term, wildfire-like threat, although being less fatal [6]. This novel betacoronavirus, SARS-CoV-2, shares 79% genome sequence identity with SARS-CoV and 50% with MERS-CoV2 [1]. To date, more than 10,5 million SARS-CoV-2 RNA genomes are uploaded in the Global Initiative on Sharing All Influenza Data, known as GISAID, as the scientific community races against time to provide as much information for the virus and succeed in the development of therapeutic agents and stronger vaccines [4].

It is still uncertain where the pandemic originated from, with research tracking the initial cases in the Huanan South China Seafood Market, where snakes, birds and other animals such as bats were sold, as many patients were traced back there. Although at first, a suspected bat origin -due to a 96% genome sequence identity to a bat coronavirus, was outlined and the pangolins were demonstrated as natural host of the virus, human to human transmission became more strongly supported and the disease

rapidly spread worldwide [4]. According to the European Centre for Disease Prevention and Control (ECDC), as of 17th week of 2022 512,690,034 cases of COVID-19 and 6,252,316 deaths have been reported worldwide since 31st December 2019. European continent was among the ones with highest number of cases (210,861,802), with France and Germany representing the leading countries [8].

SARS-CoV-2 is replicated mainly in the upper respiratory tract, herein supporting the high infectivity, and transmission occurs via respiratory droplets and aerosols, whilst -regardless the clinical manifestation- it is easily, specifically and sensitively detected in the nasal passages of infected subjects. Viral RNA is also present in lower respiratory tract in much higher concentrations, much lesser in the gastrointestinal tract or peripheral blood of critically ill patients. It can also be transmitted by asymptomatic and presymptomatic individuals, while uncertain infection periods and inhomogeneous susceptibility of the population all form the perfect set up for a pandemic to unfold [11, 12].

In some cases, transmission via direct contact to infected surfaces is documented, although the virus cannot live long outside a host, and it is uncertain whether this in fact was the route of infection. Although viral RNA decays gradually, viable virus has been isolated for up to 3 hours in aerosols and up to 72 hours from surfaces, the longest viability being reported on plastics and stainless steel. Transmission to domestic animals and between them has been documented. It is hypothesized but not proven or documented that the virus can be transmitted with sexual, fecal–oral, or bloodborne transmission [13].

Patients were at the beginning mostly adults with a male predominance, with transmission associated to health care environments being predominant, as well. Nowadays a rising number of children are being infected around the world and most health personnel infections are classified as community transmissions. The most common risk factors for severe disease are age, cardiovascular disease, chronic respiratory disease, diabetes mellitus, immunodeficiency, cancer and autoimmune diseases [14]. There have been described more than 60 predictors of COVID-19 severity, with the most important being age, C-reactive protein, D-dimer, serum albumin, body temperature, Sequential Organ Failure Assessment (SOFA) score and diabetes mellitus, with the last one being the most consistent comorbidity predicting morbidity [15]. Simultaneously, the well-established “immunosenescence” of the elderly, meaning the immunological deficiencies in both innate and adaptive immune response due to aging, makes this group highly susceptible to severe COVID-19, same as influenza and other viral infections [16].

Pathophysiology

As mentioned above, coronaviruses are zoonotic viruses of the Coronoviridae family, infecting domestic and companion animals as well as human causing mild to sever respiratory disease. There are 7 HCoVs (Human Coronaviruses) that have been identified to affect humans, namely the α-type HCoV-

229E and HCoV-NL63, the β-type HCoV-HKU1, SARS-CoV, MERS-CoV, HCoV-OC43 and 2019-nCoV, causing the COVID-19 outburst. They are of different pathogenicity with SARS-CoV, MERS-CoV and SARS-CoV-2 being the worst in terms of ability to cause severe acute respiratory syndrome and death. SARS-CoV-2 is a single-stranded RNA virus with a size of 29,891 bases, whose genome encodes 29 proteins for purposes of virion assembly, replication, cell invasion and infection [17].

Four structural proteins form the accommodation of its' genome, all surrounded by a lipid envelope made of (S) spike, (E) envelope and (M) membrane proteins. The last two are necessary for virion assembly, while the spike protein is essential for virus entry and recognition from the host. The large protrusions forming the spike protein on the surface of the virus are common in the Coronaviridae family and are the reason for the name "CORONA", as they resemble crowns. A receptor binding domain (RBD) contained in the S protein, binds to the human angiotensin- converting enzyme 2 (ACE2), allowing membrane fusion and endocytotic insertion of the virus in the human cells. That RBD is the most variable region of the virus' genome [18].

The simplest model of infection suggests that, after SARS-CoV-2 affects the host, innate immune response is triggered to release proinflammatory cytokines, in which epithelial and endothelial microvascular cells are susceptible to, leading to cell edema, apoptosis and increased alveolar permeability. Concomitantly, the virus causes viremia entering the blood stream, deranging the renin-angiotensin-aldosterone system (RAAS) and gradually leading to multi-organ damage, sepsis and death [9].

Further inside this model, post-mortem findings of COVID-19 patients document increased lung weight due to oedema and congestion, while there is microscopic evidence of mucus and cell debris deposits in the bronchi, with diffuse damage and fluid and fibrin filled alveoli. Lung parenchyma appears remodeled by hyperplastic and necrotic pneumonocytes with disrupted cell membranes and predominant microangiopathy and microthrombi. Similar to the observations of SARS virus and H1N1 influenza virus, the lung is infiltrated by leukocytes, while, perivascular T-cells and macrophages are found in the alveoli, as well as haemophagocytosis is confirmed in the pulmonary lymph nodes, all suggesting the initiation of cytokine release syndrome [18].

This cytokine release syndrome theory was already elucidated from animal models, as in the course of a coronavirus infection, it has been shown that the severity of the disease was associated to immune dysregulation and excessive inflammatory response rather than the virus titer. Therefore, old nonhuman primates were more likely to develop severe illness upon infection to younger ones, involving rapid viral replication, vigorous cytokine response and over induction of epithelial and endothelial apoptosis [19]. The hypothesis of cytokines playing the key role during a viral infection response, is long known, with relevant evidence suggesting a delay in the innate immune response in respiratory epithelial cells, dendritic cells (DCs), and macrophages, secretion of low levels of antiviral interferons

(IFNs) and very high levels of proinflammatory cytokines, such as interleukin IL-1β, IL-6, and tumor necrosis factor (TNF) and chemokines (C-C motif chemokine ligand (CCL)-2, CCL-3, and CCL-5) [20].

As with SARS-CoV-2 and MERS-CoV, the main cause of death in SARS-CoV-2 is development of acute respiratory distress syndrome (ARDS), evidently attributed to several proinflammatory cytokines and chemokines, along with immunopathological changes in the lungs. Alveolar fluid is reabsorbed by type I pneumocytes in the interstitium, while surfactant is secreted by type II pneumonocytes, resulting in increased permeability. Lung injury is mediated by the inflammatory cell- rich protein fluid filling the alveolar space causing collapse and de-recruitment. The initial immune response consists of neutrophil apoptosis, expansion of resident fibroblasts, reformation and regrowth of the alveolar epithelium differentiating type II to type I alveolar cells. This proliferative phase of ARDS might be prolonged or even impaired, consequently preventing recovery and resulting in impaired gas exchange, increased work of breathing and ventilation to perfusion abnormalities, finally leading to respiratory failure [21]. Computed Tomography (CT) imaging has shown that the lung is not uniformly affected by ARDS, with the greatest damage observed in the dorsal and basilar regions of the lung in terms of edema, parenchymal densities and consolidation [22]. As the remaining, available for gas exchange, lung volume shrinks under the weight of flooded alveoli, lung compliance decreases, and endothelial cell injury progresses to vasculopathy. In COVID-19, autopsy evidence indicates greater oxidative stress and faster formation of macro- and micro-thrombosis, with vascular dilation and angiogenesis greater than with H1N1 influenza or the earlier SARS, with a predominance of monocytes and lymphocytes, whereas in classic bacterial ARDS infiltration by neutrophils predominates [23].

As with any “invader”, immunological defense against SARS-CoV-2 starts with activation of the innate immune system. The RBD of S protein binds to ACE2 of the human cell, allowing the virus to enter it with multiple ways -endocytosis or viral escape from the endosome cathepsin L (CTSL) mediated, or direct fusion of the cell membrane with the virus’ envelope. Afterwards pattern recognition receptors (PRRs), such as Toll-like receptors (TLRs), recognize pathogen-associated molecular patterns (PAMPs) of the virus, leading to excess transcription of nuclear factor-kappa B (NF-κB) and interferon regulatory factors (IRFs) in cascade of events that results in proinflammatory cytokine production and release. In this stage, the critically ill COVID-19 patients exhibit a fulminant cytokine release with significantly increased IL-6 levels and decreased CD4+ T- and CD8+ T-cells, and natural killer (NK) cells, as an immunosuppressive state. This dysregulated response of the immune system during the viral infection generates secondary hemophagocytic lymphohistiocytosis, which is reported in approximately 3.7–4.3% of sepsis cases. Levels of IL-1β, IL-6, IL-8, and soluble tumor necrosis factor receptor 1(TNF-1), are strongly and positively correlated to severity of COVID-19, as demonstrated in a recent retrospective study [24].

While the infection progresses, the adaptive immune system is also activated with a consequent release of cytotoxic T-cells and production of viral specific antibodies by B-cells. T-helper type 1(Th1) response is activated by the above cytokines (IL-1B, IFN-γ, IL-10, IL-6, monocyte chemoattractant protein 1 (MCP-1)), being the key event to the start of a normal strong specific immunity [23, 24]. However, SARS-CoV-2 infection is characterized by lymphopenia with reduced CD4+ T-cells, CD8+ T-cells, and B-cells and high levels of T-helper type 2 (Th2) cell-secreted cytokines (such as IL-4 and IL-10), which inhibit the inflammatory response. With the virus being present in both the spleen and lymph nodes, induction of adaptive immunity in COVID-19 is attributed to direct cytotoxic effect in lymphatic organs, reduced haematopoiesis, haemophagocytosis and lymphocyte sequestration in the lungs or other organs [21, 25]. It has been shown that severity of COVID-19 is related to the levels of granulocyte colony-stimulating factor (GCSF), IP-10, MCP-1, macrophage inflammatory protein-1A and TNF-α, when patients from general wards are compared to the ones in the intensive care unit (ICU) [25]. Combining all the above findings to the excessive production of cytokines in COVID-19, has highlighted the development of cytokine release syndrome as a basic severity and mortality- related pathophysiological complication.

Cytokine release syndrome (CRS) is a systemic inflammatory response, first described in the early ‘90s, as immunotherapy in hematology, rheumatology and oncology started to develop. A series of infections and certain drugs can trigger the response, such as T-cell-engaging immunotherapies, a growing field in the treatment of hematologic malignancies [26]. The syndromes’ pathophysiology remains unclear, with a massive release of a wide range of cytokines by immune and non-immune cells (ex. endothelial), most consistently IL-1, IL-6, IL-10, IFNγ. IL-6 may be the most important cytokine, as its’ release induces a pleiotropic response, activating cellular and innate immunity, as well as Th2 and Th17 cell differentiation. Moreover, it stands out as the initial component of cytokine storm in COVID-19, as it binds to sIL-6R (soluble IL-6 Receptor), entering almost any human cell and causing vascular growth factor (VGF) to produce and even more pro-inflammatory cytokines to be excreted (like IL-1) [27]. Similarly but earlier n the process, IL-1 induces gene expression and cytokine release in macrophages and dendritic cells, participating in both non and specific immunity, stimulating the continuous secretion of a pro-inflammatory complex, toxic to the lung and all organs. This metabolic cellular abnormality is the main cause of septic shock and initiation of ARDS, gastrointestinal and neurological disorders, all detrimental to the course of COVID-19, supporting the inhibition or lack of formation of IL-1 as an interesting therapeutic target to prevent hemodynamic changes, septic shock and organ inflammation [28, 29].

Two last hallmarks in the pathophysiology of CRS are the activation of endothelial cells and the overwhelming activation of the complement system, which both organize a harmful acute and chronic inflammation, endothelial cell dysfunction, and intravascular coagulation. Coagulatory dysfunction and

thrombotic vasculopathy are amongst the commonest and more serious dysregulations addressed in COVI-19 patients [30]. SARS-CoV-2 might also directly damage the endothelium other than ensuing cytokine mediated inflammation. Alongside, dysregulation of RAAS and sepsis itself are implicated inτo COVID-19 associated hypercoagubility, remodeling of small vessels, and subsequent changes in capillary density, organ perfusion and metabolism [31]. Research progresses further to support the vascular endothelium as the key organ in the course of COVID-19, hence common risk factor-comorbidities in the severely ill patients are hypertension, diabetes mellitus, cardiovascular disease, all diseases with underlying endothelial impairment [32]. Moreover, cardiovascular events occur often as a complication of this viral infection, either directly as described above or indirectly (perivascular cell damage), affecting coronary microvessels and causing myocardial injury. Microscopically, ACE2 levels decrease locally in the small coronary vessels. Subsequently, the imbalanced bradykinin system and systemic inflammation form an environment of increased coronary blood flow, moving preexisting atherosclerotic plaques towards embolic events. Last but not least, oxidative stress and over-activation of sympathetic nerve system are possible pathophysiologic mechanism for CV events during COVID-19 [33, 34].

Pending information rise each every day as COVID-19 continues to thrive worldwide and researchers work unstoppably, with the most recent information highlighting a subset of autoimmunity and autoinflammation in COVID-19, a theory confirmed by common complications of autoimmune etiology, such as Guillain–Barré syndrome and its variants, immune thrombocytopenic purpura (ITP), antiphospholipid syndrome (APS) and thrombotic thrombocytopenic purpura (TTP) [35].

Clinical Features and Diagnostic Tools

Infection by SARS-CoV-2 is categorized as asymptomatic, presymptomatic and symptomatic. The viral RNA is detected up to 6 days early before onset of symptoms, reaching peak concertation alongside with the beginning of symptoms, while it becomes undetectable in the upper respiratory tract about 15 days after the symptoms start. In the lower respiratory tract, the viral load reaches higher concentrations, which also peak later and remain longer than in the upper tract. Approximately 33% of those testing positive, will remain asymptomatic and as many as half of those will develop symptoms later. The vast majority of patients (over 80%) suffer a mild disease. Symptomatic cases are further divided into critical, severe, and non-severe. Critical cases (up to 5%) are ones presenting with ARDS, multiorgan injury, sepsis, septic shock or other conditions requiring advanced support and life sustaining therapies, mechanical ventilation and/or vasopressors. Severe (over 15%) are those cases presenting with hypoxia, respiratory failure, signs of respiratory distress (accessory muscle use, inability to speak in full sentences) or CT findings of lung involvement over 50%. Any patient not meeting criteria for critical or severe disease is categorized as non-severe [36, 37].

In the early stages of the pandemic, high rates of hospitalization and mortality were suggested by studies, but nowadays, novel therapeutic agents, getting more and more acquainted with COVID-19 itself and most importantly vaccination strategies, have allowed risks of hospitalization, mechanical ventilation, and mortality to fall. Specifically, initial mortality rates were reaching up to 20%, but as our knowledge and research progresses, ICU survival rates rise variably from 58% to 80%, ICU admission requirement falls to 35%, and all case – of either severity- fatality rate is under 2%. This percentage is strongly dependent on age and rises to 6.4% over the age of 60 years, 13% over 80 years and 25% over 90 years [38].

The main symptoms of COVID-19 include respiratory and other systemic symptoms. The first ones commonly include sore throat (5-14%), rhinorrhea (4-24%), cough (up to 82%) and dyspnea (up to 64%). Systemic symptoms of SARS-CoV-2 infection usually are fever (44-98%), headache (about 10%), diarrhea, nausea, vomiting, skin rash, fatigue, anosmia, ageusia and a plethora of other manifestations ranging from cardiovascular and gastrointestinal to even neurological disorders. Headaches, neuropathy, myalgia, encephalitis, encephalopathy are frequently observed as a result of the virus' neuroinvasive tropism, to the transsynaptic cells of the olfactory bulb and pulmonary sensorial receptors. Hepatic and renal impairment can also occur either directly due to the disease or as a result of the applied therapeutic interventions [39, 40].

Imaging in COVID-19 consists mainly of chest X-ray (CXR) and CT scan. Frequent CXR findings in severe disease are multifocal opacities of the interstitial space (72%), or alveolar opacities usually affecting both lungs, with two-third of patients exhibiting bilateral findings. The CT scan, on the other hand, has the advantage of diagnosing morphological abnormalities of the lung in the early stages of infection, and has proven an important technique for SARS-CoV-2 negative patients with low viral load. It was proposed as a diagnostic tool but was quickly proven ineffective, missing out those cases where the lung parenchyma is not involved, eventually exposing health care workers to the virus. It is now considered a way for stratification of patients and a supplemental diagnostic tool [41].

Hospitalized patients with COVID-19 pneumonia share common laboratory findings such as leukopenia (25%), leukocytosis (25%), lymphopenia (63%), elevated aminotransferases and lactate dehydrogenase (LDH) and thrombocytopenia. Inflammation cascade is depicted also in the increased inflammatory markers, such as serum C-reactive protein, IL-6 and ferritin levels. Those findings have been found to be strongly associated with clinical severity and mortality risk. Finally, as a result of myocardial injury or coexistence of pulmonary embolism or both, troponin and d-dimmer levels are usually high in COVID-19 patients [42].

The wildfire like spread of the SARS-CoV-2 pandemic created the need for quick, easy to use, sensitive and specific novel diagnostic tests, with many being designed and proposed over the last 3 years. Nowadays three types of tests dominate disease management and public health control. The first

one detects viral RNA using molecular or nucleid acid amplification tests (PCR), the second detects viral proteins (antigen tests) and the last one finds specific host antibodies produced either via infection or via vaccination (serology tests). Out of three, molecular tests present the highest specificity and sensitivity in diagnosing acute infection and are recommended by the WHO. Respectively to the technical restrictions or the amount of time needed for a result, PCR remains the "gold standard" in the diagnosis of COVID-19. Rapid antigen tests, also indicating acute infection, with a clinical sensitivity higher during the first week of illness, reaching up to 80% according to test brand, and a specificity over 97%, could be considered even a replacement of RT-PCR, if immediate decisions about patient care can be made. They are easier to perform, cost-effective and provide a fast result, mainly for those who are most likely spreaders of the virus. Rapid antigen detection tests are a valuable tool to ensure safe travel, schooling, social activities, and are currently considered as standard of care in screening individuals being at enhanced risk in the community, such as health care providers. The third category of tests, the serology ones, detect antibodies against SARS-CoV-2, indicating past infection and after vaccine roll-out, can only be of population surveillance use, as means of public policy. Serology tests do not provide proof of immunity, as it is still unclear how long the last one lasts. No matter the availability, it is of cardinal importance to choose the right test, in the right specimen at the right timing [11, 40, 42, 43].

Poor prognostic comorbidities in the course of COVID-19 infection include age, obesity, diabetes, cardiovascular disease, cancer, autoimmune diseases, chronic obstructive pulmonary disease (COPD) or history of transplantation. Specifically, obesity is independently associated with increased mortality and need for mechanical ventilation, even four times higher than the general population. Clinical prognostic factors associated with higher mortality, longer hospitalization and ICU admissions are numerous with the most common being initial oxygen saturation < 88%, lymphopenia, thrombocytopenia, C-reactive protein (CRP) > 200 mg/L, ferritin >2500 ng/mL, D-dimer >2500 ng/mL, elevated troponin and lactate dehydrogenase, acute kidney injury and acute hepatic impairment [44].

Complications

It is well understood that COVID-19 is not only a "flu-like disease" or simply "a virus causing ARDS", but a viral infection with acute and chronic multiorgan complications, indicative of its dynamics and diversity, like no other coronavirus. One of the most important complications addressed during a SARS-Cov-2 infection are the cardiovascular ones. The true epidemiology of these adverse effects is difficult to establish. While the hospitalized and severely ill patients with ARDS and possible under mechanical ventilation might develop cardiac complications due to therapeutic strategies, in the non-hospitalized, mild cases such condition seems to be underdiagnosed. The most frequent effect is myocarditis, with an estimated incidence of 22 cases per 100000 and many difficulties in its definite diagnosis. Cardiac injury in general, is reported in 19-18% of COVID-19 patients, a percentage

including myocardial infarction, heart failure, arrhythmias, and thromboembolic disease, all associated with increased length of hospital stay and mortality. Roughly, 1 out of 4 patients is affected, with hypotension, tachycardia, acute coronary syndromes and pulmonary embolism, regardless of preexisting coronary artery disease, diabetes, hypertension, obesity and chronic kidney disease. The most frequently encountered arrhythmia (20%) newly found in COVID-19 patients is atrial fibrillation, while cases of acute heart failure sometimes unmask a subclinical preexisting heart condition [45, 46].

Coagulation is also commonly disturbed in the course of SARS-CoV-2 infection in many ways, with a prevalence up to 79%, varying from laboratory findings alone (elevated d-dimer, fibrinogen, low platelet count) to venous thromboembolism, pulmonary embolism, disseminated intravascular coagulation (DIC) or sepsis induced coagulopathy (SIC). This state of sever hypercoagulability is depicted by the fact that many patients have been reported to develop such complications despite receiving prophylactic heparin [47]. Another group of COVID-19 complications are thrombotic microangiopathies, characterized by the triad of thrombocytopenia, microangiopathic hemolytic anemia and end-organ capillary thrombosis [48].

Reportedly, renal and liver impairment are also common in COVID-19. Acute kidney injury and exacerbation of chronic kidney disease have a prevalence of 5-23% and include elevation of blood creatinine and urea, microscopic hematuria, proteinuria and histopathological changes [49]. On the other hand, liver injury has a more complex pathophysiology, with the infection causing a mild and self-limiting injury, but cardiac injury and therapeutic targets also playing an important role in its course. The severity of the injury is associated with the severity of the disease in acquaintance with ARDS, acute cardiac injury and acute kidney injury. Coexistence of other viral hepatitis, cirrhosis or use of hepatotoxins (statins, antibiotics, paracetamol, non-steroid anti-inflammatory agents, antiviral agents, tocilizumab) should also be taken into account [50].

Another aspect of complications to be considered during COVID-19 are the neurological ones, with an estimated of 2.3% incidence of acute ischemic stroke and others reportedly being cerebral venous (sinus) thrombosis, epilepsy, meningitis, encephalitis and meningoencephalitis, acute myelitis, Guillain–Barré syndrome (GBS), posterior reversible encephalopathy syndrome (PRES) [51]. There is also a wide variety of autoimmune disorders that have been related to SARS-CoV-2. Other than a known -during infection- predisposition to systemic autoimmunity (cytokine storm syndrome), reactive arthritis, connective tissue disorders, drug-induced lupus, haemolytic anemia, immune thrombocytopenia, cutaneous vasculitis and pulmonary fibrosis are among the autoimmune complications of the disease. Moreover, autoimmune diseases might flare in the course or after COVID-19. The off-label use and withdrawal of antirheumatic agents in the treatment (like tocilizumab or anakinra), might paradoxically induce autoimmunity due to transient immunosuppression followed by inappropriate immune reconstitution [52].

Last but not least, a distinct condition presenting after the acute phase of COVID-19 is “long COVID syndrome”. It encompasses an extremely broad spectrum of cardiopulmonary and neurologic symptoms, associated with a prolonged inflammatory reaction. COVID-19 survivors may suffer from long-term pulmonary complications, such as dyspnea, chronic cough, hyperventilation syndrome, impaired exercise capacity and inhaled medication or oxygen dependent restricted pulmonary disease. Ongoing cardiovascular inflammation and endothelial damage contribute to the development of hypotension (1 in 2 patients), tachycardia (3 in 4 patients), derangement of existing hypertension, cardiomegaly, bradycardia, and postural tachycardia syndrome (POTS) [53]. Impairment of the glucometabolic control is the most important consequence of COVID-19, increasing the predisposition to cardiovascular events and presenting as glucose homeostasis impairment, hyperlipidemia, hypertriglyceridemia and/or alterations in thyroid function. In regard to neuropsychiatric complication, long COVID is characterized by fatigue, chronic widespread musculoskeletal pain, disturbed sleep, anxiety, migraine, manifestations of vast importance to the patients’ normal return to work and social life. Even though the syndrome is yet to be defined and is underdiagnosed, it is among the COVID-19 complications that need special attention by physicians [54, 55].

Treatment

Since the emergence of COVID-19, the entire scientific community has unstoppably worked trying to deconstruct specific particularities of the virus and organize clinically applicable therapeutic options. As the virus spreads rapidly across the universe, causing millions of deaths, profound negative pressure on healthcare systems and a worldwide socioeconomic crisis, clinicians are faced with desperation, hence therapeutic options are scarce and lack evidence base. Even with the support of tenuous data and hypotheses, clinical practice reached for last resort solutions, subsequently proven harmful, like the initial wide use of hydroxychloroquine or the suggestion for avoidance of systemic corticosteroids. Two years later, still numerous clinical trials, randomized and non-randomized controlled trials and relevant meta-analyses are issued, laboratories all over the world conduct novel research, while clinicians try to formulate the results and unify them in clinical practice guidelines.

Several therapeutic protocols and agents have been evaluated for the treatment of COVID-19, as healthcare systems around the world experience immense stress and the public health crisis is depicted in business closures, trade disruption, tourism industry devastation and social distancing. The goal is to support the patients with pharmacological and non-pharmacological interventions that will firstly significantly decrease all-cause mortality, along with intubation rates and post-COVID-19 syndrome prevalence. A first approach that seemed promising at first was the repurposing of the antiviral agents used in ARDS due to SARS-CoV-1 and MERS-CoV, such as antiretroviral, immunoglobulins and convalescent plasma, a tactic proven ineffective. The clinicians and researchers, then, turned their

attention to other repurposed drug regimens, in forms of immunosuppressants, as the pathophysiology of the cytokine storm in COVID-19 became clearly established. The idea was to reuse known drugs with established pharmacokinetics and pharmacodynamics and simultaneously reduce the time and cost of developing a new agent amidst an ongoing pandemic. On the other hand, intensivists worked sleeplessly to develop oxygen support regimens, expanding the use of non-mechanical ventilation techniques and teaching unspecialized personnel to use them. Updated practice guidelines are being published frequently by various societies worldwide, as research advances, to assist practitioners.

Non-Pharmacologic Interventions

As mortality in COVID-19 is mainly attributed to ARDS and its subsequent hypoxemic respiratory failure, initiation of respiratory support, timing and form are the most important component of the diseases therapy. Noninvasive support is preferred and when applicable can be delivered via low-flow nasal cannula (LFNC), simple face masks, Venturi masks, non-rebreather masks, high-flow nasal cannula (HFNC) or non-invasive mechanical ventilation (NIMV). The goal is to treat the patient's hypoxia without needing to proceed to mechanical ventilation (intubation), a step that significantly increases mortality and secondary complications. It is a tactic thoroughly researched in ARDS management and cautiously used in its' treatment for the past 10 years [56].

More specifically, based on the cause and severity of the acute respiratory failure, a different device might be applicable. LFNC, simple face masks and Venturi masks are generally used for hypoxemic patients with no need of mechanical support. On the other hand, NIMV delivers simultaneously supplemental oxygen at a prescribed fraction of inspired oxygen (FiO2) and mechanical support using a tight-fitting mask (nasal, oronasal, full-face), producing either continuous positive airway pressure (CPAP) or bilevel positive airway pressure ventilation (BiPAP). NIMV, therefore, improves gas exchange and alveolar recruitment and decreases the work of breathing. Although the use of NIMV in exacerbations of COPD, cardiogenic pulmonary oedema and in weaning from invasive mechanical ventilation is well established, evidence remains unclear in acute respiratory failure. ARDS treatment apparently has better recovery rates using CPAP than BiPAP, which is, among others, better tolerated by the patients. Nevertheless, both techniques inhibit mobilization, restrict communication and nutrition and are described as dysphoric. The overwhelming number of patients requiring ventilation support during the pandemic, has led to a massive usage of NIMV even in non-ICU beds and was the motive for a lot of research. Recent meta-analysis supports the application of NIMV outside the ICU in patients with COVID-19 [57].

On a similar page of avoiding intubation, high flow nasal cannula (HFNC), a device previously mostly used in neonatal ICUs, is frequently used in COVID-19 with many data supporting its efficiency. The device delivers high flows of oxygen and humidified air through a wide bore nasal cannula and is

feasible in adults experiencing acute respiratory failure, or at risk of acute respiratory failure. In a former study it was supported that HFNC may result in less treatment failure in comparison to standard oxygen therapy, a result not seen when compared to NIMV [58]. Another recent meta-analysis shows that HFNC application reduces 28-day mortality and length of hospital stay but does not confer a significant risk reduction in avoidance of intubation compared to NIMV [59]. The devices' efficiency, non-inferiority to NIMV and better tolerability profile are the main reason that it is the main NIMV broadly used in COVID-19 patients with acute respiratory failure.

When all non-invasive measures fail, invasive mechanical ventilation (IMV) through intubation is the only solution. Even though it is publicly considered a lifesaving step, it is associated with multiple complications, such as secondary infections, prolonged sedation with associated neurocognitive disorders, increased ICU stay, increased mortality and increased prevalence of post-extubation related life-long disability. The regional differences from ICUs treating COVID-19 patients around the world, in terms of mortality rates, support the need for optimal ventilation strategy protocols [60]. Another important aspect is the timing of intubation. Although several practice guidelines recommend early intubation in COVID-19 associated ARDS, it is still unclear, as large meta-analysis evidence showed that this may have no effect on mortality rates, suggesting withholding intubation tactics [61]. The topic remains unclear, but it is unanimous that IMV should by all means be avoided when possible.

Extracorporeal membrane oxygenation (ECMO) is a salvage strategy for severe ARDS patients in whom IMV fail to obtain sufficient oxygenation, using the cardiopulmonary bypass technology to perform gas exchange. It was shown early in the course of the pandemic that ECMO reduced the in-hospital mortality, with rates similar to those with non-COVID-19 ARDS [62]. In such an event of ECMO initiation, advanced age, other comorbidities, vasopressor support, active bleeding, low pre-ECMO pH are predictors of worse outcome, while the duration of MV prior to ECMO and total ECMO duration appearing to be similar between survivors and non-survivors [63].

Finally, a very famous technique of non-pharmacological support in patients suffering from COVID-19 ARDS, both intubated and non-intubated, is prone positioning. Its' potential efficacy is yet to be well-defined in terms of contraindications and duration, but there has been a rising interest with cohort studies and trials in the run. Prone positioning in awake patients is easily applicable and less time consuming in comparison to intubated ones, and acts with the same pathophysiological mechanism of gravity-assisted diversion of pulmonary blood flow to dorsal regions recruiting more alveoli. Evidently, it is still unclear how often, for how long and which patient is eligible. Recent meta-analyses in intubated COVID-19 patients exhibit potential benefits in oxygenation and mortality rate, but in the non-intubated, prone positioning does not appear to have a significant effect on critical care admission or incidence of intubation [64, 65].

Pharmacologic interventions

The usage of **corticosteroids** in serious infections and in ARDS has -for years- been a continuous controversy, but during the pandemic, the absence of potent therapies and the rising numbers of critically-ill patients have acted as a stimulus for researchers to address this flaming matter. The pathophysiology of ARDS is based on an innate immune cell mediated response causing lung alveoli damage. It has long been hypothesized that corticosteroids due to their anti-inflammatory and antifibrotic properties are ideal agents against the ongoing pulmonary and systemic damage of hyperinflammation in ARDS, regardless its' etiology. Despite the fact that many randomized controlled trials have shown beneficial effects with the use of corticosteroids in ARDS, observational data in certain subtypes of ARDS, such as in influenza, suggest that they may raise mortality and increase opportunistic infections. Of note, those trials were mostly performed in patients with ARDS ventilated with a non-protective regimen, hence being uncertain of the result of a lung protective regimen in combination with steroids [66]. These data resulted in the addition of a recommendation for corticosteroid use in patients with ARDS and PaO2/FiO2 ratio < 200 by the 2017 SCCM (Society of Critical Care Medicine) and ESICM (European Society of Intensive Care Medicine) guidelines for the Diagnosis and Management of Critical Illness-Related Corticosteroid Insufficiency (CIRCI) [67].

While clinical practice so far was variable and many questions regarding initiation of treatment, type of steroid, optimal dosage and duration of therapy remained unanswered, the lack of other therapies in COVID-19, pushed research down the path of the easy to find and use, cost effective and well-established glucocorticoids. Subsequently, as of July 2020 more than 60 studies for the treatment of COVID-19 with corticosteroids had been registered at ClinicalTrials.gov. At the same time, it has been shown that COVID-19 ARDS can be treated similarly to non-COVID-19 ARDS as far as corticosteroid regimens is concerned [68]. The hypothesis of a beneficial steroid therapy in COVID-19 ARDS is based on its' potency to downregulate the cytokine storm and is well known from autoimmune disease patients, where flares of their disease, macrophage activations syndrome (MAS), cytokine release syndrome (CRS), are mainly treated with high doses of steroids. With the addition of COVID-19 randomized controlled trials and their large numbers of patients, data concerning ARDS in general are becoming more precise, while subgroup analyses about certain populations, comorbidities and administration regimens are more comprehensive.

Most COVID-19 treatment protocols suggested the use of dexamethasone, as a result of a large meta-analysis published in the early 2020, supporting its' advantages [69]. Dexamethasone is 20 to 30 times stronger than the natural hormone cortisol and up to 5 times stronger than prednisone, producing a strong anti-inflammatory effect, but the weakest mineralocorticoid effects in contrast to the rest steroids. With a biological half-life of 36 to 54 hours, its' pharmacokinetic allows daily one-dosage regimens.

One of the largest RCTS in England, during the first year of the pandemic, the UK-based Randomized Evaluation of COVID-19 Therapy (RECOVERY) trial, randomized 6425 patients to a daily dosage of 6 mg dexamethasone or standard of care, ultimately suggesting an overall reduction in mortality of 2.8%, with a much greater benefit for patients under IMV at the time of randomization (mortality 29.3% for dexamethasone vs 41.4% for usual care, rate ratio 0.64 [95% CI, 0.51-0.81])[69]. The RECOVERY trial showed an absolute reduction in the risk for death by 12.1% in mechanically ventilated patients assigned to low-dose dexamethasone [70]. In a meta-analysis conducted by the WHO Collaboration, also in the early stages of the pandemic, administration of corticosteroids was associated with lower all-cause mortality at day 28 and with no suggestion of an increased risk of serious adverse events. The analysis suggested similar odds for mortality with either dexamethasone or hydrocortisone, while no evidence supporting high over low dose was found [71]. Similarly, in the subgroup analyses, no difference in mortality was found between younger and older individuals, men and women, short and long duration of symptoms. On the other hand, lower mortality was demonstrated in patients not receiving vasoactive medication at randomization than in those who were receiving under vasopressors [71].

All these data moved towards the conclusion that dexamethasone is beneficial in severe COVID-19, making it an important step in almost all treatment protocols around the world. In the latest meta-analysis, corticosteroid treatment had no impact on mortality in 18190 COVID-19 patients and researchers concluded that dexamethasone, upon early initiation, may reduce mortality rate by 5%, while the risk of ICU admission and IMV initiation and duration of hospitalization, are comparable between those receiving steroids and controls [72]. Researchers speculate that a certain group of patients with hyperinflammatory response is that will benefit most, whilst more and more data from RCTs all around the world await to be investigated upon.

A treatment option that received substantial interest, especially for mild to moderate COVID-19 patients that are not hospitalized, are inhaled corticosteroids. The PRINCIPLE study, was an open-label, multicentre, multi-arm, randomized controlled trial, that included non-hospitalized patients older than 65 years or older than 50 years with comorbidities. It established a positive effect of inhaled budesonide in time needed to recover and proposed a possible reduction of hospital admissions and deaths. Nevertheless, it is still unknown which the right dosage is and what is the exact mechanism of inhaled corticosteroids in reducing time to self-reported recovery. Moreover, trial participants are scarcely vaccinated and of older age, so important questions about the impact of inhaled steroids on younger populations and vaccinated individuals remain. Recent meta-analysis provides a promise for inhaled corticosteroids, particularly in the elderly, and confirms reduction in recovery time [73-75].

Although it is profound medical knowledge that antibiotics are not to be used to treat viral infections and antibiotic resistance grows yearly upon unnecessary antibiotics' prescription, tons of

boxes of them have been prescribed during the pandemic, mostly as a treatment towards the doctors' fears, of complications in patients under quarantine with no physical examination and follow-up. **Azithromycin** is the most widely used antibiotic in the management of COVID-19, due to its' established anti-inflammatory and immunoregulatory effects in the bronchial epithelial cells and the knowledge derived from its' use in other viral pneumonias being associated with improved outcome. It is a broad-spectrum antibiotic, cost effective, easy to find, with rapid absorption after oral intake and long half-life (68 hours). The only concerning adverse effects is QT prolongation and cardiotoxicity. Various trials and studies evaluated the use of this drug in non-hospitalized patients, with PRINCIPLE trial showing no evidence of any benefit in mild disease [76]. Similarly, in patients with moderate COVID-19 not requiring hospitalization, the addition of azithromycin had no impact on the risk of hospital admission or death, compared to standard of care [77]. Evidence is consistent from the relevant meta-analyses, with data from all continents, presenting strong evidence that the use of azithromycin is of no benefit but also of no harm [78, 79].

Colchicine, a frequently used and well known to physicians anti-inflammatory drug, was also tested and widely prescribed for SARS-CoV-2 infection. The rationale for its' use is based on colchicine's pharmacodynamic, as it inhibits interleukins and more specifically the NLRP3 (NLR Family Pyrin Domain Containing 3) inflammasome. When macrophages dendritic cells and other antigen presenting cells are invaded by a virus, this multiprotein complex (NLRP3) is activated and initiates the cytokine release response. During the course of COVID-19, there is overactivation of this complex, leading to overproduction of cytokines and multiorgan damage. Subsequently, colchicine appeared promising in the inhibition of the cytokine release syndrome, through the inactivation of the NLRP3. Various protocols have been reported worldwide with either inpatient or outpatient use of colchicine, but mostly further analyzed data concerning hospitalized patients. Apparently, colchicine reduces the length of hospital-stay and prevents clinical deterioration, decreases CRP levels and d-dimer levels, but has no effect on all-cause mortality and MV initiation. Subgroup analyses show an enhancement in these effects in diabetic and obese COVID-19 patients, creating an insight for the future for these specific groups to be further analyzed [80, 81].

Hydroxychloroquine was firstly a drug developed to treat malaria, but with the discovery of its' many other drug properties, it has become an important therapeutic agent in autoimmune, rheumatological and dermatological diseases. In the beginning of the pandemic, large expectations were based on hydroxychloroquine and misinformation led people preventively buying the drug over the counter, resulting in a sudden shortage of backlog. Soon it became evident that the possibility of cardiac adverse events from the use of this agent was greater than the benefit. Patients with COVID-19 receiving hydroxychloroquine reportedly suffered from increased incidence of cardiac complications and arrythmias, such as atrial fibrillation, atrial flutter, ventricular tachycardia, ventricular fibrillation, a

danger difficult to quantify. Nevertheless, combined data from the conducted trials do not support the use of hydroxychloroquine in the management of COVID-19 patient, as it did not change mortality or mechanical ventilation need [82, 83].

Remdesivir is a nucleotide prodrug with intracellular metabolism to active ATP, allowing it to interfere with viral-RNA-dependent RNA polymerase activity, thus it inhibits viral RNA. It is an antiviral agent originally designed for the treatment of Ebola, although the actual pharmacology and pharmacokinetics of the drug within the respiratory tract remain unknown. In the absence of other possible treatments and the increasing burden of the pandemic, it was approved on 14 November 2021 by the U.S. Food and Drug Administration (FDA) for the treatment of adults hospitalized with COVID-19 [84]. Former studies have shown a significant difference in clinical course between standard of care and administration of remdesivir for 5 days, while no significant benefit was documented with extension of administration to 10 days. On the other hand, trials showed that this drug does not accelerate recovery or viral clearance, suggesting that remdesivir's effectiveness is not associated with viral load. Large meta-analyses of relevant data conclude that it has little or no difference in mortality, but it may reduce hospitalization days, time to recovery and serious adverse events [85, 86]. It is the most widely used COVID-19 therapy, second to dexamethasone, nevertheless the WHO recently published a conditional recommendation against its use in inpatient setting, surpassing its' proven effectiveness in early stages of the disease [87].

Molnupiravir is a new antiviral agent that inhibits SARs-CoV-2 replication, acting as the isopropyl-ester prodrug of the ribonucleoside analogue β-D-N4-hydroxycytidine. It exhibited in vitro evidence of strong potency to rapidly reduce viral load in the submicromolar range and has received so on considerable attention as a possible therapeutic agent [88]. While evidence is still conflicting and research remains to advance, some studies showed a reduction in the risk of hospital admission or death by approximately 50% in the outpatient setting [89].

Another antiviral agent being tested in COVID-19 patients is **Lopinavir/Ritonavir (L/R)**, supported by its' efficacy in SARS-CoV-1, established in 2004 [90]. Both of the drug components are protease inhibitors. Lopinavir at a usual dose of 400mg is prescribed always in combination with low-dose ritonavir (100mg), which pharmacokinetically enhances its activity by inhibiting CYP450 metabolized protease inhibitors. Many protocols recommended its' use during the first months of COVID-19 emergence and observational studies and RCTs started evaluating its treatment efficacy for SARS-CoV-2. Meta-analyses with meta-regression analysis of those data found no difference in mortality or initiation of MV but showed some benefit in reduction of time to recovery and days of hospitalization [91, 92].

Last but not least, Nirmatrelvir boosted with ritonavir (marketed as **Paxlovid**) was recently added to COVID-19 therapeutics, targeting the major SARS-CoV-2 protease (Mpro). Mpro is

responsible for polyprotein processing through more than 11 cleavage sites and because it is highly conserved across SARS-CoV-2 variants, it is a strong subset in designing potent therapy protocols. It can be administrated in adult patients with mild to moderate disease, suffering from high-risk comorbidities in matters of disease progression, in the outpatient setting. Treatment must ideally start within 5 days from symptom onset, with a special attention to drug interactions, as ritonavir is a CYP450 inhibitor. Paxlovid is currently widely prescribed and used, and scientific community awaits with great interest the results from larger, forthcoming RCTs [92, 93].

The antiparasitic agent **ivermectin** can in vitro inhibit viral replication, thus supporting a molecular hypothesis that it might be active against SARS-CoV-2 in the early stages of infection as treatment or prevention after exposure. More than 30 studies have put its efficacy and safety into test with conflicting evidence, while the most important problem to surpass is that it would take major doses in humans to reproduce the in vitro positive result against the virus. It has not been officially approved for COVID-19 and can only be used in well-designed trials [94]. Evidence show that it provides the safety profile needed to be used in larger clinical trials for the management of COVID-19, although it possibly is not significantly associated with improvement in surrogate outcomes [95].

Intravenous immunoglobulin (IVIG) is a polyclonal IgG antibody derived from the blood of healthy donors, with strong anti-inflammatory properties when used in immunodeficiencies, autoimmune diseases and other inflammatory conditions. It enhances the recipients' passive immune response against common pathogens and therefore is valuable in the course of severe infections with an additional advantage of high safety and tolerability. Subsequently, it was an easy candidate therapy for severe COVID-19 [96]. IVIG treatment in mild and moderate COVID-19 patients did not exhibit any significant benefit when added to standard of care [97]. On the other hand it showed a significant benefit in critically ill patients, via reduction of mortality rate and therefore, it could be a potential treatment option in selected, critical cases [98].

On a similar treatment rationale basis, it was hypothesized that individuals who had recovered from COVID-19 could act as donors of **convalescent plasma**, in order to close the time gap between infection time and the innate triggering of a sufficient immune response. The use of convalescent plasma in COVID-19 has attracted spotlight and intrigued research, especially for immunocompromised patients. In a recent meta-analysis of 16 RCTs with over 16000 COVID-19 patients, routine use of convalescent plasma was not efficacious enough, as its administration did not confer a significant risk reduction in all-cause mortality [99]. Similar results were subsequently demonstrated by further trials [100, 101]. Despite these results, because antibody response to SARS-CoV-2 is usually already present upon hospital admission, RCTs are turning to very early administration, in an attempt to test whether it might affect disease progression, with promising results [102, 103].

The establishment of the cytokine storm as a crucial mechanism in the progression of severe COVID-19, brought to the spotlight therapeutic interventions with strong ability to inhibit signaling pathways of the inflammatory response. Janus Kinase- Signal transducer and activator of transcription (JAK-STAT) is one of the most important cellular pathways, activated in survival, differentiation, and proliferation of a normal innate response, controlling the duration and quantification of cytokine release and signaling. **JAK inhibitors** (JAK1/JAK2) were developed and are commonly used for diseases with overacting JAK signaling, like autoimmune diseases such as rheumatoid arthritis (RA), psoriasic arthritis, ulcerative colitis and atopic dermatitis or myeloproliferative disorders.

In particular, **baricitinib** is a JAK1/JAK2 inhibitor used in the treatment of RA that appeared from the beginning safe and effective against SARS-CoV-2. Obviously, baricitinib's low plasma protein binding rate (50% in contrast to about 95% of ruxolitinib and fedranitib) and its' low interaction with CYP450 made it a great candidate to be co-administrated safely with the rest COVID-19 regimens [104]. Based on many observational studies and a clinical trial conducted by the National Institute of Allergy and Infectious Diseases (ACTT-2), exhibiting benefit, in terms of severity, mortality and survival, from the addition of JAK inhibitors in COVID-19 patients, on November 19, 2020, the US FDA issued an emergency authorization for Baricitinib-Remdesivir coadministration in COVID-19 hospitalized patients [105]. Meta- analyses of the data after 1 year of using baricitinib, are promising, since it is significantly associated with mortality reduction, decreased risk of ICU admission, decreased risk for initiation of mechanical ventilation and increased discharge oxygenation index. Those results appear to be dosage dependent, while baricitinib appears neutral to causing secondary infections, sepsis or septic shock [106, 107].

The medical community is faced daily, not only with severely ill COVID-19 patients, but with a continuously rising number of mild to moderate cases, especially after broad vaccination strategies. It is important to stratify those patients based on comorbidities and address their outpatient care and therapy with preventive towards deterioration tactics. For milder non-hospitalized patients, **neutralizing monoclonal antibodies (mABs)**, such as bamlanivimab, have been developed, under the rationale of passive immunotherapy. mABs are laboratory-manufactured molecules, programmed to mimic a normal immune response against an invader, directly attacking them, thus winning time over other therapies. It is a promising technique, originally developed for cancer treatment and other infections.

The neutralizing monoclonal antibodies target the spike protein in the surface of SARS-CoV-2 and prevent the virus from attaching to the human cells. Synthesis of the results from large RCTs assessing their efficacy in COVID-19 appears promising. The rate of COVID-19 related hospitalization and the rate of emergency department visits was significantly lower with mABs, indifferent in the subgroup analyses of the administration regimen [108]. Finally, mortality was significantly lower in the patients receiving mABs, whereas no significant raise in any or serious adverse events was observed.

The effect on all-cause mortality was not confirmed in a recent meta-analysis, although the outcome narrowly missed statistical significance [109]. So far, 4 neutralizing monoclonal antibodies, namely bamlanivimab with or without added etesevimab, casirivimab with added imdevimab, sotrovimab and bebtelovimab, have received emergency authorization by the FDA, for outpatient use only as a single-dose intravenous infusion.

As it was thoroughly analyzed in the pathophysiology section, cytokine release syndrome is the primary pathway of multi-organ damage and severe disease in SARS-CoV-2 infection. Hyper-inflammation is mirrored in the elevated ferritin and CRP levels and is orchestrated by several cytokines with IL-1, IL-6, IL-10, GCSF, MCP1 being the most important. This knowledge has turned the spotlight towards **immunomodulatory agents** as possible therapies in COVID-19 with a raising research interest and many clinical trials on the run, since they carry along a good safety profile and approval for the treatment of autoimmune diseases.

IL-1 blockade is succeeded in two general mechanisms, either binding to the IL-1 receptor or binding directly to IL-1. More specifically, **anakinra** is a recombinant IL-1 receptor antagonist, currently used in autoinflammatory diseases by rheumatologists, gastroenterologists, and dermatologists and has proven effective in secondary Hemophagocytic lymphohistiocytosis (sHLH) or macrophage activation syndrome (MAS), while **canakinumab** is a human anti-IL-1ß monoclonal antibody. These immunosuppressants act decreasing the over-regulation of IL-1 and IL-6, curtailing the development of the cytokine storm, all combined with a remarkable safety profile and short half-life. In this setting IL-1 antagonists were included in COVID-19 management regimens and many RCTs have been published strengthening their use in severely ill hospitalized patients.

Early data from meta-analysis of observational studies indicating clinical benefit, was recently confirmed by SAVE-MORE, a double blinded, placebo-controlled, randomized trial [110]. SAVE-MORE showed reduction in 28-day mortality risk in 594 patients, with no effect on intubation risk and bloodstream infections, with the administration of subcutaneous anakinra 100mg once daily for 10 days. Although earlier in the pandemic, this therapeutic option was faced with great optimism and first studies showed significant association with increased ventilator free days and decreased ICU admissions, their use remains conflicting [10,111]. Later on, although immunosuppressants appear to reduce mortality in COVID-19 patients, anakinra and canakinumab probably have a small or no impact on clinical improvement but with an acceptable safety profile, as a recent Cochrane meta-analysis has demonstrated [112, 113].

In a similar pathway, cytokine release syndrome has been successfully treated with IL-6 receptor inhibitors (**tocilizumab** and sarilumab) and IL-6 inhibitors (siltuximab), hence many RCTs were designed to test their efficacy and safety in COVID-19 severe cases. In a prospective meta-analysis conducted by the WHO in 2021, a total of 10930 patients enrolled in relevant RCTs, exhibited lower

all-cause mortality 28 days after randomization with IL-6 antagonists administration compared to standard of care. A significant reduction in the risk for initiation of mechanical ventilation, vasopressor support and ECMO was also demonstrated, while there was no increased risk for secondary infections compared to standard of care [114]. More recent analyses are consistent with the above results, suggesting that tocilizumab has a significant benefit in moderate to severe COVID-19, as far as 28-day mortality, mechanical support initiation, adverse effects and hospitalization duration is concerned [115].

Vaccination against COVID-19

From the emergence of COVID-19, researchers and pharmaceutical companies have been working restlessly to develop a strong and safe vaccine. It is an effort to decrease infection rate and cases of severe illness, to relieve the "overwhelmed" public healthcare systems and limit some of the collateral socioeconomic crisis components. Most importantly, it is an effort to save lives and prevent further harm. More than 2 years into the pandemic, several vaccines have been authorized by regulatory authorities and the WHO, based on large RCTs results, with efficacy results having a median follow-up time of only 2-3 months. The ideal vaccine should have an acceptable safety profile and proven efficacy, preventing infection or transmission or both for at least 6-months after administration.

The first vaccine to receive Emergency Use Authorization was the BNT162b2 (Comirnaty®: BioNTech and Pfizer) on December 2020 after confirmation of 95% efficacy against severe illness in adults [116]. Since then, according to the WHO's latest report on vaccine development on 24 May 2022, 160 COVID-19 vaccine studies are in different clinical trial phases and an additional of 198 vaccines are at preclinical stage [117]. Moreover 65.8% of the world population has received at least one dose of a COVID-19 vaccine, with a total of 11.79 billion doses having been administered globally, while 6.45 million people are vaccinated each day [118].

Despite the proven efficacy of the administrated vaccines and the undoubtable harm of the pandemic, hesitancy of the public regarding their safety results in refusing or delaying vaccination. Nevertheless, it has been demonstrated that every 10% increase in vaccine coverage, can reduce mortality rate by a significant 7.6% [119]. Simultaneously, the purpose of the vaccine itself, in means of effectiveness, is to reduce severity, mechanical ventilation and mortality. Concerning these surrogate outcomes, all vaccines irrespectively, offer a significant risk reduction. In simple words, compared with no vaccine, all types and brands of vaccines are effective in preventing ARDS and MV [120]. Research has established vaccine efficacy and safety for children, as well, with vaccination now routinely being applied for children over 5 years of age, while trials in younger infants and children are in progress [121].

As far as duration of immunity is concerned, it appears that immunological memory gained from infection persists more than 6 months. T-cell immunity may last longer, up to years, but the precise duration and protectivity of a certain antibody titer is yet to be defined [122]. Likewise, research is running

against time, trying to evaluate the duration of SARS-CoV-2 vaccine efficacy across different outcomes. Although vaccine protection against infection and mild COVID-19 symptom development appears to wane after about 6 months, efficacy against severe disease remains high. As countries worldwide continue to face surges of COVID-19, public policies turn to booster doses to the immunocompromised, the elderly and the health-care providers, in an attempt to avoid new outbreaks [123-125].

PURPOSE

The purpose of this systemic review and meta-analysis is to assess the safety and efficacy of Inteleukin-1 antagonists widely used for the treatment of moderate to severe COVID-19 disease, with special emphasis in surrogate outcomes, retracting data from the so far available randomized control trials (RCTs). Namely we will be setting death due to COVID-19 disease as the primary endpoint, and the use of either invasive mechanical ventilation (IMV), or extracorporeal membrane oxygenation (ECMO), or non-invasive mechanical ventilation (NIMV), or high flow nasal cannula (HFNO) as secondary endpoints. Moreover simultaneously, we will be assessing major safety outcomes such as secondary bacterial infections, cytopenias, acute renal injury, liver dysfunction attributed to the initiation of the treatment.

METHODS

The present systematic review and meta-analysis was conducted according to the Preferred Reporting Items for Systematic Reviews and Meta-Analyses (PRISMA) guidelines [126]. The present protocol has been registered at the PROSPERO database (University of York), with the following registration number: CRD42022324746.

We searched PubMed, Cochrane Library, clinicaltrials.gov, European Union (EU) Clinical Trials Register and medrxiv.gov databases from inception to 1st April 2022 for randomized controlled trials (RCTs) enrolling hospitalized adult subjects with documented SARS-CoV-2 infection, assigned either to an IL-1 antagonist (either anakinra or canakinumab) or control (placebo or active comparator), regardless of disease severity. Our exclusion criteria were a. observational studies, b. case series or case reports and c. studies performed in the pediatric population.

We utilized the following search terms: “anakinra”, “kineret”, “canakinumab”, “interleukin-1 receptor antagonist”, “anti-IL-1β monoclonal antibody”, “coronavirus disease-2019”, “COVID-19”, “SARS-CoV-2”, “severe acute respiratory distress syndrome coronavirus 2”, combined with the use of Boolean operators “AND” and “OR”. We used both free-text words and Medical Subject Headings (MeSH) terms. We did not imply any filter regarding study setting, study sample, publication language or publication date. Complete search strategy is provided in supplementary appendix (supplementary table 1).

We set as primary efficacy outcome the death in the context of SARS-CoV-2 infection. We set as secondary efficacy outcome the need for invasive mechanical ventilation (IMV) or initiation of extracorporeal membrane oxygenation (ECMO) and the composite of non-invasive mechanical ventilation or initiation of high-flow oxygen (HFO). We also assessed major safety outcomes, with

emphasis on secondary bacterial infections (bacterial sepsis and septic shock), neutropenia and transaminasemia.

After de-duplication and assessment of eligible studies at title and abstract level for potential inclusion in our systematic review, two independent reviewers (A.D. and D.P.) extracted the data of interest from the eligible reports, by using a pilot tested, data extraction form. Among the extracted information, we included the following: first author, year of publication, year of study conduction, study setting, study sample and main baseline demographic characteristics of enrolled participants.

We sought to conduct certain subgroup analyses, according to the type of administered IL-1 antagonist (anakinra or canakinumab), the administration of antiviral agents (e.g. remdesivir) and the administration of corticosteroids, if such data were made available by the authors.

Differences were calculated with the use of risk ratio (RR) or mean difference (MD), for dichotomous and continuous variables respectively, with 95% confidence interval (CI), after implementation of the Mantel-Haenszel (M-H) random effects formula. Statistical heterogeneity among studies was assessed by using I^2 statistics. Heterogeneity was considered to be low if I^2 was between 0% and 25%, moderate if I^2 was between 25% and 50%, or high if I^2 was greater than 75% [127]. All analyses were performed at the 0.05 significance level, while they were undertaken with RevMan 5.3 software [128].

Two independent reviewers (D.P. and A.D.) assessed the quality of the included RCTs, by using the Revised Cochrane risk of bias tool for randomized trials (RoB 2.0) for the primary efficacy outcome [129]. Discrepancies between reviewers were solved by discussion, consensus, or arbitration by a third senior reviewer (D.M.).

RESULTS

Study selection process is depicted in the corresponding flow diagram, as provided below (figure 1). Seven RCTs were finally included in the present systematic review and meta-analysis [109, 130-135]. Two trials assessed the efficacy and safety of canakinumab [130, 131], while the rest five evaluated the efficacy and safety of anakinra, compared to control or active comparator, in patients with COVID-19 [109, 132-135]. Therefore, we pooled data from seven trials in a total of 2120 enrolled subjects.

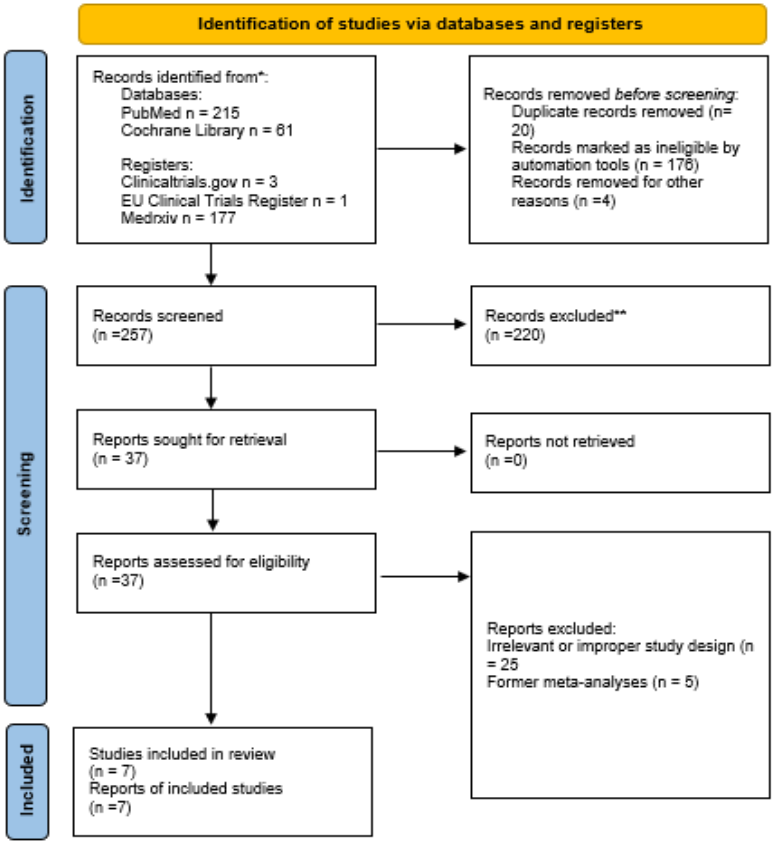
A detailed description of participants' baseline characteristics of interest across the eligible RCTs is provided in supplementary table 2.

As demonstrated in supplementary table 2, all studies enrolled subjects with documented SARS-CoV-2 infection, aged between 49 to 71 years old, with the majority of them (more than two-thirds) being male.

Enrolled participants were overweight or obese, with body mass index ranging from 26.8 to 30.8 kg/m^2, with significant co-morbidities. Specifically, relative frequency of hypertension ranged from 37 to 75% of diabetes mellitus from 16.3 to 66.7% of cardiovascular disease from 11 to 37% of chronic obstructive pulmonary disease from 3.7 to 21.4% and of chronic kidney disease from 0.5 to 43%.

According to given data regarding the pO2/FiO2 ratio of enrolled participants, the vast majority of them had established moderate acute respiratory distress syndrome (ARDS) at the time of randomization, with the corresponding value ranging from 106 to 220 mm Hg. Inflammatory markers, such as CRP and ferritin levels were significantly elevated, according to data reported across the selected RCTs.

Concerning the usage rates of medication of interest prior to randomization to IL-1 antagonist or placebo, corticosteroids were administered to 12-89% of enrolled subjects, while usage rates of remdesivir ranged from 4 to 75%, indicating a significant heterogeneity in the treatment algorithms followed across the eligible RCT. A detailed description of standard of care across the selected RCTs is provided in table 1.



*Consider, if feasible to do so, reporting the number of records identified from each database or register searched (rather than the total number across all databases/registers).

**If automation tools were used, indicate how many records were excluded by a human and how many were excluded by automation tools.

Figure 1. Flow diagram depicting the study selection process.

Table 1. Standard of care across the eligible randomized controlled trials.

STUDY	STANDARD OF CARE
CORIMUNO- ANA-1 (2021)	Antibiotic drugs, antiviral drugs, corticosteroids, vasopressor, support, anticoagulants, at the discretion of treating physicians
Carrichio et al. (2021)	Anti-viral treatment, corticosteroids and/or supportive care
Cremer et al. (2021)	Antivirals related to COVID-19, corticosteroids, use of convalescent plasma, and other immunosuppressive agents
Declerq et al. (2022)	Hydroxychloroquine, dexamethasone
Derde et al. (2021)	Anti-viral treatment, corticosteroids, antibiotics, and/or supportive care
Kharazmi et al. (2022)	Remdesivir, lopinavir/ritonavir, interferon, favipiravir, and corticosteroid
Kyriazopoulou et al. (2021)	Dexamethasone, remdesivir, antibiotics

Surrogate efficacy outcomes

COVID-19 related mortality

Administration of IL-1 antagonists failed to produce a significant decrease in the risk for COVID-19 death (RR = 0.93, 95% CI; 0.70 – 1.22, I^2 = 28%, p = 0.22), as shown in figure 2. Neither anakinra (RR = 0.95, 95% CI; 0.66 – 1.36, I^2 = 45%, p = 0.77) nor canakinumab (RR = 0.74, 95% CI; 0.39 – 1.41, I^2 = 0%, p = 0.36) had a significant effect on this surrogate outcome. No difference between the two treatment options was documented ($p_{subgroup}$ = 0.51).

Invasive mechanical ventilation

Administration of IL-1 antagonists did not result in a significant effect on the risk for COVID-19 related IMV (RR = 1.05, 95% CI; 0.77 – 1.42, I^2 = 41%, p = 0.13), as shown in figure 3. Again, neither anakinra (RR = 1.05, 95% CI; 0.64 – 1.70, I^2 = 56%, p = 0.86), nor canakinumab produced a significant effect on the prespecified (RR = 0.91, 95% CI; 0.61 – 1.38, I^2 = 28%, p = 0.67). No difference between the two treatment options was documented ($p_{subgroup}$ = 0.68).

Non-invasive mechanical ventilation or high-flow oxygen

IL-1 antagonists similarly did not reduce the risk for the composite outcome of non-invasive mechanical ventilation or HFO initiation (RR = 1.03, 95% CI; 0.65 – 1.62, I^2 = 0%, p = 0.9), as shown in figure 4. Neither anakinra (RR = 1.04, 95% CI; 0.62 – 1.76, I^2 = 0%, p = 0.88) nor canakinumab (RR = 0.99, 95% CI; 0.40 – 2.44, I^2 = 0%, p = 0.98) showed a significant effect on the prespecified outcome. No difference between the two treatment options was documented ($p_{subgroup}$ = 0.92).

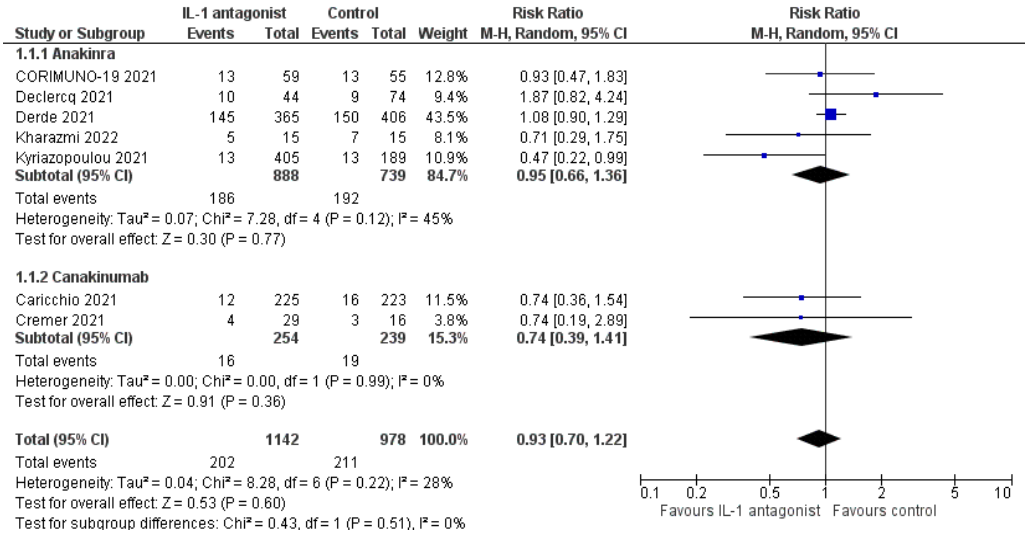


Figure 2. Effect of IL-1 antagonists compared to control on the risk for COVID-19 death.

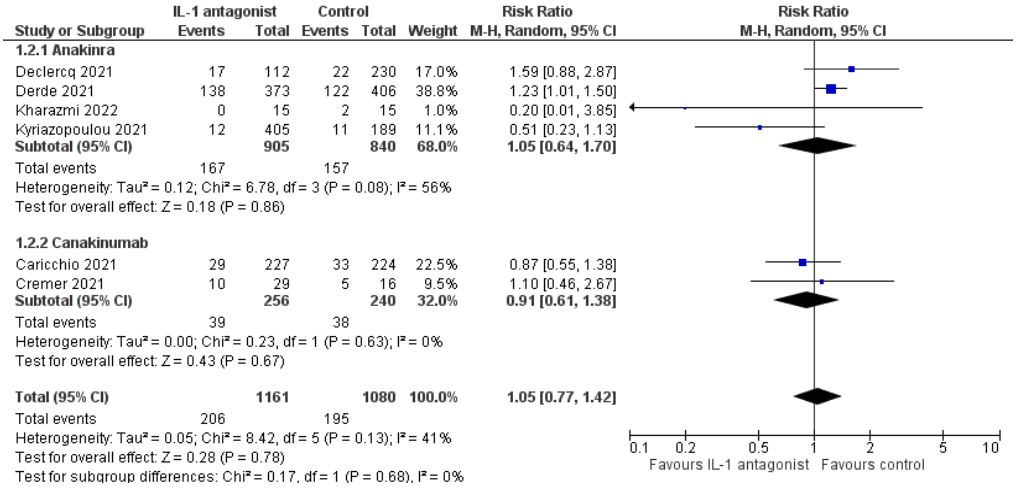


Figure 3. Effect of IL-1 antagonists compared to control on the risk for invasive mechanical ventilation due to COVID-19.

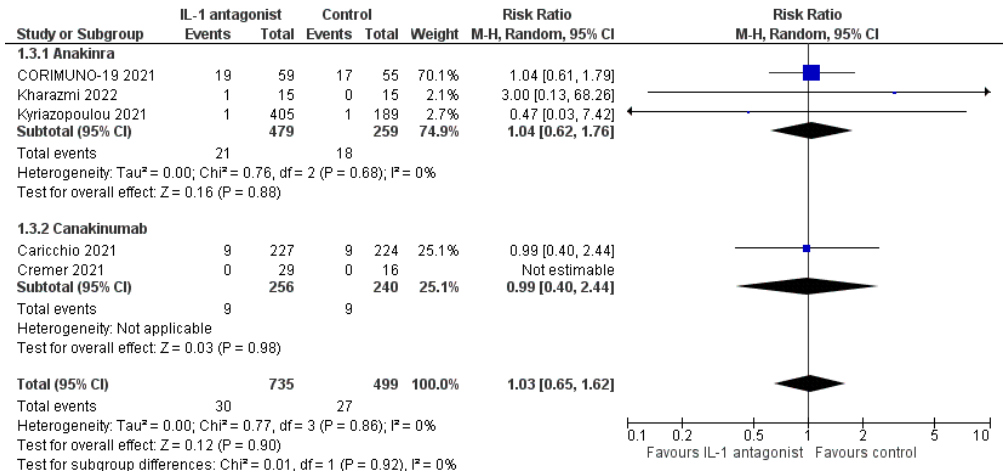


Figure 4. Effect of IL-1 antagonists compared to control on the risk for non-invasive mechanical ventilation or initiation of HFO due to COVID-19.

Safety outcomes

Bacterial sepsis

Regarding this safety outcome of interest, we pooled data from two trials with anakinra since the rest did not make corresponding data available. Overall, anakinra did not result in a significant increase in the risk for bacterial sepsis (RR = 1.82, 95% CI; 0.83 – 3.99, I^2 = 0%, p = 0.14), as depicted in figure 5.

Bacterial septic shock

For this prespecified safety outcome, only three trials assessing the safety and efficacy of anakinra in patients with COVID-19 reported relevant data of interest. Overall, anakinra did not increase the risk for bacterial septic shock (RR = 1.27, 95% CI; 0.29 – 5.45, I^2 = 65%, p = 0.75), as shown in figure 6.

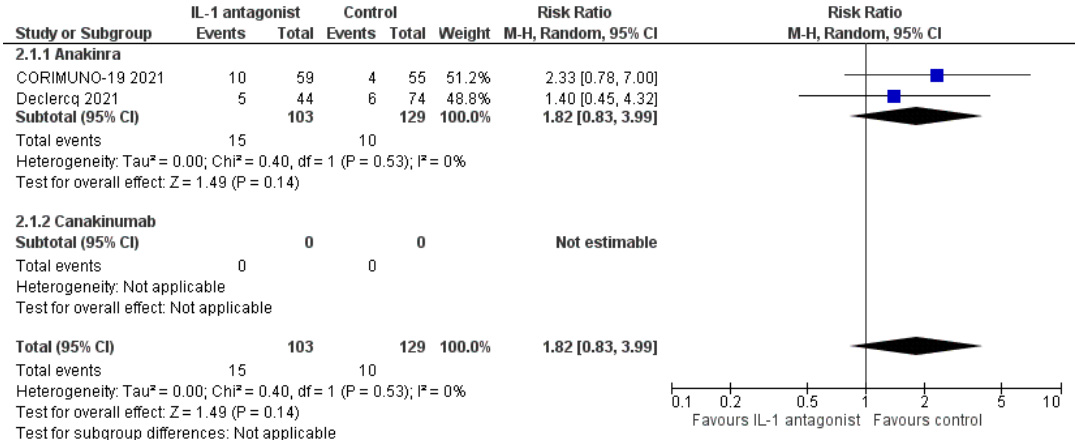


Figure 5. Effect of IL-1 antagonists compared to control on the risk for bacterial sepsis in patients with COVID-19.

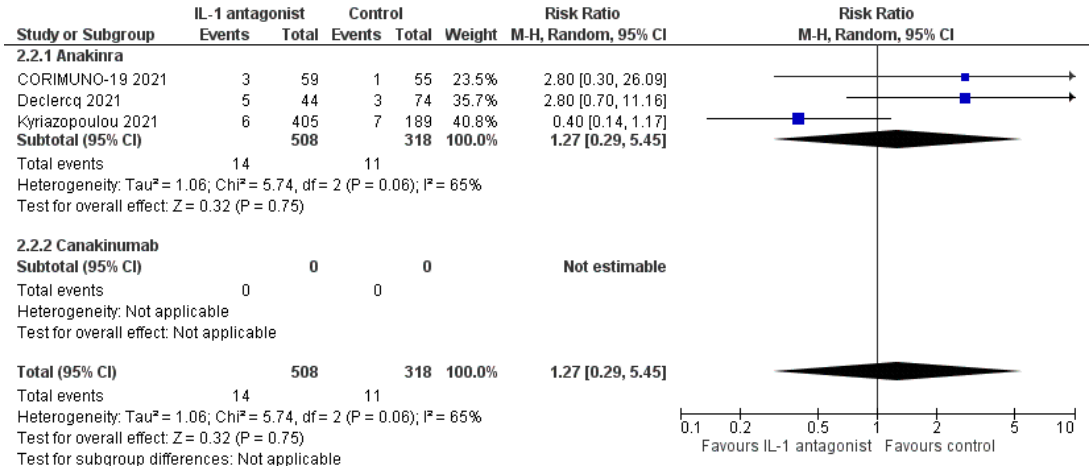


Figure 6. Effect of IL-1 antagonists compared to control on the risk for bacterial septic shock in patients with COVID-19.

Neutropenia

For this prespecified safety outcome, only two trials assessing the safety and efficacy of anakinra in patients with COVID-19 reported relevant data of interest. Overall, anakinra did not increase the risk for neutropenia (RR = 4.58, 95% CI; 0.83 – 25.39, I^2 = 0%, p = 0.08), as shown in figure 7.

Anemia

Regarding this safety outcome, we pooled data from two trials with anakinra and one trial with canakinumab. Overall, treatment with IL-1 antagonists did not increase the risk for anemia among subjects with documented COVID-19 (RR = 0.75, 95% CI; 0.53 – 1.06, I^2 = 0%, p = 0.11), as shown in figure 8. No subgroup difference between the two treatment options was identified ($p_{subgroup}$ = 0.55).

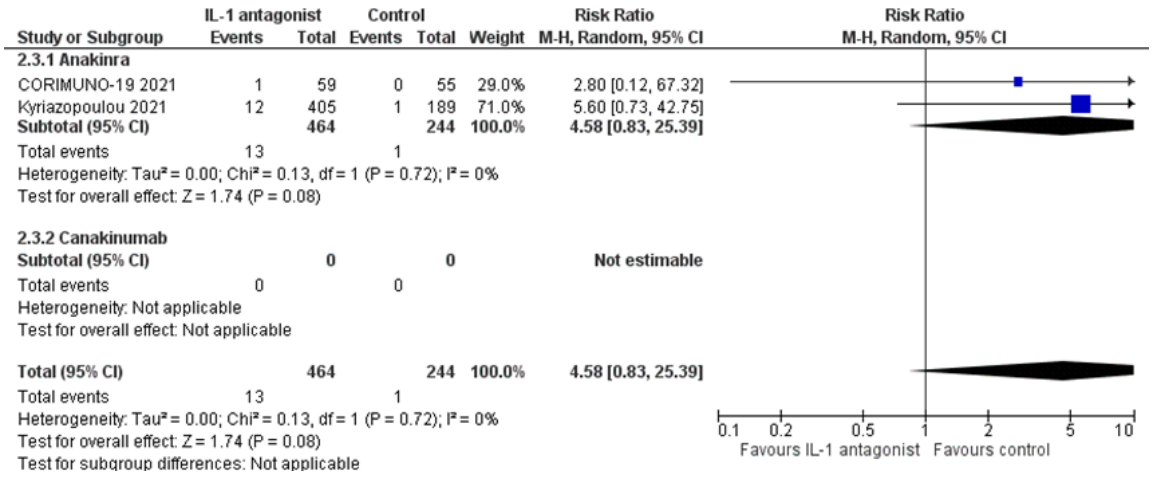


Figure 7. Effect of IL-1 antagonists compared to control on the risk for neutropenia in patients with COVID-19.

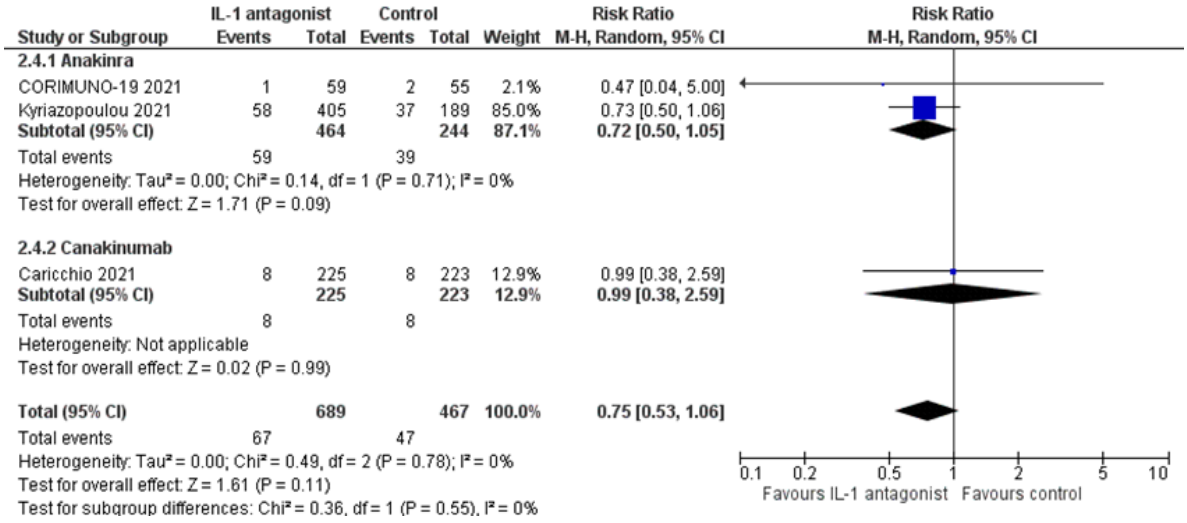


Figure 8. Effect of IL-1 antagonists compared to control on the risk for anemia in patients with COVID-19.

Thrombocytopenia

Concerning this safety outcome of interest, we pooled data from two trials comparing anakinra with usual care. Overall, treatment with anakinra did not increase the risk for thrombocytopenia (RR = 0.91, 95% CI; 0.30 – 2.71, I^2 = 0%, p = 0.86), as shown in figure 9.

Transaminasemia

Regarding this safety outcome, we pooled data from two trials with anakinra. Overall, treatment with anakinra did not increase the risk for transaminasemia among subjects with documented COVID-19 (RR = 2.64, 95% CI; 0.22 – 31.16, I^2 = 70%, p = 0.44), as shown in figure 10.

Acute kidney injury or acute renal failure

Regarding this safety outcome, we pooled data from three trials with anakinra and one trial with canakinumab in adult subjects with COVID-19. In total, IL-1 antagonist administration did not result in a significant effect on the risk for acute kidney injury or acute renal failure (RR = 0.71, 95% CI; 0.38 – 1.34, I^2 = 0%, p = 0.3), as depicted in figure 11.

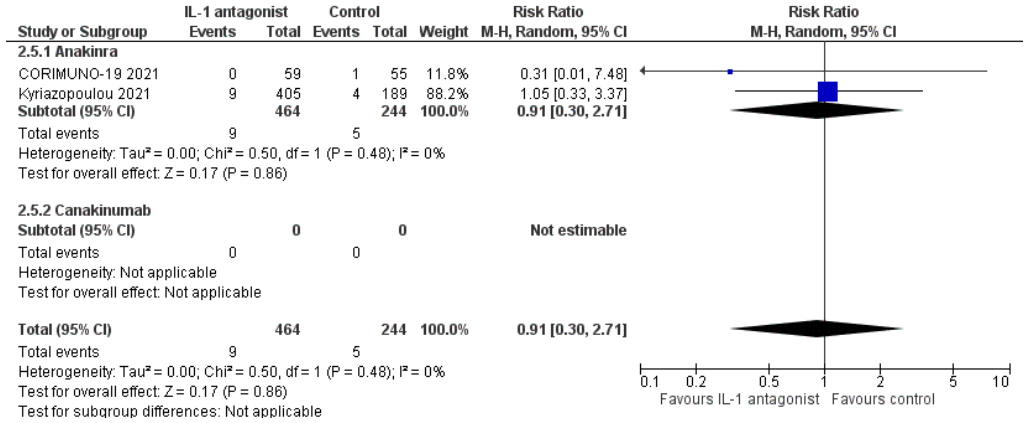


Figure 9. Effect of IL-1 antagonists compared to control on the risk for thrombocytopenia in patients with COVID-19.

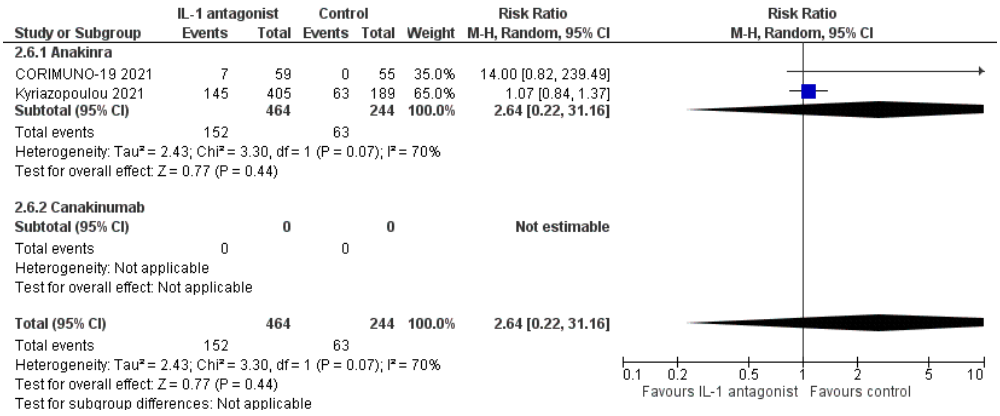


Figure 10. Effect of IL-1 antagonists compared to control on the risk for transaminasemia in patients with COVID-19.

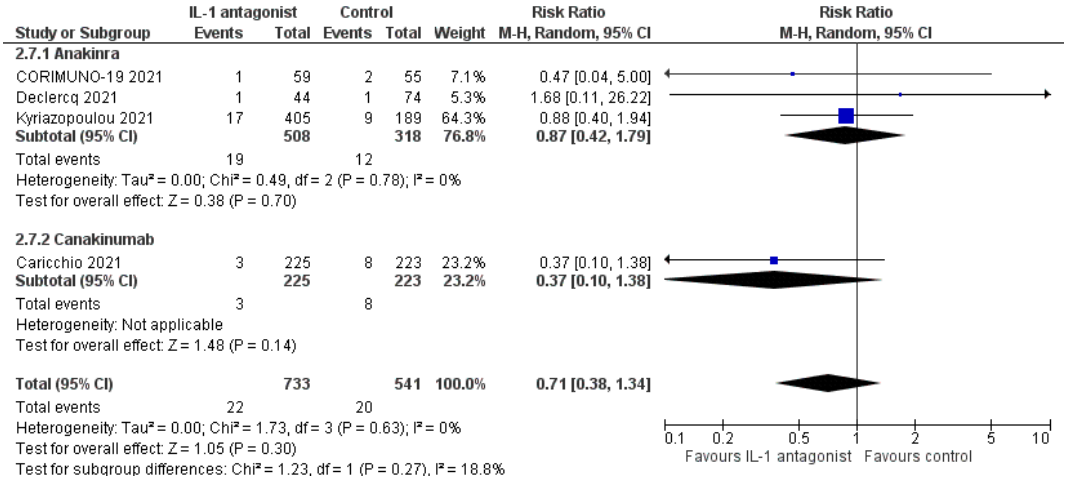


Figure 11. Effect of IL-1 antagonists compared to control on the risk for acute kidney injury or acute renal failure in patients with COVID-19.

Publication bias

Inspection of the corresponding funnel plot (figure 12) did not reveal any asymmetry, therefore there is no evidence of significant publication bias. Since we included less than ten RCTs in our meta-analysis, we did not apply the formal Egger's test for the assessment of publication bias.

Risk of bias assessment

We implemented the RoB2 tool for the assessment of risk of bias across the eligible RCTs, included in the present meta-analysis. As shown in table 2, overall risk of bias is considered to be low.

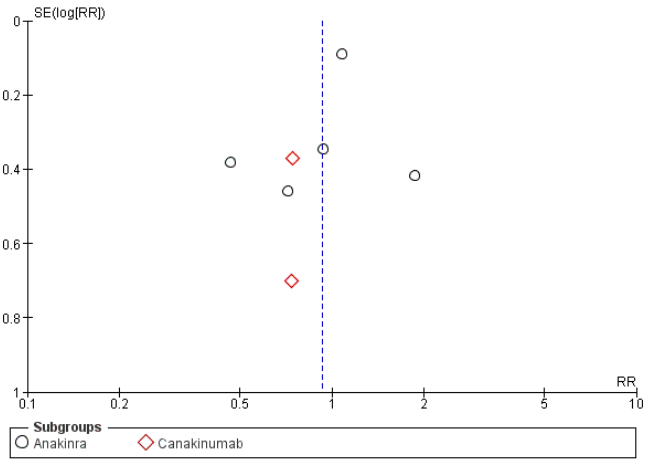


Figure 12. Funnel plot for the visual assessment of publication bias in the present meta-analysis

Table 2. Risk of bias across the trials included in the systematic review and meta-analysis

Study	Randomization process	Deviations from intended interventions	Missing outcome data	Measurement of the outcome	Selection of the reported result	Overall
CORIMUNO-ANA-19 (2021)	Low	Low	Low	Low	Low	Low
Carrichio et al. (2021)	Low	Low	Low	Low	Low	Low
Cremer et al. (2021)	Low	Low	Low	Low	Low	Low
Declerq et al. (2022)	Low	Low	Low	Low	Low	Low
Derde et al. (2021)	Low	Low	Low	Low	Low	Low
Kharazmi et al. (2022)	Some concerns	Low	Low	Low	Low	Low
Kyriazopoulou et al. (2021)	Low	Low	Low	Low	Low	Low

DISCUSSION

In the present updated meta-analysis of relevant RCTs we demonstrated that IL-1 antagonists do not exert any significant effect on "hard" outcomes in COVID-19, such as in-hospital mortality, need for invasive mechanical ventilation and requirement for non-invasive mechanical ventilation. Of note, neither anakinra nor canakinumab provide any significant effect on the above-mentioned surrogate endpoints. However, utilization of IL-1 antagonists in hospitalized patients with severe COVID-19 seems to be relatively safe, across a number of safety endpoints, including bacterial sepsis and septic shock, cytopenia, acute kidney injury and transaminasemia.

The results of our meta-analysis are opposite to those of relevant meta-analyses of observational studies, strongly supporting the use of anakinra in patients with severe COVID-19 [136, 137]. In specific, Kyriazopoulou et al. [136] have formerly shown that anakinra led to an impressive reduction in the odds for COVID-19 related death by 68% (OR = 0.32, 95% CI; 0.20-0.51), regardless of comorbidities, ferritin concentrations, or the baseline PaO2/FiO2 ratio. Of note, authors documented that anakinra resulted in a significant decrease in the odds for COVID-19 related death by 64% for patients with ferritin levels > 1000 ng/mL (OR = 0.36, 95% CI; 0.19-0.69), while the corresponding odds reduction for patients with ferritin levels lower than 1000 ng/mL was 33% (OR = 0.67, 95% CI; 0.32-1.42), confirming the catalytic role of anakinra in the suppression of hyper-inflammation [136]. In addition, Kyriazopoulou et al. [136] showed a significant decrease in the odds for COVID-19 death in patients with

severe ARDS (pO2:FiO2 ratio <100), equal to 75% (OR = 0.25, 95% CI; 0.13-0.48). However, authors also documented the synergistic effect of anakinra and dexamethasone on mortality benefit, while no significant effect was shown when anakinra was administered without dexamethasone [136].

Similar results were generated by Barkas et al. [137], who showed in their meta-analysis of observational studies that anakinra decreased the odds for mortality by 68% (OR = 0.32, 95% CI; 0.23–0.45) and the odds for invasive mechanical ventilation by 62% (OR= 0.38, 95% CI; 0.17–0.85), compared to standard of care, while no significant increase in the odds for major adverse events, such as secondary bacteremia (OR= 1.07, 95% CI; 0.42–2.73) or liver dysfunction (OR= 0.75, 95% CI; 0.48–1.16), was documented. What is more, authors found a significant decrease in the odds for invasive mechanical ventilation by 62% (OR = 0.38, 95% CI; 0.17-0.85) [137].

Another meta-analysis of non-randomized cohort studies by Pasin et al. [138] showed an outstanding reduction in the risk for COVID-19 death by 74% with anakinra compared to control (RR = 0.26, 95% CI; 0.14-0.48). Need for invasive mechanical ventilation was found to be significantly decreased by 55% in patients treated with anakinra compared to control (RR = 0.45, 95% CI; 0.25-0.82), whereas no significant increase in the risk for transaminasemia, secondary bacterial infections, or venous thromboembolism was shown [138].

Regarding canakinumab, a former mixed meta-analysis of both RCTs and observational studies by Ao et al. [139] demonstrated that canakinumab may exert a favorable effect on COVID-19 related death, by decreasing the corresponding odds by 44% (OR=0.56, 95%CI; 0.35 - 0.90). However, these results seem to be mainly driven by the relevant observational studies, whereas quantitative synthesis of RCTs confirms a non-significant effect of canakinumab on COVID-19 related death [139].

In addition, a recently published meta-analysis by Naveed and colleagues [140] showed that anakinra treatment in patients with COVID-19 produces a large and significant decrease in inflammatory biomarkers, namely C-reactive protein and ferritin levels, and in d-dimmer levels, another significant prognostic marker in COVID-19, confirm the anti-inflammatory effect of this agent in patients with COVID-19.

Of course, all the above-mentioned meta-analyses of observational studies were preliminary, prior to the publication of the relevant RCTs, whose results were rather contradictory, both for anakinra and canakinumab, suppressing the initial enthusiasm about the use of IL-1 blockers for severe COVID-19. In another preliminary pooled analysis of 3 non-randomized studies, it was shown that anakinra might provide a significant risk reduction for COVID-19 death compared to tocilizumab, an IL-6 targeting monoclonal antibody [141]. However, recent evidence retrieved from high-quality RCTs showed that IL-6 inhibitors compared to standard of care result in a significant decrease in the risk for COVID-19 death by 15% (HR = 0.85, 95% CI; 0.77-0.93) [142].

Despite the promising role of IL-1 blockers for severe COVID-19, based on the crucial role of IL-1 in the cytokine storm mediating major complications of the disease [143, 144], current evidence does not support the hypothesis that their addition to standard of care in patients hospitalized with severe COVID-19 can produce any significant treatment benefit, although it is a rather safe treatment option. Previous trials have found that IL-1 receptor blockage may produce an impressive reduction in the risk for death for patients with sepsis induced multiorgan dysfunction syndrome, with concurrent hepatobiliary dysfunction or disseminated intravascular coagulation as features of macrophage activation syndrome (MAS) [145]. Therefore, since COVID-19 is also associated with the development of MAS [146], anakinra might be an efficacious treatment option for patients with severe COVID-19 complicated by MAS. However, present meta-analysis cannot answer this interesting research question, due to the absence of individual participant data across the eligible RCTs.

We consider as major strengths of our meta-analysis the fact that included only RCTs, which are considered as the highest level of evidence, after a thorough and meticulous searching in medical databases and grey literature sources. However, we recognize as main limitation of the present meta-analysis the lack of access to individual participant data, which could permit us to conduct subgroup analyses for the assessed outcomes, according to baseline characteristics of specific interest, such as co-morbidities, pharmacotherapy, or status of prior vaccination. In addition, the results of the present meta-analysis cannot be generalized to newer SARS-CoV-2 variants, such as the omicron variant.

CONCLUSION

In the present updated meta-analysis of relevant RCTs, we failed to document any treatment benefit with IL-1 blockers in hospitalized patients with COVID-19, as added to standard of care, despite being a safe treatment option. Current evidence does not support their administration in patients with severe COVID-19. Other treatment options, such as IL-6 inhibitors, seem to provide better results in terms of morbidity and mortality in patients with COVID-19. National treatment algorithms for COVID-19 should be amended accordingly. However, there might be specific indications for the use of IL-1 blockers for patients with COVID-19, such as the rare complication of MAS. Of course, their safety and efficacy against newer SARS-CoV-2 variants should also be tested in future, well-designed RCTs.

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SUPPLEMENTARY APPENDIX

Supplementary table 1. PubMed search strategy.

MEDLINE (January 1966 to April 2022)

Search	Query	Items found
#1	Search anakinra	6804
#2	Search kineret	6818
#3	Search canakinumab	939
#4	Search ilaris	940
#5	Search interleukin-1 receptor antagonist	6979
#6	Search IL-1RA antagonist	7448
#7	Search anti-IL-1β monoclonal antibody	130
#8	Search #1 OR #2 OR #3 OR #4 OR #5 OR #6 OR #7	9794
#9	Search COVID19 [MeSH]	148834
#10	Search SARS-CoV-2[MeSH Terms]	118838
#11	Search severe acute respiratory distress syndrome coronavirus 2	2944
#12	Search #9 OR #10 OR #11	151461
#13	Search #8 AND #12	215

Supplementary table 2. Baseline demographic characteristics of enrolled subjects across eligible randomized controlled trials.

	Carrichio et al.	Cremer et al.	Mariette et al.	Derde et al.	Kharazmi et al.	Kyriazopoulou et al.	Declercq et al.
Year of publication	2021	2021	2021	2021	2022	2021	2021
Setting	Inpatient	Inpatient	Inpatient	Inpatient	Inpatient	Inpatient	Inpatient
Number of analyzed subjects	454	45	114	779	30	594	118
IL-1 antagonist	Canakinumab	Canakinumab	Anakinra	Anakinra	Anakinra	Anakinra	Anakinra
Age (years)	CANA: 59 (49, 69)	CANA 300 mg: 70.7 (64.7, 74.6) CANA 600 mg: 66.4 (63.5, 72.9)	Anakinra: 67.0 (55.5, 74.3)	Anakinra: 59.8 (11.9)	Anakinra: 49.3 (19.1)	Anakinra: 62 (11.4)	Anakinra: 65 (54, 70)
	Placebo: 57 (50, 68)	Placebo: 68.2 (56.1, 83.3)	Placebo: 64.9 (59.5, 78.3)	Placebo: 61.1 (12.9)	Placebo: 59 (1.8)	Placebo: 61.9 (12.1)	Placebo: 63 (56, 73)

Sex (male/female)	CANA: 135/92	CANA 300 mg: 9/5 CANA 600 mg: 11/4	Anakinra: 43/16	Anakinra: 269/104	Anakinra: 8/7	Anakinra: 235/169	Anakinra: 37/6
	Placebo: 132/95	Placebo: 13/3	Placebo: 37/18	Placebo: 285/121	Placebo: 11/4	Placebo: 108/81	Placebo: 53/19
Body mass index (kg/m^2)	CANA: 29.9 (26.5, 34.8)	CANA 300 mg: 28.3 (25.8, 32.0) CANA 600 mg: 29.2 (28.0, 46.4)	Anakinra: 27.4 (24.9, 32)	Anakinra: 29.7 (26.3, 35.3)	Anakinra: 28.2 (3.6)	Anakinra: 29.4 (5.5)	Anakinra: 29 (27, 32)
	Placebo: 30.8 (27, 34.7)	Placebo: 29.2 (24.0, 42.9)	Placebo: 26.8 (24.7, 31.5)	Placebo: 30.9 (27.1, 34.9)	Placebo: 28 (4.9)	Placebo: 29.8 (5.6)	Placebo: 28 (25, 31)
Hypertension (%)	CANA: 53%	CANA 300 mg: 64.3% CANA 600 mg: 73.3%	NR	NR	Anakinra: 13.3%	NR	Anakinra: 37%
	Placebo: 59%	Placebo: 75%			Placebo: 53.3%		Placebo: 42%
Diabetes mellitus (%)	CANA: 35%	CANA 300 mg: 28.6% CANA 600 mg: 66.7%	Anakinra: 32%	Anakinra: 33.8%	Anakinra: 20%	Anakinra: 16.3%	Anakinra: 35%
	Placebo: 37%	Placebo: 43.8%	Placebo: 27%	Placebo: 37.4%	Placebo: 53.3%	Placebo: 14.8%	Placebo: 29%
Cardiovascular disease (%)	CANA: 21%	NR (data shown for CAD, stroke)	Anakinra: 37%	Anakinra: 11.2%	NR	NR (data shown for CAD, HF)	Anakinra: 26%
	Placebo: 19%		Placebo: 25%	Placebo: 11.7%			Placebo: 18%
Chronic kidney disease (%)	CANA: 10%	CANA 300 mg: 14.3% CANA 600 mg: 40%	Anakinra: 8%	Anakinra: 1.1%	NR	Anakinra: 2.2%	Anakinra: 14%
	Placebo: 7.5%	Placebo: 43.8%	Placebo: 5%	Placebo: 2.1%		Placebo: 0.5%	Placebo: 8%
Chronic obstructive pulmonary disease (%)	CANA: 8.8%	CANA 300 mg: 21.4% CANA 600 mg: 20%	Anakinra: 10%	Reported as combined COPD/Asthma	NR	Anakinra: 3.7%	NR
	Placebo: 5.7%	Placebo: 12.5%	Placebo: 5%			Placebo: 4.8%	
Asthma (%)	CANA: 7.9%	NR	NR	Reported as combined COPD/Asthma	NR	NR	NR
	Placebo: 7.5%						
PO2/FiO2 (mm Hg)	CANA: 180.1 (112.3, 261.9)	CANA 300 mg: 160 (77, 246)	NR	Anakinra: 106 (84, 148)	NR	Anakinra: 239 (18, 302)	Anakinra: 114 (80, 243)

		CANA 600 mg: 148 (73, 203)					
	Placebo: 179.6 (127.5, 268.8)	Placebo: 117 (66, 210)		Placebo: 118 (89, 169.5)		Placebo: 223 (168, 297)	Placebo: 149 (95, 254)
C-reactive protein (mg/L)	CANA: 89 (47, 153)	CANA 300 mg: 122 (64, 153) CANA 600 mg: 127 (108, 197)	Anakinra: 121 (77, 198)	Anakinra: 112 (70, 189)	Anakinra: 123.7 (49)	Anakinra: 50.5 (25.2, 100.2)	Anakinra: 150 (96, 215)
	Placebo: 77 (42, 136)	Placebo: 176 (150, 199)	Placebo: 120 (87, 192)	Placebo: 129 (71, 208)	Placebo: 105.1 (51)	Placebo: 51.4 (25.2, 98.5)	Placebo: 120 (77, 190)
Ferritin (mg/L)	CANA: 681 (304, 1271)	CANA 300 mg: 998 (857, 1626) CANA 600 mg: 740 (448, 1969)	Anakinra: 1479 (444, 2334)	NR	Anakinra: 780.5 (311.9)	Anakinra: 558.9 (294.1, 1047)	Anakinra: 1886 (1294, 2544)
	Placebo: 631 (305, 1160)	Placebo: 1246 (768, 2355)	Placebo: 1151 (847, 2530)		Placebo: 599.5 (365.4)	Placebo: 628.6 (293.5, 1062.3)	Placebo: 1606 (1205, 2730)
Corticosteroids (%)	CANA: 41%	CANA 300 mg: 21.4% CANA 600 mg: 53.3%	Anakinra: 12%	Anakinra: 85.9%	Anakinra: 73.3%	Anakinra: 84.4%	Anakinra: 67%
	Placebo: 32%	Placebo: 62.5%	Placebo: 15%	Placebo: 65.9%	Placebo: 53.3%	Placebo: 88.9%	Placebo: 60%
Remdesivir (%)	CANA: 22%	CANA 300 mg: 35.7% CANA 600 mg: 66.7%	NR	Anakinra: 29.5%	Anakinra: 13.3%	Anakinra: 73.6%	Anakinra: 7%
	Placebo: 20%	Placebo: 37.5%		Placebo: 26.1%	Placebo: 26.6%	Placebo: 74.6%	Placebo: 4%
Other antiviral agents (%)	NR	NR	Anakinra: 2%	NR	Anakinra: 60%	NR	NR
			Placebo: 4%		Placebo: 80%		
Azithromycin (%)	CANA: 37%	NR	Anakinra: 19%	NR	NR	Anakinra: 18.8%	NR
	Placebo: 37%		Placebo: 25%			Placebo: 18.5%	

*Data is presented as mean (standard deviation), median (interquartile range), absolute numbers or relative frequency (%), unless otherwise stated.

**CANA: canakinumab, CAD: coronary artery disease, HF: heart failure, NR: not reported