Philippine COVID-19 Living Clinical Practice Guidelines
Institute of Clinical Epidemiology, National Institutes of Health, UP Manila
In cooperation with the Philippine Society for Microbiology and Infectious Diseases
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EVIDENCE SUMMARY

Should LDH, CRP, and Ferritin be used to guide immunotherapy in patients with COVID-19?

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RECOMMENDATIONS

There is insufficient evidence to recommend the use of specific cut-off values of CRP, LDH and Ferritin to guide the initiation of immunotherapy in patients with COVID-19 *(Very low certainty of evidence)*

Consensus Issues
The evidence base included studies that investigated the levels of LDH, CRP, and ferritin that are correlated with progression to severe disease, poor outcomes, or mortality, rather than improved survival with immunotherapy. Thus, the panel unanimously decided that insufficient evidence remains to use specific cut-off values of these markers to guide immunotherapy. Additionally, the panel cited the availability of other parameters that may be used to guide initiation of immunotherapy besides these markers.

PREVIOUS RECOMMENDATION

There is insufficient evidence to recommend the use of specific cut-off values of CRP, LDH, and ferritin to guide the initiation of immunotherapy in patients with COVID-19. *(Very low quality of evidence)*

Previous Consensus Issues
It was noted that varying cut-off values of LDH, CRP, and ferritin were used across the studies to predict mortality and severity of disease. Thus, no specific levels could be recommended to guide initiation of immunotherapy for COVID-19 patients.

Key Findings

- Moderate quality of evidence showed significant correlation of CRP >10 mg/L with mortality (four studies) and poor outcome (ten studies).
- High quality of evidence from seven studies showed significant correlation between elevated LDH and progression to severe disease.
- Moderate quality of evidence from 18 studies showed significant correlation between LDH >250 U/L and poor outcome.
- Low quality of evidence from four studies did not find significant correlation between elevated LDH and mortality.
- Very low quality of evidence showed significant correlation between elevated CRP and progression to severe disease (one study) and ICU admission (one study) in pediatric patients.

Introduction

Severe COVID-19 disease is characterized by an immunologic and hyperinflammatory response known as the cytokine release syndrome (CRS), whereby elevated pro-inflammatory cytokines, such as IL-2, IL-6, IL-10, and TNF-α cause multiple organ dysfunction, and possibly death. This uncontrolled immune response serves as the basis of immunotherapy in severe to critical COVID-19.[1]

Determining the onset of CRS is largely a clinical dilemma, as testing for the cytokines that herald it are not readily available in most institutions, and when they are, come at a high cost. Meanwhile, other markers of inflammation, such as lactate dehydrogenase (LDH), C-reactive protein (CRP), and ferritin have also been observed to be elevated in COVID-19 and, in some earlier cohort studies, were found to be associated with disease severity, progression, and mortality.[2] These are more readily available and less costly compared to testing for cytokines.

Current international society recommendations have utilized these inflammatory markers as guides for initiation of immunotherapy. For instance, the recommendation for initiation of tocilizumab is a CRP level of $\geq$75mg/L [3–6] and/or a ferritin level of $\geq$1,000 ng/ml.[7] Serial monitoring of LDH, ferritin, and CRP are also recommended for patients undergoing immunotherapy.[7,8] However, these cut-off levels were based on earlier cohort studies on patients with COVID-19 from Wuhan, China.[9] Subsequent systematic reviews and meta-analyses [10–13] have consistently revealed the value of CRP and LDH in predicting severe outcomes in COVID-19. However, the cut-off points vary between studies, making it difficult to generalize.

A previous review for the COVID-19 Living Clinical Practice Guidelines by Ibañez et al. [14] reported varying CRP, LDH, and ferritin cut-off values for disease severity, progression, and mortality in early observational studies and did not recommend specific biomarker levels for the initiation of immunotherapy. This review sought to build on previous evidence to determine the cut-off values of CRP, LDH, and ferritin associated with COVID-19 severity, progression, and mortality in pediatric and adult patients based on pooled data in systematic reviews and meta-analyses.

Review Methods

A literature search was done for published studies from 14 May 2021 up to 19 November in MEDLINE, the Cochrane COVID-19 Living Study Register, and the COAP COVID-19 Living Evidence Database. The MEDLINE search was done using the following terms: “CRP” OR “C-reactive protein” OR “hsCRP” OR “ferritin” OR “ferritin” [Mesh] OR “lactate dehydrogenase” OR “LDH” AND “Mortality” OR “Mortality” [Mesh] OR “Severity” OR “Severity” [Mesh] AND COVID-19. The Cochrane and Living Evidence databases were searched with combinations and derivations of the terms “CRP,” “LDH,” “ferritin,” “COVID-19,” “mortality,” and “severity.”

Included in this review were systematic reviews and meta-analyses that recruited adult and pediatric hospitalized patients diagnosed with COVID-19 and reported baseline CRP, ferritin, and LDH values, and the following outcomes: mortality, disease severity upon admission, and progression to more severe stages (e.g., need for mechanical ventilation or ICU admission). Excluded from this review were studies that did not report an effect estimate (e.g., odds ratio, hazards ratio) for the biomarkers of interest, studies not written in English, conference abstracts, and preprint studies. The respective corresponding authors were contacted via e-mail for studies with inaccessible full-text articles, supplementary materials, or presented data.

Methodological quality of the included systematic reviews was assessed using the appraisal criteria by Dans et al. [15] Certainty of evidence for outcomes with cut-off values was determined using GRADE approach.

Results

Characteristics of Included Studies

A total of 4,076 titles and abstracts were retrieved from the database search, among which were ten meta-analyses that satisfied the inclusion and exclusion criteria. Eight reviews [16-23] were of adult patients or had no specific age criteria, while two [24-25] were of pediatric patients. Eight reviews [17,19-25] measured baseline CRP levels, six reviews [17,20-23,25] measured LDH levels, and four reviews [16,18,19,21] measured ferritin levels. Regarding outcomes, seven reviews [17-19, 21-24] used mortality, eight reviews [16-19,21-22,24-25] used severe disease or manifestations thereof such as intensive care unit (ICU) admission or mechanical ventilation, two reviews [23-24] used progression to severe disease, and two reviews [20,22] used a composite of poor outcomes. All the included meta-analyses reviewed observational studies; notably, one review was entirely of case series.[23] Regarding effect measures, two reviews [20,24] used odds ratio (OR) with biomarker cut-off values, two reviews [23,25] used OR with no specific cutoff-value, one review [17] used difference of medians, and the remaining six used standardized mean difference (SMD) or weighted mean difference (WMD).

Appendix 3 shows a summary of the characteristics of included studies.

Risk of Bias Assessment

Based on the Painless Evidence-Based Medicine approach, all the included meta-analyses closely approximated the research question posed in this review. The determination of biomarker levels either was coincident with or preceded the stated outcome in all included reviews. Unbiased criteria were used to determine biomarker levels and outcome in all reviews. One review [24] did not report patient group comparisons for its included studies, while the remaining reviews reported discrepancies between groups. There was no mention of follow-up rate for any review.

Biological applicability issues were noted. Three reviews [19,21,23] had body of literature that mainly comprised of Chinese studies thereby affecting generalizability. Another review [18] had between-study heterogeneity for severe/critical disease attributed to differences in age and comorbidities, and heterogeneity for mortality attributed to differences in age and proportion of patients with male sex. On the other hand, the reviews on pediatric patients included one study [24] where males had higher prevalence of underlying childhood diseases than females, which may distort the correlation between biomarkers and outcomes. No socio-economic applicability issues were mentioned in any of the studies.

Appendix 4 shows the detailed risk of bias assessment of the included studies.

Summary of Results of Included Studies

A. Outcome: Mortality

CRP

Six reviews [17,19,21-24] discussed CRP and mortality. One meta-analysis [23] with five studies and 1,565 patients used OR to report a significant correlation between increased CRP and mortality (OR 2.48, 95% CI 1.37, 4.50). The upper limit of the locally defined reference range per study was used as the cut-off point. Four reviews [17,19,21,22] reported that CRP levels were significantly higher in non-survivors than in survivors. One review on children and adolescents [24] with two studies and 72 patients showed that CRP levels were not significantly different between survivors and non-survivors (WMD -8.89, 95% CI -59.98, 42.20).

Ferritin
Three reviews [18,19,21] discussed ferritin and mortality. All three reported significantly higher levels of ferritin in non-survivors compared to survivors.

LDH
Four reviews [19,21-23] discussed LDH and mortality. One review [23] of four studies with 1,791 patients did not find a significant correlation between increased LDH and mortality (OR 5.23, 95% CI 0.91, 30.06). The three remaining reviews reported significantly higher levels of LDH in non-survivors compared to survivors.

Table 1 of Appendix 5 shows a summary of the association of CRP, ferritin, and LDH with mortality.

B. Outcome: COVID-19 severe disease
CRP
Four reviews [18-19,22,25] discussed CRP and severe disease. One review on pediatric patients [25] did not find a significant correlation between CRP levels and severe disease (OR 2.17, 95% CI 0.51, 3.84) with no stated cut-off value. The other three reviews reported significantly higher levels of CRP between patients with severe and non-severe disease

Three reviews [17,22,24] discussed CRP and ICU admission. One review on pediatric patients [24] reported a significant correlation between CRP >10 mg/L and ICU admission (OR 8.00, 95% CI 1.60, 39.97) based on one study (54 patients) with very low-quality evidence. This review also reported significantly increased CRP for pediatric patients admitted to the ICU, which is also reflected in the remaining two reviews.

One review [18] reported a significant increase in CRP levels in patients with acute respiratory distress syndrome (ARDS). One review on pediatric patients [24] reported a significant increase in CRP levels in patients requiring respiratory support.

Table 2 of Appendix 4 shows the effect estimates on the association of CRP, ferritin, and LDH with COVID-19 severe disease.

Ferritin
Four reviews [16,18,19,21] discussed ferritin and severe disease on admission. All four reported significantly higher levels of ferritin in non-survivors compared to survivors.

One study [18] reported significantly increased ferritin levels for patients who underwent ICU admission and mechanical ventilation compared to those who did not.

Table 3 of Appendix 5 shows the effect estimates on the association of ferritin with severe disease on admission.

LDH
Three reviews [21-22,25] discussed LDH and severe disease. One review [25] on pediatric patients with 52 studies and 203 patients did not find a significant correlation between increased LDH and severe disease (OR 1.60, 95% CI 0.92, 4.51). The other two reviews reported significantly higher levels of CRP between patients with severe and non-severe disease. Table 2 of Appendix 5 shows the effect estimates on the association of LDH with severe disease on admission.

Two reviews [17,22] reported significantly higher levels of LDH in patients requiring ICU admission.

Table 3 of Appendix 5 shows the effect estimates on the association of LDH with ICU admission.

C. Outcome: Disease progression

CRP

Two reviews [23,24] discussed CRP and progression to severe disease. One review [24] with five studies and 1,565 patients reported a significant correlation between increased CRP and progression to severe disease (OR 5.57, 95% CI 4.41, 7.04) based on locally defined reference ranges. One review on pediatric patients [23] reported a significant correlation between CRP ≥80 mg/L and progression to severe disease (OR 11.0, 95% CI 4.37, 31.37) based on one study (250 patients) with very low-quality evidence. The latter also reported significantly higher CRP levels in patients who progressed to severe disease compared to those who did not (WMD 33.29, 95% CI 11.12, 55.33).

Ferritin

No review discussed ferritin and progression to severe disease.

LDH

One review [23] with seven studies and 1,337 patients reported a significant correlation between increased LDH levels and progression to severe disease (OR 4.12, 95% CI 2.88, 5.90) with no stated cut-off value.

Table 4 of Appendix 5 shows the effect estimates on the association of CRP and LDH with progression to severe disease.

D. Outcome: Composite poor prognosis

CRP

Two reviews [20,22] discussed CRP and composite poor outcome. One review of 20 studies with 3,313 patients [20] reported a significant correlation between CRP >10 mg/L and composite poor outcome (OR 3.97, 95% CI 2.89, 5.45). Another review [22] reported a significant increase in CRP in patients with poor outcomes.

Ferritin

No review discussed ferritin and composite poor outcome.

LDH

Two reviews [20,22] discussed LDH and composite poor outcome. One review of 18 studies with 3,641 patients [20] reported a significant correlation between LDH >250 U/L and composite poor outcome (OR 5.48, 95% CI 3.89, 7.71). Another review [22] reported a significant increase in LDH in patients with poor outcomes.

Table 5 of Appendix 5 shows the effect estimates on the association of CRP and LDH with composite poor prognosis.

Certainty of Evidence Assessment

The evidence for the correlation of CRP >10 mg/L with poor outcome (20 studies) and with mortality (4 studies) were assessed to have moderate certainty of evidence based on serious limitations in inconsistency. The evidence for the correlation of CRP >10 mg/dL with pediatric ICU admission (1 study), and CRP ≥80 mg/L with progression to severe disease in pediatric patients (1 study) had very low certainty of evidence based on serious limitations in risk of bias, inconsistency, and imprecision.

The evidence on the correlation of LDH with the progression to severe disease (7 studies) was rated to have high certainty of evidence. The correlation of LDH >250 U/L with poor outcome (18 studies) had moderate certainty of evidence based on serious limitations in inconsistency,

while the correlation of LDH with mortality (4 studies) had low certainty of evidence based on serious limitations in inconsistency and imprecision.

Recommendations from Other Groups

The latest recommendations from the **United States National Institutes of Health** [4] and the **Infectious Disease Society of America** [5] for the use of tocilizumab (April 2021) are heavily based on the results of the RECOVERY trial [3], which reports significant benefit for tocilizumab for patients with systemic inflammation, defined as **CRP ≥75 mg/L**.

The **National Institute for Health and Care Excellence (NICE)** in the United Kingdom (March 2021) recommends the use of tocilizumab as well as sarilumab, a novel IL-6 inhibitor, for adult patients requiring supplemental oxygen and **CRP ≥75 mg/L**.[6]

The **National Institute for Infectious Diseases in Italy** (February 2020) recommended the use of **ferritin, CRP and LDH for serial monitoring** during infusion of immunotherapy (such as Tocilizumab) and failure to reduce levels by 50% from baseline levels warrant a second dose of therapy.[8]

The **Kuwaiti Ministry of Health** issued a guideline in April 2020 recommending the use of tocilizumab for adults with severe or critical COVID-19 suspected to have cytokine release syndrome, as shown by **high CRP (≥100 mg/L)** or **ferritin (≥1,000 ng/ml)** levels, in addition to **high LDH (≥200 U/L)** levels, elevated **D-dimer (>250 ng/ml)**, and the need for ICU supportive care with mechanical ventilation for ≤48 hours.[26]

Local guidelines from the Philippines last July 2020 stated the use of **ferritin >1,000 ng/ml** as one of the indications for initiating Tocilizumab. Furthermore, they stated serial monitoring of ferritin, CRP, and LDH during therapy.[7]

Research Gaps

As of December 2021, there were two ongoing clinical trials on inflammatory phenotypes in COVID-19. Studies on the outcomes of immunotherapy initiated at specific levels of LDH, CRP, and ferritin, as well as the trends in the level of these biomarkers on subsequent monitoring would provide more direct evidence as to the role of these biomarkers in optimal COVID-19 management. Additionally, analyses on the levels of LDH, CRP, and ferritin at which a second dose of immunotherapy (e.g., tocilizumab) would lead to optimal outcomes should be undertaken.

Appendix 7 lists the characteristics of the ongoing trials.

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Appendix 1. Evidence to Decision

Table 1. Summary of initial judgements prior to the panel discussion (N = 9)

FACTORS	JUDGEMENT						RESEARCH EVIDENCE/ADDITIONAL CONSIDERATIONS
Problem	No	Yes (9)					Severe COVID-19 disease is characterized by an immunologic and hyperinflammatory response known as the cytokine release syndrome (CRS), whereby elevated pro-inflammatory cytokines,such as IL-2, IL-6, IL-10, and TNF-α cause multiple organ dysfunction, and possibly death. This uncontrolled immune response serves as the basis of immunotherapy in severe to critical COVID-19.
Certainty of Evidence	High	Moderate (6)	Low (3)	Very low			Moderate quality of evidence showed significant correlation between CRP>10 mg/L and mortality (4 studies) and poor outcome (10 studies). High quality of evidence from seven studies showed significant correlation between elevated LDH and progression to severe disease. Moderate quality of evidence from 18 studies showed significant correlation between LDH >250 U/L and poor outcome. Low-quality evidence from 4 studies does not find significant correlation between elevated LDH and mortality. Very low quality of evidence showed significant correlation between elevated CRP and progression to severe disease (1 study) and ICU admission (1 study) in pediatric patients.
Accuracy	Very Accurate	Accurate (6)	Inaccurate (2)	Very Inaccurate	Uncertain (1)		
Values	Important uncertainty or variability (3)	Possibly important uncertainty or variability (6)	Possibly NO important uncertainty or variability	No important uncertainty or variability			No evidence found.
Resources Required	Uncertain	Large cost (2)	Moderate Cost (5)	Negligible cost or savings (2)	Moderate savings	Large savings	Immunohistochemical CRP costs Php 600.

FACTORS	JUDGEMENT						RESEARCH EVIDENCE/ADDITIONAL CONSIDERATIONS
Certainty of evidence of required resources	No included studies (4)	Very low	Low (2)	Moderate (2)	High (1)		
Cost effectiveness	No included studies (5)	Favors using the markers (3)	Does not favor either using the markers or the comparator (1)	Favors comparison			
Equity	Uncertain (4)	Reduced (2)	Probably no impact (1)	Increased (1)			
Acceptability	Uncertain (5)	No	Yes (4)				
Feasibility	Uncertain (2)	No	Yes (7)				

Appendix 2. Search Yield and Results

Database	Search terms	Number of entries
MEDLINE	("CRP" OR "C-reactive protein" OR "hsCRP" OR "ferritin" OR "ferritin" [Mesh] OR "lactate dehydrogenase" OR "LDH") AND ("Mortality" OR "Mortality" [Mesh] OR "Severity" OR "Severity" [Mesh]) AND COVID-19	871
Cochrane COVID-19 Study Register	("CRP" OR "C-reactive protein" OR "hsCRP" OR "high-sensitivity C-reactive protein" OR "high sensitivity C-reactive protein" OR "ferritin" OR "serum ferritin" OR "lactate dehydrogenase" OR "LDH") and ("Mortality" OR severity OR severe OR ICU OR "intensive care unit" OR complication OR complications) and ("COVID-19" or COVID or coronavirus OR "coronavirus disease-19" OR "coronavirus disease 19")	1971
COVID-19 Living Evidence Database	((CRP) OR (C-reactive protein) OR (hsCRP) OR (ferritin) OR (lactate dehydrogenase) OR (LDH)) AND ((Mortality) OR (Severity)) AND (COVID-19)	1234

Appendix 3. Characteristics of Included Studies

Study ID	Patient population	Study types	Studies (sample size)	Biomarker (cut-off if stated)	Outcome	Effect estimate[b]
Hossein Kazemi 2021	Patients with COVID-19	Retrospective, prospective, descriptive observational studies	18 (2459)	Ferritin	Severity	MD
Katzenschlager 2021	Patients with COVID-19	Retrospective, prospective cohort	88 (69762)	CRP, LDH	Severity, mortality	DoM
Kaushal 2021	Adult patients with COVID-19	Retrospective, prospective, ambispective studies	163 (n/a)	Ferritin	Severity, mortality	SMD
Mahat 2021	Patients with COVID-19	Cohort, case control, cross-sectional studies; case series	83 (17299)	CRP, Ferritin	Severity, mortality	SMD
Malik 2021	Hospitalized patients with COVID-19	Retrospective, prospective studies	32 (10491)	CRP, LDH	Composite[a]	OR
Melo 2021	Adult patients with COVID-19	Retrospective, prospective studies	40 (9833)	CRP	Severity, mortality	MD
Shi 2021	Pediatric patients with COVID-19	Cohort studies, case-control studies; case series	56 (79104)	CRP	Severity, progression, mortality	OR, WMD
Wang 2021	Adult patients with COVID-19	Retrospective studies, cohort studies, case-control studies	32 (7739)	CRP, LDH	Severity, mortality, composite[a]	SMD
Zhang 2021	Patients with COVID-19	Retrospective studies	34 (344431)	CRP, LDH	Progression, mortality	OR
Zhou 2021	Pediatric patients with COVID-19	Case series	52 (203)	CRP, LDH	Severity	OR

Legend: DoM, difference of medians; MD, mean difference; OR, odds ratio; SMD, standardized mean difference; WMD, weighted mean difference.

[a]Composite outcome (severe disease, ICU admission, mechanical ventilation, mortality)

[b]As reported by study authors

Appendix 4. Risk of Bias Assessment

	Hossein Kazemi 2021	Katzenschlager 2021	Kaushal 2021	Mahat 2021	Malik 2021	Melo 2021	Shi 2021	Wang 2021	Zhang 2021	Zhou 2021
I. APPRAISING DIRECTNESS										
Did the study provide a direct enough answer to your clinical question in terms of patients (P), exposure/intervention (E) and outcome (O)?	P - Patients with COVID-19 E - HLH score parameters (hemoglobin, WBC, neutrophils, lymphocytes, platelets, AST, ferritin, fibrinogen, fever) O - Severe COVID-19	P - Patients with COVID-19 E - COVID-19 symptoms, comorbidities, treatment regimens, laboratory values (WBC, neutrophils, lymphocytes, D-dimer, CRP, LDH, troponin I) O - Hospitalization, intubation, ICU admission, mortality	P - Adult patients with COVID-19 E - Serum ferritin level O - RT-PCR positive COVID-19, severe/critical disease, mortality, ICU admission, mechanical ventilation, liver/heart/kidney involvement, thrombotic complications	P - Patients with laboratory-confirmed COVID-19 E - Inflammatory biomarkers O - Severe disease, mortality	P - Hospitalized patients with COVID-19 E - Inflammatory biomarkers O - Composite of poor outcomes (ICU admission, O2St <90%, mechanical ventilation, severe disease, mortality)	P - Adult patients with COVID-19 E - Inflammatory biomarkers O - Severe disease, mortality	P - Pediatric patients with COVID-19 E - Age, sex, comorbidities, symptoms, laboratory findings O - Mortality, ICU admission, respiratory support, progression to severe/critical disease	P - Adult patients with COVID-19 E - CRP, LDH, serum amyloid A O - Poor outcome (severe disease, mortality, need for ICU care, ARDS)	P - Patients with COVID-19 E - CRP O - Progression to severe disease, mortality	P - Pediatric patients with COVID-19 E - Age, sex, comorbidities, symptoms, chest radiography features, laboratory biomarkers O - Severe disease
II. APPRAISING VALIDITY										
Did exposure precede outcome in the study?	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
Were the patient groups being compared sufficiently similar with respect to baseline characteristics? If not, were statistical adjustments made?	No; Meta-regression using random effects model	No; Meta-regression with random-effects model (Quantile Estimation approach)	No; Univariate meta-regression for categories with >10 studies or with high heterogeneity	No; Random/fixed-effects meta-regression	No; Meta-regression using random effects model	No; Meta-regression using random-effects model	Not stated; Random/fixed-effects meta-regression	No; Random/fixed-effects meta-regression	No; Random/fixed-effects meta-regression	No; Logistic regression analysis
Were unbiased criteria used to determine exposure in all patients?	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
Were unbiased criteria used to detect outcome in all patients?	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
Was follow-up rate adequate?	Not stated	Not stated	Not stated	Not stated	Not stated	Not stated	Not stated	Not stated	Not stated	Not stated

	Hossein Kazemi 2021	Katzenschlager 2021	Kaushal 2021	Mahat 2021	Malik 2021	Melo 2021	Shi 2021	Wang 2021	Zhang 2021	Zhou 2021
III. APPRAISING RESULTS										
1. How strong is the association between exposure and outcome? 2. How precise is the estimate of the risk?	Ferritin (ng/mL): MD 437.25 (100.37, 774.13)	ICU admission: CRP - DoM 56.41 (39.8, 73.02) LDH - DoM 140.4 (81.04, 199.6) Mortality: CRP - 69.1 (50.43, 87.77) LDH - 189.49 (155, 223.98)	Severe/critical disease: SMD 0.882 (0.738, 1.026) Mortality: SMD 0.734 (0.530, 0.938) ICU admission: SMD 0.674 (0.515, 0.833) Mechanical ventilation: SMD 0.430 (0.258, 0.602)	Severe disease: CRP - SMD 1.14 (0.97, 1.32) Ferritin - SMD 0.71 (0.60, 0.81) Mortality: CRP - SMD 1.18 (0.80, 1.55) Ferritin - SMD 0.95 (0.74, 1.17)	CRP >10 mg/L: OR 3.97 (2.89, 5.45) LDH >250 U/L: OR 5.48 (3.89, 7.71)	Severe disease: CRP - MD 53.54 (39.79, 67.29) LDH - MD 153.58 (87.09, 220.08) Ferritin - MD 654.4 (383.48, 925.33) Mortality: CRP - MD 58.48 (43.45, 73.61) LDH - MD 230.99 (192.29, 269.70) Ferritin - MD 853.43 (601.20, 1105.67)	Mortality: CRP: WMD -8.89 (-59.98, 42.20) ICU admission: CRP >10 mg/dL: OR 8.00 (1.60, 39.97) CRP: WMD 60.04 (23.83, 96.26) Respiratory support: CRP: WMD 18.20 (7.31, 29.09) Progression to severe/critical disease: CRP ≥ 80 mg/L: OR 11.0 (4.37, 31.37) CRP: WMD 33.29 (11.25, 55.33)	CRP: Composite poor outcome: SMD 0.98 (0.85, 1.11) Severe disease: SMD 0.94 (0.78, 1.11) Mortality: SMD 0.84 (0.64, 1.05) Need for ICU care: SMD 1.50 (1.30, 1.69) ARDS: SMD 0.78 (0.20, 1.36) LDH: Composite poor outcome: SMD 1.18 (1.00, 1.36) Severe disease: SMD 1.05 (0.81, 1.29) Mortality: SMD 1.22 (0.94, 1.51) Need for ICU care: SMD 1.56 (1.02, 2.10)	CRP progression: OR 5.57 (4.41, 7.04) CRP mortality: OR 2.48 (1.37, 4.50) LDH progression: OR 4.12 (2.88, 5.90) LDH mortality: OR 5.23 (0.91, 30.06) *cut-off values = upper limit of locally defined reference range	CRP: OR 2.17 (0.51-3.84) LDH: OR 1.60 (0.92-4.51)
IV. ASSESSING APPLICABILITY										

	Hossein Kazemi 2021	Katzenschlager 2021	Kaushal 2021	Mahat 2021	Malik 2021	Melo 2021	Shi 2021	Wang 2021	Zhang 2021	Zhou 2021
Are there biologic issues that may affect applicability of treatment?	Not stated *Comorbidities may affect increase/decrease of acute-phase reactants and/or risk of progression to severe disease	Not stated	High heterogeneity for studies involving severe/critical disease attriuted to differences in age and comorbidities; High heterogeneity for studies involving mortality attriuted to differences in age and male sex	Majority of studies from China, affecting generalizability	No data on changing levels of biomarkers during disease course, which may predict clinical course	Most studies involve Asian patients, which may affect generalizability	Males have higher prevalence of underlying childhood diseases than girls	Not stated	All included studies involving CRP and LDH were done on Chinese patients	Not stated
Are there socio-economic issues affecting applicability of treatment?	Not stated *Socioeconomic status may affect progression to severe disease	Not stated	None stated	None stated	None stated	None stated	None stated	Not stated	None stated	Not stated

Appendix 5. Summary of Findings

Table 1. Association of CRP, ferritin, and LDH with mortality.

Biomarker	Meta-analysis Number of studies (sample size)	Population age group	Biomarker cut-off (mg/L)	Effect Estimate[a]	95% CI	Heterogeneity (I^2, %)	Interpretation (Significant? Yes/No)
CRP and mortality	**Katzenschlager 2021** 34 studies	Not stated	None	DoM 56.41 (39.8, 73.02)		95.99%	Yes
	Mahat 2021 19 studies (n=4318)	Not stated	None	SMD 1.18 (0.80, 1.55)		94%	Yes
	Melo 2021 15 studies (n=3755)	Adults	None	MD 17.93 (11.89, 23.98)		99%	Yes
	Shi 2021 2 studies (n=72)	Pediatric patients	None	WMD -8.89 (-59.98, 42.20)		0%	No
	Wang 2021 10 studies (n=3035)	Adults	None	SMD 0.84 (0.64, 1.05)		Not stated	Yes
	Zhang 2021 4 studies (n=1855)	Not stated	Upper limit of locally-defined reference range	OR 2.48 (1.37, 4.50)		73%	Yes
FERRITIN and mortality	**Kaushal 2021** 57 studies	Adults	None	SMD 0.734 (0.530, 0.938)		97.29%	Yes
	Mahat 2021 11 studies (n=2554)	Not stated	None	SMD 0.95 (0.74-1.17)		76%	Yes
	Melo 2021 9 studies (n=2088)	Not stated	None	MD 853.43 (601.20, 1105.67)		94%	Yes
LDH and mortality	**Katzenschlager 2021** 23 studies	Not stated	None	DoM 189.49 (155, 223.98)		75.03%	Yes
	Melo 2021 10 studies (n=2622)	Adults	None	MD 230.99 (192.29, 269.70)		96%	Yes
	Wang 2021 12 studies (n=4000)	Adults	None	SMD 1.22 (0.94, 1.51)		Not stated	Yes
	Zhang 2021 4 studies (n=1791)	Not stated	Upper limit of locally-defined reference range	OR 5.23 (0.91, 30.06)		85%	No

[a] Effect estimates as reported by study authors

Table 2. Association of CRP, ferritin, and LDH with severe disease.

Biomarker	Meta-analysis Number of studies (sample size)	Population age group	Biomarker cut-off (mg/L)	Effect Estimate[a]	95% CI	Heterogeneity (I^2, %)	Interpretation (Significant? Yes/No)
CRP and severe disease	**Mahat 2021** 44 studies (n=7898)	Not stated	None	SMD 1.14 (0.97, 1.32)		90%	Yes
	Melo 2021 14 studies (n=4138)	Adults	None	MD 53.54 (39.79, 67.29)		97%	Yes
	Wang 2021 11 studies (n=1909)	Adults	None	SMD 0.94 (0.78, 1.11)		Not stated	Yes
	Zhou 2021 52 studies (n=203)	Pediatric patients	Not stated	OR 2.17 (0.51, 3.84)		Not stated	No
FERRITIN and severe disease	**Hossein Kazemi 2021** 5 studies (n=264)	Not stated	None	MD 437.25 (100.37, 774.13)		68.20%	Yes
	Kaushal 2021 39 studies	Adults	None	SMD 0.882 (0.738, 1.626)		85.6%	Yes
	Mahat 2021 9 studies (n=1609)	Not stated	None	SMD 0.71 (0.60, 0.81)		5%	Yes
	Melo 2021 6 studies (n=3470)	Adults	None	MD 654.4 (353.48, 925.33)		96%	Yes
LDH and severe disease	**Melo 2021** 10 studies (n=2622)	Adults	None	MD 230.99 (192.29, 269.70)		96%	Yes
	Wang 2021 12 studies (n=4000)	Adults	None	SMD 1.22 (0.94, 1.51)		Not stated	Yes
	Zhou 2021 52 studies (n=203)	Pediatric patients	Not stated	OR 1.60 (0.92, 4.51)		Not stated	No

[a] Effect estimates as reported by study authors

Table 3. Association of CRP, ferritin, and LDH with ICU admission, mechanical ventilation, and ARDS.

Biomarker	Meta-analysis Number of studies (sample size)	Population age group	Biomarker cut-off (mg/L)	Effect Estimate[a]	95% CI	Heterogeneity (I^2, %)	Interpretation (Significant? Yes/No)
CRP and ICU admission	**Katzenschlager 2021** 10 studies	Not stated	None	DoM 56.41 (39.8, 73.02)		76.56%	Yes
	Shi 2021	Pediatric patients	CRP >10 mg/dL 1 study (n=54)	OR 8.00 (1.60, 39.97)		100%	Yes
			None 6 studies (n=365)	WMD 60.04 (23.82, 96.26)		38.6^	Yes
	Wang 2021 4 studies (n=802)	Adults	None	SMD 1.50 (1.30, 1.69)		Not stated	Yes
CRP and need for respiratory support	**Shi 2021** 1 study (n=37)	Pediatric patients	None	WMD 18.20 (7.31, 29.09)		100%	Yes
CRP and ARDS	**Wang 2021** 2 studies (n=121)	Adults	None	SMD 0.78 (0.20, 1.36)		Not stated	Yes
FERRITIN and ICU admission	**Kaushal 2021** 21 studies	Adults	None	SMD 0.674 (0.515, 0.833)		80.32%	Yes
FERRITIN and mechani-cal ventilation	**Kaushal 2021** 8 studies	Adults	None	SMD 0.430 (0.258, 0.602)		32.79%	Yes
LDH and ICU admission	**Katzenschlager 2021** 23 studies	Not stated	None	DoM 189.49 (155, 223.98)		75.03%	Yes
	Wang 2021 3 studies (n=762)	Adults	None	SMD 1.56 (1.02, 2.10)		Not stated	Yes

Table 4. Association of CRP, ferritin, and LDH with progression to severe disease.

Biomarker	Meta-analysis Number of studies (sample size)	Population age group	Biomarker cut-off (mg/L)	Effect Estimate[a]	95% CI	Heterogeneity (I^2)	Interpretation (Significant? Yes/No)
CRP and progression	**Shi 2021**	Pediatric patients	CRP ≥ 80 mg/dL 1 study (n=250)	OR 11.0 (4.37, 31.37)		100%	Yes
			None 5 studies (n=347)	WMD 33.29 (11.25, 55.33)		94.3%	Yes
	Zhang 2021 5 studies (n=1565)	Not stated	Upper limit of locally-defined reference range	OR 5.57 (4.41, 7.04)		35%	Yes
LDH and progression	**Zhang 2021** 7 studies (n=1337)	Not stated	Upper limit of locally-defined reference range	OR 4.12 (2.88, 5.90)		49%	Yes

Table 5. Association of CRP, ferritin, and LDH with composite poor outcome.

Biomarker	Meta-analysis Number of studies (sample size)	Population age group	Biomarker cut-off (mg/L)	Effect Estimate[a]	95% CI	Heterogeneity (I^2)	Interpretation (Significant? Yes/No)
CRP and composite poor outcome	**Malik 2021** 20 studies (n=3313)	Not stated	CRP > 10 mg/L	OR 3.97 (2.89, 5.45)		52%	Yes
	Wang 2021 24 studies	Adults	None	SMD 0.98 (0.85, 1.11)		86.6%	Yes
LDH and composite poor outcome	**Malik 2021** 20 studies (n=3313)	Not stated	LDH >250 U/L	OR 5.48 (3.89, 7.71)		67%	Yes
	Wang 2021 25 studies	Adults	None	SMD 1.18 (1.00, 1.36)		85.4%	Yes

Appendix 6. GRADE Certainty of Evidence Profile

Number of studies	Certainty assessment						Impact	Certainty	Importance
	Study design	Risk of bias	Inconsistency	Indirectness	Imprecision	Other considerations			
CRP >10 mg/L and poor outcome									
20	observational studies	not serious	serious	not serious	not serious	none	All included studies were graded as having low (65%) and moderate (35%) risk of bias using the Newcastle-Ottawa Scale. Heterogeneity was 52%, marked by the authors as significant. Cut-off values were determined based on four component studies with thresholds of 3, 5, 8, and 15 mg/L.	⊕⊕⊕◯ MODERATE	IMPORTANT
CRP >10 mg/dL and ICU admission in pediatric patients									
1	observational studies	serious	serious	not serious	serious	none	Review authors cited the included study as limited in study design. Heterogeneity was 100%. The included study had a less than optimal information sample size (n=42).	⊕◯◯◯ VERY LOW	IMPORTANT
CRP and mortality									
4	observational studies	not serious	serious	not serious	not serious	none	Included studies were all rated 8/9 on the Newcastle-Ottawa Scale, indicating low risk of bias. Heterogeneity of 73% was considered significant. Sample size (n=1855) met optimal sample size requirement. Only one of the included studies reported a CRP cut-off (>10 mg/L). None of the studies reported effects in multivariate analysis.	⊕⊕⊕◯ MODERATE	IMPORTANT
CRP ≥ 80 mg/L and progression to severe disease in pediatric patients									
1	observational studies	serious	serious	not serious	serious	none	Review authors cited the included study as limited in study design. Heterogeneity was 100%. The included study had a less than optimal information sample size (n=250).	⊕◯◯◯ VERY LOW	IMPORTANT

Number of studies	Certainty assessment						Impact	Certainty	Importance
	Study design	Risk of bias	Inconsistency	Indirectness	Imprecision	Other considerations			
LDH >250 U/L and poor outcome									
18	observational studies	not serious	serious	not serious	not serious	none	All included studies were graded as having low (67%) and moderate (33%) risk of bias using the Newcastle-Ottawa Scale. Heterogeneity was 67%, marked by the authors as significant. Cut-off values were determined based on three component studies with thresholds of 225, 440, and 550 U/L.	⊕⊕⊕◯ MODERATE	IMPORTANT
LDH and mortality									
4	observational studies	not serious	serious	not serious	serious	none	Risk of bias score of the studies was 8 via NOS, indicating low risk of bias. Heterogeneity was significant at 85%. The CIs crossed the clinical decision threshold (OR 5.23, 95% CI 0.91-30.06). Two of the studies enumerated LDH thresholds at 245 and 250 U/L respectively. None of the studies reported effects in multivariate analysis.	⊕⊕◯◯ LOW	IMPORTANT
LDH and progression to severe disease									
7	observational studies	not serious	not serious	not serious	not serious	none	Risk of bias scores of the studies were 7-8 via NOS, indicating low risk of bias. Heterogeneity was not significant at 49%. Two studies reported LDH threshold of 245 U/L, two studies reported 250 U/L, and one reported 445 U/L. Two of the studies used multivariate analysis.	⊕⊕⊕⊕ HIGH	IMPORTANT

Appendix 7. Characteristics of Ongoing Clinical Trials

Clinical Trial Identifier (Location)	Official Title	Methodology	Outcome Measures	Population	Estimated Date of Completion
NCT04818866 United States	International Study of Inflammation in COVID-19 (ISIC)	Diagnostic Test: SuPAR, C-reactive protein, Ferritin, D-Dimer, Procalcitonin, Interleukin-6, Lactate Dehydrogenase	Primary: In-hospital incidence of death, need for mechanical ventilation and need for renal replacement therapy Secondary: length of hospitalization, acute kidney injury, cardiovascular events, mortality, need for renal replacement therapy, need for mechanical ventilation within 6 months	n=2500 18 years and older	December 30, 2022
NCT04867161 Czech Republic	Superinfection and Hyperinflammatory Phenotype in COVID-19 (Coronavirus Disease 2019) Pneumonia Patients (SUPER-HI)	Observational prospective cohort study Laboratory sampling, bronchoalveolar lavage	Primary: To investigate the role of inflammatory markers (CRP, PCP, PSP, IL-6) as diagnostic tools for superinfection in COVID-19 pneumonia patients. Secondary: To assess the association between hyperinflammatory phenotype and course of illness and mortality rates in COVID-19 pneumonia patients.	n=300 18 to 99 years	December 31, 2022