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Review

Klotho Deficiency in Severe COVID-19: A Unifying Hypothesis

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Abstract: COVID-19 is characterized by a striking similarity to premature aging. Its clinical manifestations range from asymptomatic to critical illness. No single central agent has been demonstrated so far. We present Klotho, an antiaging protein, as a key factor in COVID-19 pathophysiology. There is epidemiological evidence that both acute and chronic uses of Klotho agonists have a beneficial effect in reducing COVID-19 severity and mortality. A review of the PubMed epidemiological, clinical, and mechanistic evidence supports a role for Klotho deficit as a central determinant of severe COVID-19. Clinical data support the idea that chronic use of Klotho agonists protects against severe COVID-19 and that its acute use may be beneficial. We propose a unifying hypothesis that low Klotho levels play a key role in severe COVID-19, while increasing Klotho levels can have a beneficial effect through the prevention of acute kidney injury (AKI) and potential antiviral effects. Further research is needed.

Keywords: COVID-19; Klotho; Klotho-related peptide 1; metformin; statins; vitamin D; acute kidney injury

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1. Introduction

SARS-CoV-2 is the third important pathogenic coronavirus to affect the human population over a span of two decades. This virus causes a disease called COVID-19, which caused a catastrophic pandemic responsible for 775 million cases and over seven million deaths globally [1]. The clinical manifestations of COVID-19 disease range from an asymptomatic condition to severe life-threatening disease, with symptoms that may include acute respiratory distress disorder, hypercoagulability, acute kidney injury, severe inflammation, and cytokine storm [2]. In addition, a broad spectrum of aging-related risk factors can lead to severe COVID-19 cases. Low levels of Klotho, an antiaging protein, have been suggested as a central agent capable of explaining the diverse risk factors and clinical manifestations of COVID-19 [3].

α-Klotho is a glycoprotein first discovered in 1997 through an accidental knockout of its gene in transgenic mice [4]. Rodents with a total knockout of this gene developed an aging phenotype, characterized by infertility, lung emphysema, low bone mineral density, vascular calcifications, and premature death, among others. The human α-Klotho gene maps to 13q13.1, has five exons and four introns, and encodes a 3036-nucleotide mRNA [5]. This gene also encodes for β-Klotho and Klotho-related peptide, Krp. In this paper, we will refer only to α-Klotho as simply Klotho. Klotho's first identified function was as a single 130 kDa transmembrane co-receptor for fibroblast growth factor 23 (FGF23) [6], which is an endocrine FGF necessary for the urinary excretion of phosphate. Klotho is expressed mainly in the distal convolute tubules (and to a lesser extent in the proximal tubules) and choroid plexus. Membrane-bound Klotho has two extracellular domains (KL1 and KL2), a single-pass transmembrane segment and a short intracytoplasmic tail [5]. Klotho was

later found to have hormone properties through membrane shedding as soluble Klotho, as well as paracrine and endocrine functions. Soluble Klotho is found in serum, urine and cerebrospinal fluid. In serum, soluble Klotho can be measured via enzyme-linked immunosorbent assay (ELISA), but the best method to measure serum Klotho is through immunoprecipitation–immunoblot (IP-IB) assay, which has a consistent correlation with the glomerular filtration rate [7].

The most well-known functions are antiaging, anti-inflammatory, antifibrotic, and antitumoral activities [8]. These functions are mediated by Klotho inhibition of the following signaling pathways:

- Transforming growth factor beta (TGF-β), responsible for tissue fibrosis [9];
- NF-κβ, an important inflammatory pathway [10,11];
- Wnt/β-catenin, a tumoral pathway when augmented [12];
- Insulin/insulin-like growth factor 1 (IGF1)/phosphoinositide 3-kinase (PI3K) signaling pathway, which increases oxidative stress [13] and is also a highly conserved pathway whose inhibition extends the life span in animals from *C. elegans* to *Drosophila* to mice [14];
- Akt/mTOR pathway, as its inhibition by Klotho upregulates autophagy [15–20].

Soluble Klotho's main intracellular signaling involves the regulation of these pathways [21].

As previously mentioned, an almost-absolute deficiency of Klotho induced a (humanized) aging phenotype in rodents; these animals did not live more than 100 days [4]. This phenotype was almost completely rescued by the exogenous Klotho expression in limited organs. Conversely, Kurosu et al. showed that overexpression of Klotho induces a longevity phenotype that outlived wild-type animals by 30% in male rodents and by 19% in female rodents [14]. Mechanistically, for such a purpose, the authors showed that Klotho overexpression inhibits the autophosphorylation of insulin and IGF-1 receptors, thereby inhibiting insulin and IGF-1 signaling, a highly conserved cellular signaling pathway across species. This inhibition is causally linked to an increased lifespan [22]. However, more recently it has been shown that there is at least another pathway, presumably independent from insulin/IGF-1 signaling, for premature aging in Klotho deficiency. The mice originally described by Kuro-o and colleagues as exhibiting an almost-absolute deficiency of Klotho [4] were severely hyperphosphatemic (almost twice the serum phosphate levels of the wild-type animals). Ohnishi and Razzaque [23] have shown that phosphate toxicity in mice lacking Klotho is in large part responsible for the premature aging phenotype and that decreasing the serum phosphate levels through genetic manipulation with NaPi2a$^{-/-}$/klotho$^{-/-}$ double-knockout mice rescued the phenotype, with the mice regaining fertility and body weight, decreasing ectopic tissue calcifications and increasing lifespan. Therefore, the canonical pathway of Klotho in FGF23/phosphate homeostasis [6] is required for membrane-bound Klotho aging-suppressive actions.

In preclinical studies, Klotho has proven to ameliorate several health conditions, such as decreasing the damage in acute kidney injury (AKI) and halting the progression to chronic kidney disease [24], alleviating acute lung injury induced by oxidative stress [25], decreasing the injury in paraquat poisoning [26], and improving cognitive functions in rodents [27] and in non-human primates [28].

Klotho is expressed mainly in the renal distal tubular cells, and secondarily in the choroid plexus of the brain and in the parathyroid glands [29]. Klotho levels decrease with aging [30], in acute kidney injury (AKI) and in chronic kidney disease (CKD) [31]. AKI is the principal cause of a systemic deficit of Klotho, not only renal Klotho [31,32].

Advanced age and existing chronic kidney disease (CKD) are among the strongest risk factors for COVID-19-related mortality. Analysis of the OpenSAFELY [33] database of more than 17 million subjects highlighted that the risk of COVID-19-related mortality was 20-fold greater in patients over age 80 in comparison to patients 50 years of age; pre-existing CKD increased the risk of COVID-19-related mortality by more than double, and organ transplantation (including kidney transplantation) increased the risk three-fold.

These estimates were fully adjusted and substantially higher than those for obesity, type 2 diabetes mellitus, and chronic heart disease. But not only chronic kidney disease increases COVID-19 severity. There are also clinical data that the occurrence of AKI during an episode of COVID-19 dramatically increases the risk of mortality. Chen et al. [34] found in a previous meta-analysis that AKI is associated with a COVID-19-related mortality rate of 76%. The same is true for the other human pathogenic coronaviruses: AKI induces a mortality rate of 68% in MERS and 86% in SARS. Therefore, there is convincing epidemiological evidence that both chronic and acute kidney diseases increase the predisposition to COVID-19 severity and mortality. The kidneys, in particular, the distal, and to a lesser degree, the proximal convolute tubules, are the main source of systemic soluble Klotho [35]. Klotho decreases early and substantially in AKI [29], as assessed by preclinical data [31,32] and by clinical data on human urinary samples of critically ill patients when compared with well-matched controls [36], although most studies are small [29]. Houssen et al. [37] have found that critically ill COVID-19 patients have higher serum Klotho levels than other groups and that there is a moderate negative correlation between serum Klotho levels and COVID-19 severity (Pearson correlation $r = -0.451$, $p < 0.001$). This correlation was found to be higher than the correlation of IL-1β with disease severity. The authors concluded that Klotho levels can be a marker of disease severity in COVID-19.

Therefore, we hereby hypothesize that both acute and chronic low Klotho levels may be potential mediators of these epidemiological and clinical observations in COVID-19.

At a molecular level, Klotho can inhibit the important inflammatory pathway of NF-κβ induced by several mechanisms. Klotho can inhibit NF-κβ activation mediated by lipopolysaccharide (LPS) [11,38], by indoxylsulphate in uremic conditions [39], and by cyclosporin A [10]. In addition, Klotho can inhibit angiotensin II axis upregulation and its consequences [40], pointing toward the association between low Klotho levels and renin–angiotensin axis activation [41]. Angiotensin II activates NF-κβ [42] and this has been shown to play a key role in severe COVID-19 pathophysiology [43]. Klotho can also inhibit the IGF1R/PI3K/AKT signaling pathway [13,44], and the mechanistic (previously mammalian) target of rapamycin (mTOR) is downstream of this cascade [45,46]. In addition, Klotho directly inhibits Akt/mTOR signaling [15–20]. This may be relevant to COVID-19 disease because it has been shown that mTOR plays a key role in RNA viruses' replication, including SARS-CoV-2 [47,48]. Klotho can also inhibit the NLRP3 inflammasome pathway, which is relevant for COVID-19 [49,50].

We present the hypothesis that low Klotho levels play a key role, leading to severe COVID-19 cases, particularly through inducing AKI during a COVID-19 episode. Low Klotho may also play a role in predisposition to SARS-CoV-2 infection. Given this hypothesis, we further propose that Klotho upregulation and supplementation may decrease mortality in COVID-19 disease through its protective capacity against AKI and possible antiviral effects.

Several well-known medications have been shown to increase Klotho gene expression and therefore Klotho levels in both preclinical and clinical studies. These drugs include metformin, SGLT2 inhibitors, active vitamin D analogs, statins, and mTOR inhibitors [29,51]. If it is true that sufficient levels of Klotho play a protective role against severe COVID-19, then patients who chronically take these medications should experience a protective effect against severe COVID-19. In addition, if Klotho has antiviral properties, medications that increase Klotho levels may show antiviral (anti-SARS-CoV-2 replication) effects. These clinically relevant possibilities demand clinical studies on the survival benefit derived from the administration of Klotho (or related peptides) or Klotho-increasing drugs in severe COVID-19 cases.

2. Materials and Methods

A comprehensive literature review of the PubMed database was performed. This publication did not require original research with human subjects so no informed consent was needed. The available evidence is presented according to a hierarchy from lower to

higher levels of confidence for metformin, statins, and vitamin D upon reviewing (a) preclinical data, (b) meta-analysis of observational studies, (c) meta-analysis of randomized controlled trials (RCTs), and (d) NIH guidelines, when available. The statistical analysis includes the pooled estimates with confidence intervals. The heterogeneity is presented, and where it is substantial, the pooled estimates from the random effects analysis are reported. Experimental evidence of the effects from the supplementation of full-length Klotho or Klotho-related peptides in animal models of COVID-19 is presented. Where available, results from Mendelian randomization studies, an econometrics-derived technique that increases causal inference and decreases bias through the implementation of single-nucleotide polymorphisms (SNPs) as instruments for exposure, are discussed [52].

3. Results

3.1. Evidence for Metformin

3.1.1. Preclinical Data for Metformin

Metformin, a primary antidiabetic drug, has been shown to increase serum Klotho levels in preclinical studies [53]. Metformin has also been shown to decrease inflammatory markers such as IL-6 and TNF-alpha [54], two cytokines that play an important role in COVID-19 [55]. Interestingly, metformin has been shown to display anti-RNA virus activity, including Zika and hepatitis C viruses, and recently, SARS-CoV-2 virus titers in a subset of cell lines of human lung tissue; the mechanism appears to be inhibition of protein translation via inhibition of mTOR [56–60]. Given that this mechanism involves mTOR inhibition, this effect may be mediated at least partially through Klotho, as Klotho suppression blunts the metformin inhibition on mTOR [53]. Of note, Klotho phosphorylates AMPK [61,62] and, according to Howell et al. [56], the mTOR inhibition of metformin at low doses is AMPK-activation-dependent. However, at high doses of metformin, the mTOR inhibition is AMPK-independent [56], suggesting other independent mechanisms that require further research.

Metformin also decreases insulin resistance [63]. This is an important addition as it has been suggested that there is a bidirectional association between insulin resistance and COVID-19 severity. Insulin resistance has been shown in several reports to be associated with COVID-19 severity. For example, in the OpenSAFELY database, comprising more than 17 million participants, those with obesity and diabetes mellitus had an increased risk of COVID-19 severity [33], and it is well known that an enlarged adipose tissue mass induces systemic insulin resistance [64]. On the other hand, some small studies have shown that acute infection by SARS-CoV-2 is associated with transient hyperglycemia and markers of insulin resistance [65,66]. We believe this could be a potential alternative secondary mechanism of metformin's beneficial effects on COVID-19, most likely independent of Klotho. Some classical genetic papers on rodents have shown that an almost-absolute Klotho deficiency is associated with hypoglycemia and increased insulin sensitivity [4,67], and conversely, Klotho overexpression increases the lifespan but increases insulin resistance, mainly in male rodents, and IGF-1 resistance in female rodents [14] via inhibition of insulin and IGF-1 signaling. In addition, clinical data from a cross-sectional survey in NHANES (n = 6371 participants) have revealed a positive and strong correlation (r = 0.9) between serum Klotho levels and insulin resistance, as assessed by HOMA-IR measurements [68]. It is important to add that metformin's contribution to insulin sensitivity in COVID-19 is currently not very clear, as other insulin sensitizers, such as glitazones, which also act partly via AMPK activation, have been shown to be mortality-neutral in COVID-19, as assessed by meta-analysis [69].

3.1.2. Meta-Analysis of Observational Studies of Metformin

A history of metformin use prior to SARS-CoV-2 infection is strongly associated with a reduced mortality risk due to COVID-19 [70]. From the analysis of an electronic database involving patients from 12 hospitals and 60 primary care clinics, the reduced risk of mortality from COVID-19 due to metformin use was estimated at 68% (odds ratio

95% confidence interval: OR: 0.32 (0.15–0.66)). This important preliminary finding was described in overweight and obese patients with an age range between 30 and 85 years of age.

Several meta-analyses of observational data regarding the previous use of metformin have demonstrated the reduced risk of adverse events in COVID-19. Li et al. [71] analyzed the relation between previous metformin use and a reduced risk of death, intubation and hospitalization in patients with COVID-19. This study included 19 studies and found that metformin is associated with a 34% lower risk of COVID-19 mortality (OR: 0.66 (0.56–0.78), I^2 67%, random effects) and a 27% lower risk of hospitalization (OR: 0.73 (0.53–1), I^2 16%, random effects). The authors found no relation between metformin and the intubation risk.

Petrelli et al. [72] pooled the data of five systematic reviews involving 36 studies and showed that diabetic patients treated with metformin had a lower risk of severe complications of COVID-19 (ES 0.80) and COVID-19-related mortality (ES 0.69).

Ganesh and Randall [73] have reported a large meta-analysis of 32 cohort studies and found that metformin was associated with a lower COVID-19 mortality risk in both unadjusted (OR 0.61 (0.53–0.71), $p < 0.001$, I^2 70%, random effects) and adjusted models (OR 0.78 (0.69–0.88), $p < 0.001$, I^2 67%, random effects).

3.1.3. Meta-Analysis of Randomized Controlled Trials (RCTs) of Metformin

COVID-OUT was a phase 3, randomized, double-blind, placebo-controlled trial, using a two by three factorial design to test metformin, ivermectin and fluvoxamine for early outpatient treatment of SARS-CoV-2 infection [74]. The authors investigated whether early treatment with such drugs would prevent progression to severe COVID-19. All the trial patients were recruited remotely and the trial drugs were delivered to the patients at home. The eligibility criteria selected patients who were 30 to 85 years of age, overweight or obese, and had proof of SARS-CoV-2 infection within the past three days and an onset of symptoms within seven days before randomization. The patients were randomized into six groups to receive metformin plus fluvoxamine, metformin plus ivermectin, metformin plus placebo, placebo plus fluvoxamine, placebo plus ivermectin, and placebo plus placebo. The primary endpoint was severe COVID-19 within 14 days, defined as a composite of hypoxemia (less 93% oxygen saturation), emergency department visit, hospitalization, or death. It is important to note that the Food and Drug Administration (FDA) issued a warning [75] regarding the low accuracy of pulse oximeters due to spurious readings. However, this warning was issued after the trial had already begun and, therefore, the oximeter readings were an important source of ascertainment bias. The statistical plan included a secondary analysis, including only the following health components: emergency department visit, hospitalization, or death. The trial included 1323 patients in the primary analysis. This RCT showed a nonsignificant result for the primary outcome, including the oximeter readings (OR: 0.84 (0.66–1.09), $p = 0.19$) for the primary outcome. However, metformin decreased the risk of the composite outcomes of the secondary analysis (emergency department visit, hospitalization or death (OR: 0.58 (0.35–0.94), no p value available due to the lack of adjustment for multiple testing)). In the subgroup analysis, the authors found that the subgroup that benefited the most from metformin by decreasing the healthcare utilization was composed of those patients who initiated metformin very early in the course of the disease (OR: 0.45 (0.22–0.93)), namely those patients who initiated metformin within 4 days of symptoms. The authors concluded that none of the three drugs demonstrated a benefit regarding the risk of the primary outcome, but that a possible benefit for the prevention of the most severe components of the primary outcome was demonstrated for metformin. Ivermectin and fluvoxamine showed no benefit in this RCT.

3.2. COVID-OUT RCT: Metformin Antiviral Effect in SARS-CoV-2 Infection and Prevention of Long COVID

There were two additional important findings in the COVID-OUT RCT for metformin. First, the authors demonstrated that the use of metformin reduced the mean viral load

by 3.6-fold, as assessed by quantitative real PCR, relative to the placebo (p = 0.027) [76]. Those in the metformin arm had a lower risk of detectable virus at day 5 or day 10 (OR: 0.72 (0.55–0.94)). Secondly, in the same RCT and as a secondary outcome, the authors [77] demonstrated that a 14-day course of metformin reduced the cumulative incidence of long COVID at 10 months by 41% (HR: 0.59 (0.39–0.89), p = 0.012) when compared to the matched placebo. Moreover, when metformin was administered within 3 days of symptoms onset, the risk reduction of long COVID was 63% (HR: 0.37 (0.15–0.95)).

On the other hand, a group of Brazilian [78] researchers ran another RCT with metformin, recruiting patients 18 years or older with a risk factor for severe COVID-19. The researchers used extended-release metformin. The trial included 418 patients, a smaller sample size than the previous RCT. The primary outcome was hospitalization due to COVID-19. There were several secondary outcomes, such as mortality and viral clearance. A Bayesian approach was implemented for the primary outcome. The authors found no evidence of a benefit from metformin on hospitalization (RR: 1.14, credible interval: 0.73–1.81). This study also found no benefit for the secondary outcomes. However, it is important to mention that non-adherence to metformin was present in almost 50% in the interventional group, probably related to the non-titrated scheme of metformin dosing. The authors discussed whether differences with observational data may suggest that longer-term use of metformin may provide greater benefit than its acute administration. Bramante et al. [74] mentioned that extended-release formulations of metformin, which have different pharmacokinetics from immediate-release metformin, could be less efficient in relation to COVID-19 outcomes. Indeed, previous outcomes, such as polycystic ovary syndrome, have shown a different profile for extended-release formulations than the benefit seen for immediate-release formulations, which reach higher peak systemic exposure than extended-release formulations [79,80].

3.3. GRADE Criteria for Metformin

Erickson et al. [81] have synthesized the available clinical evidence in terms of metformin in COVID-19 through RCTs. The authors found three candidate RCTs and pooled the evidence; however, the substantial statistical heterogeneity precluded the implementation of a meta-analysis. They performed a systematic review and applied the Grading of Recommendations Assessment, Development and Evaluation (GRADE) criteria [82]. After weighting all the evidence, the authors concluded that the evidence for metformin as an effective therapy for preventing a severe outcome in COVID-19 was moderate. It is important to add that, as the authors of this systematic review pointed out, a distinction must be made between the observational data showing a consistent benefit of chronic use of metformin prior to SARS-CoV-2 infection, and the RCTs included in this systematic review, in which patients were randomized to metformin or placebo once the infection had already initiated. The evidence for the chronic use of metformin as a useful therapy for preventing severe COVID-19 is quite consistent.

NIH Evidence and Guidelines for Metformin Use in COVID-19

Initially, the NIH panel had concluded in the 2023 COVID-19 guidelines that there was insufficient evidence to recommend initiating metformin therapy in hospitalized patients (BIII recommendation). For those patients already on metformin therapy due to an underlying condition, the panel advised the continuous use of metformin during the disease, according to medical recommendations (AIII recommendation). However, there were new data published in September 2024 [83]. The NIH funded a large retrospective cohort study in the US and assessed type 2 diabetic adult patients exposed to metformin (75,996 individuals) versus type 2 diabetic patients non-exposed to metformin (13,336 individuals). The outcomes were death due to COVID-19 or post-acute sequalae of SARS-CoV-2 (PASC). The researchers found that those on prevalent metformin had a 13% to 21% lower incidence of PASC (long COVID) or death than those in the non-metformin group (in the N3C database, the HR was 0.79 (0.71–0.88), $p < 0.001$; in PCORnet, the HR was 0.87 (0.66–1.14, p = 0.08).

On the official NIH website [84], there is a statement that reads as follows: "*Adults who use the prescription drug metformin to treat their type 2 diabetes have a lower risk of developing long COVID or dying after a COVID-19 infection than people with diabetes who take other anti-diabetes medications, according to a large study supported by the National Institutes of Health (NIH)*".

In this regard, it is important to point out that metformin has a low but real risk of lactic acidosis during the onset of severe acute kidney injury [85] and should not be prescribed with a glomerular filtration rate of less than 30 mL/min. The mortality rate of metformin-related lactic acidosis is high, estimated at 50% [86].

The NIH panel also included a statement that the use of metformin is helpful for the prevention of long COVID, as shown in the COVID-OUT RCT and N3C database [77,84].

3.4. Evidence for Statins

3.4.1. Preclinical Data of Statins

Statins have been shown to increase Klotho gene expression in a dose-dependent manner in preclinical studies [87–89]. Moreover, simvastatin has been shown to inhibit replication of hepatitis C virus [58]. More recently, simvastatin has been shown to decrease SARS-CoV-2 viral replication in vivo, leading to the conclusion that this statin is protective against SARS-CoV-2 infection through inhibition of viral cell entry and inflammatory cytokine production [90]. Klotho has been shown to mediate the anti-inflammatory effects of statins, especially the inhibition of IL-6 [91]. Atorvastatin has been shown to inhibit the viral activity of SARS-CoV-2, including the ancestral, delta and mu variants, in vitro [92]. It is important to add that the antiviral effects of statins are partly mediated by an increase in autophagy through the inhibition of mTOR [93]. Statins have also been shown to decrease membrane lipid rafts, which is crucial for SARS-CoV-2 infection [90,93]. As a consequence, and despite an overall enhancement of ACE2 being considered to not necessarily be detrimental [90], statins displace ACE2, inducing a cellular redistribution and decreasing the effectiveness of the union of SARS-CoV-2 virus with ACE2, its receptor. In this regard, Dalton and colleagues [94] have shown that soluble Klotho is able to bind membrane lipid rafts, altering lipid organization and decreasing endocytosis. Further research is needed to establish a potential role for Klotho in interfering with lipid rafts specifically for SARS-CoV-2 internalization.

3.4.2. Meta-Analysis of Observational Studies of Statins

We have found four large meta-analyses evaluating the association of chronic use of statins and COVID-19 outcomes. Pal and coauthors [95] published a meta-analysis of data comprising 14 observational studies in almost 20,000 patients, concluding that prior and in-hospital use of statins is associated with a decrease of 49% in the risk of adverse outcomes in adjusted estimates (OR: 0.51 (0.41–0.63), $p < 0.001$; no heterogeneity found in this analysis using fixed effects).

Lao and coauthors [96] performed an even larger systematic review and meta-analysis of observational studies on statin use and COVID-19 outcomes. They included 58 studies reporting odds ratios (ORs, binary outcomes), 28 studies reporting hazard ratios (HRs, survival outcomes) and implemented a random-effects model. For the mortality outcome, the pooling of the ORs showed a significant result (OR: 0.72 (0.67–0.77); I^2 76%), meaning that the risk of mortality is 28% lower for statin users than non-users. The pooling of HRs showed a similarly significant beneficial effect of statins in decreasing the mortality risk (HR: 0.74 (0.69–0.79), I^2 79%). Statins were also associated with a lower risk of intensive care unit admission due to COVID-19 (OR: 0.94 (0.89–0.99), I^2 7%; HR: 0.76 (0.60–0.96), I^2 57%). Furthermore, statin use was also shown to decrease the requirement for mechanical ventilation (OR: 0.84 (0.78–0.92). I^2 34%; HR: 0.67 (0.47–0.97), I^2 75%).

Kollias and coauthors [97] meta-analyzed 10 studies and found that statin use was associated with a 35% risk reduction in mortality due to COVID-19 (HR: 0.65 (0.53–0.81)). Meta-analysis of a subset of six of those studies in which the authors reported the continua-

tion of statin use during hospitalization revealed an even greater risk reduction in mortality due to COVID-19, estimated at 46% (HR: 0.54 (0.47–0.62)).

Finally, Diaz-Arocutipa [98] and coauthors meta-analyzed 25 cohort studies comprising 147,824 patients to evaluate statin use and COVID-19 mortality. Meta-analyses of the adjusted estimates showed a significant mortality risk reduction for statin use, estimated at 33% when pooling the ORs (OR 0.67 (0.52–0.86), I^2 76%) and estimated at 27% when pooling the HRs (HR 0.73 (0.58–0.91), I^2 64%). Importantly, subgroup analysis of this large body of data showed that only chronic use of statins (defined as before hospital admission) significantly reduced COVID-19 mortality.

3.4.3. Meta-Analysis of Randomized Controlled Trials (RCTs) for Statins

Ren and coauthors [99] meta-analyzed seven RCTs in 2023, including 1830 participants, and found no evidence of a significant reduction in the mortality risk in COVID-19 patients due to statin therapy compared to standard of care or placebo (RR 0.92 (0.75–1.13)).

However, de Mesquita et al. [100] performed a newer and larger meta-analysis in 2024 and pooled seven randomized clinical trials, comprising 4262 patients. Through binary outcome models, the authors showed that, compared to no statin use, statin use significantly reduced the case fatality rate by 12% (RR: 0.88 (0.80–0.98), no heterogeneity). When the pooling of the effects was assessed in a survival model, the authors found similar results: a decrease in mortality of 14% compared to no statin use (HR: 0.86 (0.75–0.99), no heterogeneity). Statin use also reduced the World Health Organization COVID-19 scale of 14 days (mean difference −0.27 (−0.54–−0.01, no heterogeneity). Interestingly, these effects did not seem to be mediated through decreased inflammation as the main mechanistic pathway, as the C-reactive protein levels (CRP) were not different across randomized groups. To the best of our knowledge, there were no data on long COVID.

3.5. Statins in COVID-19: A Mendelian Randomization Analysis

From a genetic perspective, there is also evidence that suggests a causal beneficial effect on COVID-19 disease. Huang et al. [101] analyzed more than one million participants from the COVID-19 Host Genetics Initiative v4 [102], all participants of European ancestry, and used cis-eQTLs and SNPs to evaluate the evidence. The authors found by means of Mendelian randomization analyses that the increased expression of the *HMGCR* gene (the enzyme inhibited by statins) is (causally) associated with a higher risk of COVID-19 susceptibility and COVID-19 hospitalization, suggesting that statins (HMGCR inhibitors) might lower those risks.

NIH Guidelines for Statins

Given the evidence of the chronic use of statins decreasing COVID-19 adverse outcomes, in 2020, the NIH issued a statement that recommended that patients with COVID-19 who are prescribed statins for the treatment or prevention of cardiovascular disease should continue statin use [103].

3.6. Evidence for Low Serum Levels of 25OH-Vitamin D and for Vitamin D Receptor Agonists

3.6.1. Preclinical Data for Vitamin D Receptor Agonists

Vitamin D is essential for calcium and bone metabolism. Vitamin D also has important immunomodulatory effects [104]. There is epidemiological evidence that has shown an increased susceptibility to viral respiratory infections for patients with 25OH-vitamin D deficiency [105] and experimental evidence that has shown that vitamin D has antiviral effects, especially against enveloped viruses [106]. Concerning Klotho, vitamin D receptor agonists, such as calcitriol and paricalcitol, increase Klotho protein levels [107] and 1,25-dihydroxyvitamin D upregulate *Klotho* gene expression [29]. Importantly, 1,25-dihydroxyvitamin D has also been shown to play a key role in mTOR signaling, mainly through its inhibition [108].

3.6.2. Low 25OH-Vitamin D Levels and COVID-19 Infection and Severity: Meta-Analysis of Observational Studies

Two large meta-analyses have evaluated the association between 25OH-vitamin D levels and COVID-19 infection and severity. DEcclesiis et al. [109] performed a systematic review and meta-analysis including 27 observational studies (mostly cohort studies) and 205,565 participants. They applied random-effects models. The meta-analyses of seven retrospective studies (case control and cohort studies) found that lower levels of 25OH-vitamin D were associated with a higher risk of a positive COVID-19 test (RR: 1.74 (1.44–2.11), I^2 49%). In addition, the meta-analysis of 15 studies evaluating COVID-19 severity, defined as ICU admission or mechanical ventilation, showed that lower 25OH-vitamin D levels were associated with higher risk of severity (RR: 1.92 (1.39–2.64), I^2 45%). The authors also assessed the relation between vitamin D supplementation and COVID-19 severity, defined as the need for ICU admission, need for ventilation or need for intubation. The authors found that vitamin D supplementation decreased the risk of severity by 62% (RR 0.38 (0.20–0.72), I^2 47%, with no difference between trials and observational studies.

Kazemi et al. [110] performed a large meta-analysis between 25OH-vitamin D levels and COVID-19 infection, severity and mortality. For infection, the authors meta-analyzed three studies and found that lower levels of 25OH-vitamin D were associated with a higher risk of SARS-CoV-2 infection (OR: 1.77 (1.24–2.53), I^2 44.2%). For severity, six studies were meta-analyzed and the pooling showed that there was a higher risk of COVID-19 severity for patients with lower levels of 25OH-vitamin D (OR: 2.57 (1.65–4.01), no heterogeneity), meaning that vitamin D deficiency increased the risk of severe COVID-19 by 157%. The meta-analysis of four studies that implemented Cox survival models showed that there was a significant association between lower vitamin D levels and increased mortality (HR: 7.67 (3.92–15.0), no heterogeneity). Vitamin D deficiency increased the risk of mortality by over 600%. However, when the association was assessed through logistic regression models, the results were inconsistent. The authors concluded that, although there was heterogeneity between studies, most of them showed a significant association between vitamin D deficiency and SARS-CoV-2 infection, severity and mortality.

3.6.3. Meta-Analysis of Randomized Controlled Trials (RCTs) of Vitamin D Analogs in COVID-19

Because observational studies using vitamin D levels and outcomes can be hampered by the observation that patients with multi-morbidity and compromised health are often vitamin D deficient, it is important to consider intervention studies. The results of seven meta-analysis of RCTs evaluating vitamin D treatment and COVID-19 outcomes are presented according to the chronological order of the publication dates.

Varikasuvu et al. [111] performed a meta-analysis of six RCTs, comprising 551 participants, in 2022 and applied a random effects model. Vitamin D supplementation was associated with a decrease in the risk of COVID-19 severity (defined as symptoms, ICU care and mechanical ventilation) estimated at 54% (RR: 0.46 (0.23–0.93), I^2 52%). However, the authors found no association between intervention with vitamin D and mortality.

Hosseini B et al. [112] performed a larger meta-analysis of 23 studies in 2022, including both RCTs and cohort studies. The authors found that vitamin D supplementation, started prior to SARS-CoV-2 infection, did not decrease the risk of COVID-19 incidence. However, when both RCTs and cohort studies were pooled, vitamin D treatment decreased COVID-19 mortality by 48% (RR: 0.52 (0.36–0.75), I^2 54%).

Kummel et al. [113] meta-analyzed eight RCTs in 2022, including 567 patients, and did not find an association between any type of vitamin D supplementation and mortality (OR: 0.74 (0.32–1.71)). Similarly, the authors did not find evidence of a beneficial effect of vitamin D analog therapy on ICU admission or mechanical ventilation.

Meng J et al. [114] performed a larger meta-analysis of 25 RCTs in 2023, including 1828 participants. Vitamin D supplementation did not prevent COVID-19 incidence, but it reduced the rate of ICU admission (RR 0.63 (0.44–0.89)) and mechanical ventilation (RR:

0.58 (0.39–0.84)). In the overall population, intervention with vitamin D did not decrease the mortality risk; however, in the subgroup with 25OH-vitamin D deficiency, the treatment significantly decreased the mortality risk by 24% (RR: 0.76 (0.58–0.98)).

Zhong Z et al. [115] sought to evaluate the efficacy of high-dose vitamin D analog treatment in terms of COVID-19 outcomes in 2023. The authors included five RCTs involving 834 patients. High-dose vitamin D supplementation (defined as a single dose of more than 100,000 IU or a daily dose of 10,000 IU reaching 100,000 IU) did not show any effect on mortality (RR: 1.09 (0.68–1.74), no heterogeneity) or ICU admission (RR: 0.70 (0.45–1.10), no heterogeneity).

Sobczak and Pawliczak [116] meta-analyzed 13 RCTs in 2024 and found that vitamin D3 supplementation decreased the risk of ICU admission (RR: 0.73 (0.57–0.95), I^2 19.6%) and COVID-19 specific mortality (RR: 0.56 (0.34–0.91), no heterogeneity).

Finally, in the largest meta-analysis of RCTs on vitamin D supplementation in COVID-19 that we found, Yang Y [117] meta-analyzed 19 RCTs in 2024, including 2435 participants, and found that vitamin D supplementation decreased the frequency of requiring intensive care admission (OR: 0.49 (0.30–0.79), I^2 55%). In a subgroup analysis, it was shown that multiple-dose administration was much more effective than single-dose administration. Treatment with vitamin D analogs also decreased the need for mechanical ventilation (OR 0.46 (0.29–0.72), I^2 6%). The authors found no association with mortality.

3.7. Vitamin D Supplementation and SARS-CoV-2 Viral Load in RCT

It has been shown in a meta-analysis of RCTs previously mentioned [111] that vitamin D supplementation was also associated with a decrease in RT-PCR SARS-CoV-2 positivity (RR: 0.46 (0.24–0.89), no heterogeneity).

NIH Guidelines on Vitamin D on COVID-19

The NIH guidelines [118] state that there is insufficient evidence for the COVID-19 panel to recommend either for or against the use of vitamin D for the prevention or treatment of COVID-19. The authors do state in the guidelines that there are multiple observational studies that suggest that lower levels of 25OH-vitamin D are associated with worse COVID-19 outcomes, such as mortality. However, it has to be taken into account that low vitamin D levels assessed while COVID-19 has begun may reflect reverse causality.

We summarize the available evidence on antiviral agents, anti-SARS-CoV-2 agents, clinical benefits from RCTs and NIH guidelines for metformin, statins and vitamin D analogs in the following Table 1.

Table 1. Preclinical and clinical evidence on benefits from metformin, statins and vitamin D analogs in SARS-CoV-2 infection and COVID-19 mortality.

Therapeutic Agent	Evidence of Preclinical Benefit as Antiviral Agents	RCT Evidence as Antiviral Agent/Anti-SARS-CoV-2	RCT Evidence of COVID-19 Mortality Benefit	NIH Guidelines
Metformin	Zika, hepatitis C, and SARS-CoV-2. Probable mechanism: AMPK activation/ inhibition of mTOR at low doses. Ref: [56–60]	3.6-fold decrease in mean viral load (COVID-OUT RCT) Ref: [76]	Mortality reduction in 42% as secondary outcome after excluding oximeters (COVID-OUT RCT) Ref: [74]	Metformin decreases death and long COVID in diabetic patients [84]
Statins	Hepatitis C and SARS-CoV-2. Probable mechanism: displacement of ACE2 on lipid rafts and inhibition of mTOR Ref: [90,92,93]	Not available	Mortality reduction in 12% summarized in a meta-analysis of RCTs Ref: [100]	Continued chronic use of statins recommended during acute COVID-19 [103]
Vitamin D analogs	Possible benefit against enveloped viruses. Mechanisms not fully elucidated, but increased autophagy via inhibition of mTOR has been postulated. Ref: [106,108]	Meta-analysis of RCTs has shown a decrease in RT-PCR SARS-CoV-2 positivity Ref: [111]	Mortality reduction in 48% Ref: [112] Mortality reduction in 24% in subgroup with 25OH-vitamin D deficiency Ref: [114] Mortality reduction in 44% in COVID-19-specific mortality Ref: [116] However, no significant results in references [113,115,117]	Insufficient data for the use of vitamin D analogs in COVID-19. [118]

3.8. Evidence for Exogenous Klotho Therapy and Klotho-Related Peptides on COVID-19: Preclinical Data

The hypothesis that low Klotho levels play a central role in COVID-19 pathogenesis naturally leads to the need to evaluate whether the exogenous administration of Klotho protein or Klotho-related peptides proves beneficial in preventing severe COVID-19. Two independent research groups have recently found consistent results at a preclinical level.

3.8.1. Exogenous Full-Length Klotho Therapy and Mortality in COVID-19 Model Mice

Alem et al. [3] evaluated the hypothesis that an acute deficit of Klotho plays a key and central role in the pathogenesis leading to severe COVID-19. The authors performed two independent preclinical experiments in hACE2 AC70 transgenic mice, including 55 rodents. The key question was whether the application of exogenous Klotho decreased mortality in a COVID-19 model mice exposed to SARS-CoV-2. The animals were infected intranasally with SARS-CoV-2. Some cohorts received exogenous Klotho, while the control cohorts received vehicle. Through meta-analysis of the two independent experiments and after applying Weibull parametric survival models, the authors found that exogenous full-length Klotho treatment was associated with a significant decrease in mortality (HR: 0.12 (0.06–0.25), $p = 2.7 \times 10^{-8}$, no heterogeneity). When Cox models were applied, the results were more conservative but still highly significant (HR: 0.37 (0.19–0.72), $p = 0.003$, no heterogeneity). Treatment with full-length exogenous Klotho protein was able to significantly decrease the risk of mortality due to severe COVID-19 in a preclinical mouse model.

3.8.2. Klotho-Derived Peptide 1 Therapy Decreases SARS-CoV-2-Induced Acute Kidney Injury (AKI) In Vitro and In Vivo

Severe cases of COVID-19 are frequently complicated by acute kidney injury (AKI), representing up to 85% of ICU cases [119]. Remarkably, SARS-CoV-2 induces more severe AKI than other respiratory diseases, with a higher incidence of chronic kidney disease and higher mortality [120]. Autopsy series have found that SARS-CoV-2 has a large kidney tropism [121], which is shared by other pathogenic coronaviruses, such as SARS-CoV and MERS-CoV [122]. Although an older age and prevalent kidney disease are the main risk factors for severe COVID-19 [33], the molecular basis for this connection has remained largely unknown. The authors of this paper hypothesized that severe and acute Klotho deficiency play a key role in this connection, especially through AKI [123]. As Klotho is kidney-protective [24,31,32], the authors mentioned that it is conceivable that a chronic deficiency of Klotho in the elderly and those with certain other diseases predisposes these patients to more severe kidney injury during COVID-19, establishing a vicious cycle.

Acute and severe Klotho deficiency, induced by the ischemia–reperfusion approach, sensitizes model mice to develop AKI after injection of the SARS-CoV-2 N protein [123], which is the responsible for AKI in COVID-19 [124,125]. Overexpression of SARS-CoV-2 N protein markedly aggravated AKI and apoptosis markers in Klotho-deficient mice (Kl/Kl) in comparison to wild-type mice. Exogenous administration of recombinant full-length Klotho and KP1, a Klotho-derived peptide with 30 amino acids that has shown kidney antifibrotic properties [126], recapitulates the kidney protection of the full-length Klotho, inhibiting cellular apoptosis and ameliorating kidney dysfunction in vivo and in vitro. For this purpose, the authors transfected HK-2 cells with the pSARS-CoV-2 N plasmid, which induced the expression of apoptosis, fibrotic and senescence markers. These consequences were abolished by both recombinant full-length Klotho and KP1. The efficacy of KP1 was similar to full-length Klotho. The authors concluded that Klotho deficiency is a key determinant of developing AKI in COVID-19. Conversely, KP1 administration was shown to alleviate kidney tubular injury induced by the SARS-CoV-2 N protein, similarly to full-length recombinant Klotho [123].

4. Discussion

There is ample evidence suggesting that chronic use of medications that increase Klotho levels, such as metformin and statins, is associated with better outcomes and survival in COVID-19. Furthermore, although the evidence is not homogeneous, most randomized trials on metformin, statins, and vitamin D analog treatment have shown a benefit in relation to COVID-19 outcomes for those patients in the intervention arm. In general, there is stronger evidence of the mentioned Klotho-increasing agents improving the outcomes of COVID-19 if they were already being used in patients prior to SARS-CoV-2 infection than if they were used as acute treatments after infection. In this regard, the NIH guidelines have stated that patients on metformin should continue this medication during COVID-19 evolution (unless they developed an absolute contraindication), that patients on statins should continue their use in case of COVID-19, and that low levels of vitamin D seem to be associated with poorer outcomes for COVID-19 patients, according to observational studies.

Preclinical studies found that exogenous full-length Klotho and Klotho-related peptide 1 lower COVID-19 mortality and decrease renal tissue injury in SAR-CoV-2-induced AKI, respectively. The results from these two interventional studies are an important proof of concept for the hypothesis that Klotho levels are a key determinant of COVID-19 pathogenesis. Klotho supplementation therapy should be further investigated, especially since during the acute course of severe COVID-19, especially with AKI as a complication, there are contraindications to use drugs such as metformin and statins, the former due to lactic acidosis [85] and the latter due to rhabdomyolysis [127]. In addition, during the course of severe illness with acute kidney disease, the ability of the tubular cells to synthesize Klotho due to pharmacological enhancers that act as Klotho agonists is severely compromised [29], and in such instances, therapy with Klotho itself or Klotho-related peptides could be therapeutically considered instead.

It is important to notice that, according to autopsy series, the kidney involvement in severe COVID-19 is not accompanied by a large inflammatory innate immune infiltrate, highly suggesting other pathogenic mechanisms [128]. One such mechanisms could be Klotho deficiency.

We hereby present the hypothesis that chronic sufficient levels of Klotho are protective against severe COVID-19 and that, accordingly, an acute deficit of Klotho plays a central role in severe COVID-19 pathogenesis. Further investigation is required to determine if Klotho levels are also critical to the pathogenesis from infections from other coronaviruses, especially given the relevance this would possibility have in pandemic preparedness.

Limitations

One of the limitations in proving the Klotho hypothesis is that genome-wide association studies (GWAS) on severe COVID-19 cases have not found loci near to the KLOTHO locus (13q13.1, OMIM 604824) so far [129]. Another limitation in the breadth of the publications covered is that the literature search for this specific review included only English-language PubMed articles. In addition, it is possible that there is an overlap among the different meta-analysis studies reviewed, so the data may not be completely independent. Another important limitation is that the evidence of the efficacy of Klotho therapy is only available from animal models.

5. Conclusions

This review provides curated mechanistic, epidemiological, and clinical evidence that supports the hypothesis that a low level of Klotho plays a critical role in the pathogenesis toward a severe COVID-19 case. As a unifying hypothesis, the beneficial associations in terms of COVID-19 outcomes observed for patients with chronic use of Klotho agonists, the benefits suggested in some RCTs of Klotho agonists administered acutely, and the results of preclinical data on the beneficial effects of Klotho therapy on survival and kidney injury in animal studies can be integrated around Klotho as a central protective agent but also a

potential rescue therapy. In addition, Klotho may have antiviral effects, especially through the potential inhibition of mTOR. This could explain why different pharmacological classes of drugs, such as metformin, statins, and vitamin D analogs have shown antiviral effects.

We conclude that low levels of Klotho play a key role in severe COVID-19 and that both chronic administration of Klotho agonists and acute administration of Klotho or Klotho-related peptides may decrease COVID-19 severity and mortality. Further studies testing the effects of Klotho in relation to the pathogenesis initiated by other human coronaviruses are necessary.

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