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Review Article

Association of HScore Parameters with Severe COVID-19: A Systematic Review and Meta-Analysis

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What's Known

- The association of laboratory findings in severe COVID-19 with hemophagocytic lymphohistiocytosis is not clear.

What's New

- Fever, lymphopenia, thrombocytopenia, hypohemoglobinemia, hyperferritinemia, and high aspartate transferase level are common in severe COVID-19 and hemophagocytic lymphohistiocytosis.
- The leukocytosis, neutrophilia, and hyperfibrinogenemia observed in severe COVID-19 had contradictory roles in hemophagocytic lymphohistiocytosis.

Abstract

Background: Several reports have associated the severe Coronavirus disease-2019 (sCOVID-19) with secondary-hemophagocytic lymphohistiocytosis (sHLH) and proposed utilizing the hemophagocytic syndrome diagnostic score (HScore) for sCOVID-19 patients. We conducted a systematic review and meta-analysis to find the possible association of HScore parameters with severity in COVID-19 patients.

Methods: A systematic search was performed in Medline via PubMed, EMBASE, and Cochrane databases using all HScore and COVID-19 keywords. The studies were all from 2020, and the study language was limited to English. The records were screened based on inclusion/exclusion criteria. Random/fixed-effect models were employed for meta-analysis, based on the I^2 index of parameters. The pooled mean differences were estimated for continuous parameters. The pooled odds-ratio was estimated for fever. The level of significance was set at 0.05.

Results: Eighteen studies (comprising 2459 patients) out of 26151 screened studies were included in this meta-analysis. The results showed that the level of leukocyte, neutrophil, aspartate transaminase (AST), ferritin, and fibrinogen were significantly higher in sCOVID-19 patients than in non-severe ones. Significant lower levels of lymphocyte, platelet, and hemoglobin were also found in sCOVID-19 patients than non-severe patients. Fever was nearly associated with two times increased odds of sCOVID-19 (P=0.051).

Conclusion: Lymphopenia, thrombocytopenia, hypohemoglobinemia, hyperferritinemia, high levels of AST, and fever are common features of both sCOVID-19 and HLH. However, the leukocytosis, neutrophilia, and hyperfibrinogenemia found in sCOVID-19 are in contrast with HScore. Conclusively, HScore parameters could be risk factors for sCOVID-19. However, some parameters' roles are contradictory, suggesting the need for further investigation and a new way of HScore interpretation in sCOVID-19 patients.

A preprint of this study was published at https://www.researchsquare.com/article/rs-54490/v2.

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Keywords ● COVID-19 ● Hemophagocytic lymphohistiocytosis ● Systematic review ● Meta-analysis

Introduction

The pandemic coronavirus disease 2019 (COVID-19) has involved 105,394,301 cases with a mortality of 2,302,302 by February

8th, 2021.[1] There is a wide range of clinical and laboratory findings in COVID-19 patients, such as fever, dry cough, myalgia, changes in white blood cells (WBC), lymphopenia, high levels of c-reactive protein (CRP), lactate dehydrogenase (LDH), procalcitonin, ferritin, aspartate transaminase (AST), alanine transaminase (ALT), inflammatory cytokines, along with coagulative disorders manifested with high levels of fibrinogen, D-dimer, and international normalized ratio (INR), longer prothrombin time (PT), and shorter activated partial thrombospondin time (APTT).[2]

The National Health Commission of China released guidelines that stratified COVID-19 patients into four categories: mild, moderate, severe, and critical.[3] The majority of patients are asymptomatic or show mild/moderate symptoms. However, a considerable portion of the cases develop severe/critical manifestations with a high mortality rate, emphasizing the importance of biomarkers for better management of this group. A subset of COVID-19 patients is observed to develop acute respiratory distress syndrome (ARDS), threatening their lives. These severe/critical patients experienced fever, hyperferritinemia, and a massive release of inflammatory cytokines, known as a cytokine storm.[4] A cytokine storm is a syndrome identified with a systemic release of inflammatory cytokines such as interleukin (IL)-6, tumor-necrosing factor (TNF)-α, IL-1β, IL-18, interferon (IFN)-γ, and IL-33. Inflammatory cytokines, especially IL-6 in COVID-19, increase the vascular permeability leading to fluid perfusion into the alveoli. It is presumed that cytokine storm and overreaction of the immune system are major causes of ARDS and mortality in COVID-19.[5] Therefore, the administration of immunosuppressive and anti-inflammatory agents such as dexamethasone, prednisolone, naproxen, and tocilizumab (anti-IL-6 receptor antibody) is the first line of treatment in severe COVID-19 (sCOVID-19).[5, 6] Notably, such immunosuppressive modalities weaken the anti-viral immune responses as well.[7] Hence, they might aggravate the viral pathogenesis in mild and moderate patients and should be prescribed only for those with severe and hyper-inflammatory responses.[8] Thus, the identification of clinical and laboratory parameters associated with severe disease could help the clinicians manage the outcomes.

It has been reported that the hyper-inflammatory condition witnessed in sCOVID-19 could trigger hemophagocytic lymphohistiocytosis (HLH).[9] HLH is a fatal disease in which immune cells such as macrophages and lymphocytes get hyper-activated and attack the healthy cells and organs.[10] It is characterized by hyper-inflammation and a systemic release of inflammatory cytokines leading to multi-organ failure. Secondary HLH (sHLH) is not inherited and commonly occurs following autoimmune disorders or inappropriate immune responses to viral infections.[10] HLH diagnosis is based on a series of clinical and laboratory criteria called the hemophagocytic syndrome diagnostic score (HScore).[10] The HScore comprises several clinical and laboratory factors, including fever, one- or multi-lineage cytopenias, organomegaly, triglyceridemia, hyperferritinemia, hypofibrinogenemia, hypohemoglobinemia, high AST level, hemophagocytosis on bone marrow (BM) aspirate, and prolonged use of immunesuppressants.[11]

Recently, the utilization of HScore has been suggested for detecting the hyper-inflammatory syndrome in sCOVID-19 patients in order to identify the patients for whom immunosuppressive agents could decrease mortality.[9] The cytokine profile and clinical features of sCOVID-19 resemble sHLH.[9, 12, 13] A key laboratory finding in sHLH is hyperferritinemia,[14, 15] which is found in many sCOVID-19 cases.[4, 9, 12] Moreover, the presence of abnormal liver function and coagulopathy in both COVID-19 and sHLH suggests that a subgroup of COVID-19 pneumonia cases also have sHLH.[16, 17] However, there are still controversies regarding the association of sCOVID-19 and sHLH. Several studies reported the association of sCOVID-19 and sHLH, and thereby suggested the use of HScore in the clinical management of sCOVID-19 patients.[9, 12, 18, 19] Though, others are skeptical about the benefits of using HScore in sCOVID-19.[6, 20-22] This controversy highlights the necessity of investigating the possible association between HScore parameters and the severity of COVID-19 in a meta-analysis. In this systematic review and meta-analysis, we attempted to find the possible association between the parameters listed in the HScore with the severity of COVID-19 in patients.

Methods

Search Strategy

The conducted systematic review and meta-analysis followed the Preferred Reporting Items for Systematic Reviews and Meta-Analyses (PRISMA) guideline.[23] A systematic electronic search was carried out separately by two independent authors (MHK and BHKD) in Medline via PubMed, EMBASE, and Cochrane using the keywords listed in table 1. Keywords for COVID-19 were searched in title/abstract, while

Table 1: Keywords used for searching in Medline via PubMed, EMBASE, and Cochrane databases

	Searched field	Keywords
1	Laboratory and Clinical general findings	Laboratory*, Clinic*
2	Temperature	Temperature*, Fever*, Heat*
3	Cytopenia	Cytopenia*, Pan-cytopenia*, Pan cytopenia*, Leukopenia*, Anemia*, Neutropenia*, Thrombocyto*, Lymphopenia*, White blood cell*, "WBC", leukocyt*, lymphocyt*, neurophil*, monocyt*, eosinophil*, basophil*, platelet*
4	Hemoglobin	Hemoglobin,
5	Ferritin	Ferritin*, Isoferritin*
6	Serum aspartate aminotransferase	Glutamate Aspartate Transaminase*, glutamic oxaloacetic transaminase*, glutamat oxaloacetate transaminase*, "SGOT", Aspartate Aminotransferase*, Aspartate Transaminase*, Transaminase*, "AST"
7	Organomegaly	Organomegal*, Hepatomegal*, Splenomegal*, Hepatosplenomegal*
8	Triglycerides	Triglyceride*, Triglyceridemia*, Hypertriglyceridemia*, Triacylglycerol*, Triacylglyceride*
9	Fibrinogen	Fibrinogen*, Factor I, Factor 1, Factor-I, Factor-1
10	Known Immunosuppressant	Immune deficiency*, Immune-deficiency*, Immunodeficiency*, Immune-deficient*, Immunodeficient*, Immune-compromised*, Immune compromised*, mmunocompromised*, Immunosuppressive*, Immune suppressive*, Immune-suppressive*, Immunosuppressant*, Immunosuppression*, Immune suppression*, Immune-suppression*, HIV, AIDS*, Chemotherap*, Methotrexate*, Glucocorticoids*, Cortone* Cortisone*, Hydrocortisone*, Prednisone*, Deltasone*, Orasone*, Budesonide*, Entocort*, Prednisolone*, Millipred*, Methylprednisolone*, Dexamethasone*, Cyclosporine*, Neoral*, Sandimmune*, SangCya*, Azathioprine*, Azasan*, Imuran*, Mycophenolate*, Mycophenolate mofetil*, CellCept*, Myfortic*, Sphingosine 1, Sphingosine-1-Phosphate*, Sphingosine-1 Phosphate*, Phosphate inhibitor*, Fingolimod*, FTY720*, Tacrolimus*, Astagraf*, Envarsus*, Prograf*, Tofacitinib*, Xeljanz*, Sirolimus*, Rapamune*, Everolimus*, Afinitor*, Zortress*, Leflunomide*, Arava*, Abatacept*, Orencia*, Adalimumab*, Humira*, Anakinra*, Kineret*, Certolizumab*, Cimzia*, Etanercept*, Enbrel*, Golimumab*, Simponi*, Infliximab*, Remicade, Ixekizumab*, Taltz*, Natalizumab*, Tysabri*, Rituximab*, Rituxan*, Secukinumab*, Cosentyx*, Tocilizumab*, Actemra*, Ustekinumab*, Stelara, Vedolizumab*, Entyvio*, Basiliximab*, Simulect*, Daclizumab*, Zinbryta*, Antilymphocyte serum*, Antilymphocyte antibod*, Antilymphocyte Globulin*, Antithymphocyte Globulin*, Anti-thymphocyte Globulin*, Anti thymphocyte Globulin*, Antilymphocyte immunoglobulin*, Anti-rejection therap*, Anti rejection therap*, Antirejection therap*, Transplantation, Transplant*, Graft*
11	COVID-19	Covid*, sars-cov-2*, corona virus*, coronavirus*, cv 19, cv-19 2019-ncov, ncov*, Wuhan coronavirus*, Wuhan pneumonia*,

All words were searched with a star at the end to cover all possible variants of the words. The keywords in sections 1-10 were searched with an "OR" together, and the keywords for COVID-19 (section 11) were searched with an "OR" separately. The results of these two searches were then retrieved with an "AND".

the keywords related to HScore were searched in full-text/all fields. Regarding the date of COVID-19 occurrence, the records published from January 1st - June 22nd, 2020, were imported to the reference manager software EndNote X8.1 (Clarivate Analytics, Philadelphia, PA, USA) for further management, including the removal of duplicates and identification of potentially eligible records. Moreover, the references of the selected articles were reviewed to prevent the loss of data sources.

Eligibility Criteria

We systemically reviewed the studies that investigated the association of each HScore variable with severity in COVID-19 patients. Two reviewers (MHK and BHKD) independently screened the abstracts and full texts according to the eligibility criteria. A consensus or a third reviewer resolved the disagreements.

The inclusion criteria were: types of studies: retrospective, prospective, descriptive, or observational research articles reporting the relationship between HScore variables (fever, any cytopenias, hemoglobin, ferritin, AST, organomegaly, triglyceride, fibrinogen, and any immunosuppressive conditions) and severity of patients with COVID-19; subjects: patients diagnosed with COVID-19; exposure/intervention: studies classifying COVID-19 patients in severe (critical or severe) versus non-severe (moderate or mild) conditions according to the National Health Commission of China guidelines for COVID-19 diagnosis;[3] outcome indicator: odds ratios (OR) with 95% confidence intervals (CI) for fever, and mean or median with standard deviation (SD) or interquartile range (IQR)/simple range (SR), respectively, for other variables; year of study: 2020, considering that the publications on COVID-19 started from 2020; study language: English.

The exclusion criteria were: case reports/series, reviews, editorials, letters, commentaries, guidelines, perspectives, studies

with insufficient data such as studies with or without non-standard stratifications of severity, studies without a confirmatory examination of COVID-19 diagnosis, studies that only reported increases or decreases in the parameters without any clear descriptive statistics (such as mean, median, or proportion) for the parameters, studies with unavailable English full-text, and studies that only reported fever without any other parameters.

Data Extraction

Data extraction was performed by two authors, who searched and screened the records. The mean or median values (with SD and IQR/SR, respectively) of all HScore variables were extracted. After reviewing the final records, variables such as eosinophil, basophil, monocyte, triglyceride, and immunosuppression, which were available in less than five studies, were excluded from the analysis. Therefore, the following variables were included: fever, WBC, lymphocyte, neutrophil, platelet, AST, ferritin, fibrinogen, and hemoglobin. Due to the higher frequency of the means/medians of indicators, rather than ORs, and the variation in the cut-off values used for laboratory indices, means/medians with SD/IQR (or SR) were considered for the variables. Only the data for fever was considered as OR with CI.

The outcome of interest was severity status, based on which, the patients were stratified into severe and non-severe groups. The severe group comprised severe and/or critical patients with any of the following criteria: respiratory rate (RR) ≥30 breaths/min; oxygen saturation at rest≤93%; arterial partial oxygen pressure (PaO2)/ fraction of inspired oxygen (FiO2)≤300 mmHg; respiratory failure requiring mechanical ventilation; shock and organ failure requiring ICU care. The non-severe group consisted of mild and/or moderate patients with one of the following criteria: mild clinical symptoms and no radiological sign of pneumonia; fever and respiratory symptoms with pneumonia signs in radiological imaging.

Quality Assessment of Included Studies

Regarding the type of the included studies, the methodological quality of the papers was assessed using the critical appraisal checklist for cross-sectional studies (AXIS).[24] The quality score ranges 0-20.

Statistical Analysis

The meta-analysis was performed using STATA software version 16 (StataCorp, College Station, TX, USA). We used the inverse variance method to estimate the pooled mean differences (MDs) with 95% CI for cell blood parameters such as AST, ferritin, and fibrinogen. Moreover, the Mantel-Haenszel method was used to estimate the pooled OR with 95% CI for the dichotomous fever variable. I^2 and Tau^2 measures and a Q test based on Chi Square were applied to assess the heterogeneity. Depending on the I^2 index (>50% or <50%), the random-effects model using the restricted maximum likelihood method or the fixed-effects model were employed, respectively. To address heterogeneity, sensitivity analysis via the leave-one-out method and restricted maximum likelihood random-effects meta-regression were used. Meta-regression was performed for mean/median age, sex, and comorbidities (hypertension, diabetes, cardiovascular disease, renal disorders, malignancies, and respiratory disorders). Subgroup analysis was employed for WBC. Publication bias was assessed using the funnel plot; the Egger and the Begg tests for continuous variables and the Harbord test for binary outcomes were used. The significance level was set at 0.05. Since the desired effect size was not available, the mean±SD of the variables was estimated based on sample size, median, and IQR/SR according to Lou and colleagues[25] and Wan and colleagues.[26]

Results

Study Selection and Baseline Characteristics

Based on the described search strategy, a total of 26151 studies were identified in the three searched online databases. Following the removal of duplicates and screening of all records, 18 studies that met the predetermined eligibility criteria were included in the meta-analysis.[27-44] The article number and the reason for exclusion in each screening step are depicted in a PRISMA flow diagram (figure 1). Overall, 2459 patients were included in the quantitative analysis, among which 710 patients were in severe/critical conditions, and the rest (1737 cases) were classified as having a mild/moderate disease. The baseline characteristics and the quality score of the studies are presented in table 2. The mean/median or frequency of HScore parameters were extracted from the studies and are listed in table 3.

Meta-analysis

Blood Cell Parameters

The I^2 index showed that there was heterogeneity for WBC (I^2=73.52%, P<0.001), lymphocyte count (I^2=66.83%, P=0.001), and neutrophil count (I^2=64.53%, P<0.001) variables.

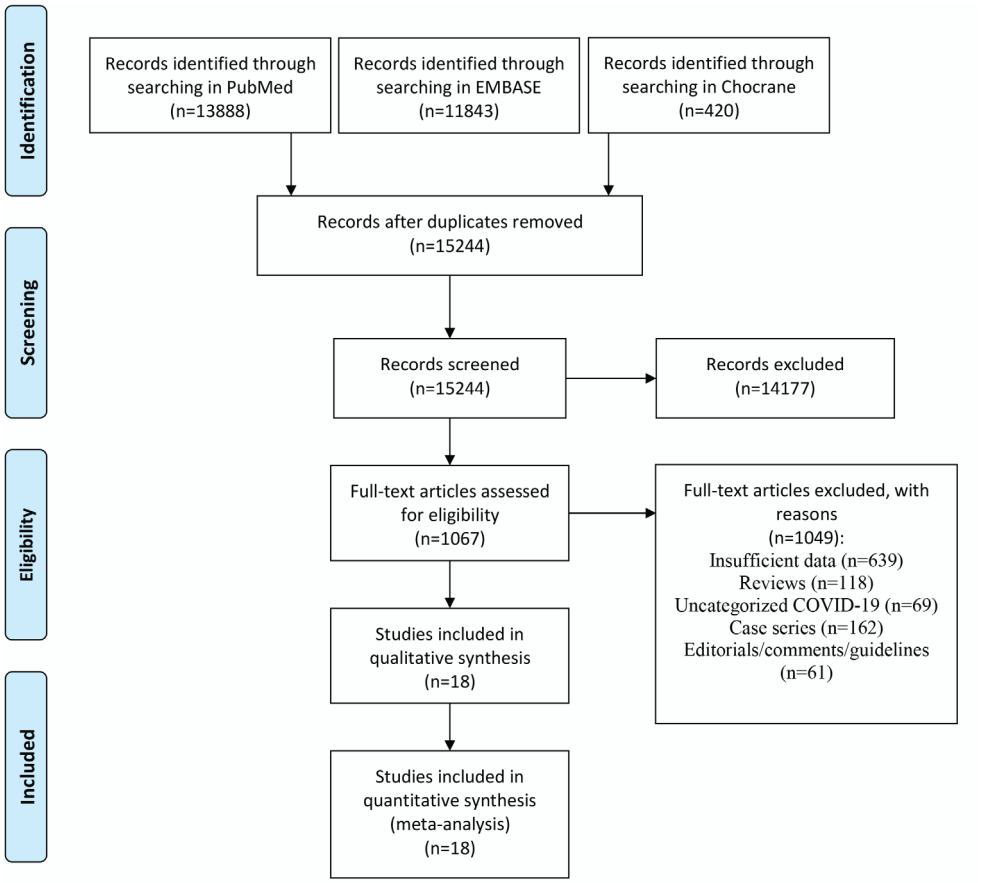

Figure 1: The PRISMA flow diagram shows the strategy of study selection. The final included studies are selected based on the inclusion and exclusion criteria according to the study selection strategy. The excluded studies and the reason for their exclusion are also illustrated.

Table 2: Baseline characteristics of the studies included in the meta-analysis

Author	Date (MM/DD)	Country	Study type	N	Quality Score
Chen and colleagues[44]	03/27	China	Cross-Sectional	21	14
Zhang and colleagues[33]	04/02	China	Cross-Sectional	115	14
Liu and colleagues[36]	04/18	China	Cross-Sectional	40	12
Zou and colleagues[32]	04/30	China	Cross-Sectional	303	14
Zhou and colleagues[27]	04/16	China	Cross-Sectional	21	15
Chen and colleagues[30]	04/28	China	Cross-Sectional	145	16
Zhao and colleagues[28]	04/29	China	Cross-Sectional	91	14
Aggarwal and colleagues[31]	04/29	USA	Cross-Sectional	16	15
Pereira and colleagues[29]	04/24	USA	Cross-Sectional	90	15
Feng and colleagues[37]	04/10	China	Cross-Sectional	422	17
Young and colleagues[34]	03/20	Singapore	Cross-Sectional	18	14
Pei and colleagues[35]	04/12	China	Cross-Sectional	200	19
Yao and colleagues[39]	04/24	China	Cross-Sectional	96	16
Li and colleagues[42]	04/12	China	Cross-Sectional	548	17
Zheng and colleagues[38]	04/29	China	Cross-Sectional	141	18
Wang and colleagues[40]	04/23	China	Cross-Sectional	45	15
Medetalibeyoğlu and colleagues[41]	08/26	Turkey	Cross-Sectional	68	12
Di Micco and colleagues[43]	05/07	Italy	Cross-Sectional	67	12

The quality score ranges 0-20, according to the Critical Appraisal Checklist for Cross-Sectional Studies, AXIS.[24] MM/DD. Month/Day; N: number

Therefore, a random-effects model was used for these variables. There was no heterogeneity for hemoglobin and platelet variables, and the fixed-effects model was executed (I^2=0%, P=0.518 and I^2=0%, P=0.509, respectively). The results of the random-effects meta-analysis showed that in twelve studies,[27, 29-31, 34, 36-41, 44] patients with sCOVID-19 had higher WBC than patients

Table 3: Mean/median or frequency of HScore parameters extracted from the included studies

Study	Country	Sample size	Men %	Severe/ non-severe patient	Age (median or mean years)	Severe cases in fever/ total fever cases (%)	WBC $\times 10^9$/L (severe/ non-severe)	Lymphocyte $\times 10^9$/L (severe/ non-severe)	Neutrophil $\times 10^9$/L (severe/ non-severe)	Platelet $\times 10^9$/L (severe/ non-severe)	Hemoglobin g/L (severe/ non-severe)	AST U/L (severe/ non-severe)	Fibrinogen g/L (severe/ non-severe)	Ferritin µg/L (severe/ non-severe)
Chen and colleagues[44]	China	21	81	11/10	56	10/20 (50)	8.3/4.5	0.7/1.1	6.9/2.7	157/175.6	136/139.5	47/24	-	1598.2/337.4
Zhang and colleagues[33]	China	115	42.6	31/84	49.52	-	-	-	-	-	-	38.9/24.4	-	-
Liu and colleagues[36]	China	40	37.5	13/27	48.7	13/36 (36.1)	6.6/3.9	0.6/1.1	4.7/2.0	186.6/181.4	123.4/127.8	51.2/25.9	6.3/4.5	835.5/367.8
Zou and colleagues[32]	China	303	52.1	26/277	51	-	-	-	-	-	-	-	4.7/4.3	-
Zhou and colleagues[27]	China	21	61.9	13/8	66.1	12/19 (63.1%)	10.68/9.3	0.7/0.8	9.5/7.8	204.2/219.2	114.1/122	52.1/56.1	5.1/4	-
Chen and colleagues[30]	China	145	54.5	43/102	47.5	39/109 (35.7)	6/5	0.9/1.3	-	192/204.5	134/139.8	28/23.5	-	-
Zhao and colleagues[28]	China	91	53.8	30/61	46	-	-	-	-	-	-	-	-	-
Aggarwal and colleagues[31]	USA	16	75	8/8	67	8/15 (53.5)	13.5/6.4	0.8/0.9	6.7/3.8	209.5/211.5	145/151	43.5/25	-	-
Pereira and colleagues[29]	USA	90	59	27/63	57	13/63 (20.6)	4.4/5.7	0.8/0.7	3.6/4.1	186/174	98/114	33/24.5	-	790/813
Feng and colleagues[37]	China	422	56.4	70/352	53	64/341 (18.7)	7.2/5.1	0.8/1.1	6/3.4	181/185	131/133	39/25	4.7/4.3	-
Young and colleagues[34]	Singapore	18	50	6/12	47	6/13 (46.1)	3.4/4.6	1.1/1.2	1.8/2.8	156/159	132/139	-	-	-
Pei and colleagues[35]	China	200	51.5	56/144	56.3	52/178 (29.2)	-	0.5/1	5.8/3	-	-	40.5/24	-	-
Yao and colleagues[39]	China	96	37.5	13/83	52	11/72 (15.2)	5.6/4.6	0.8/1.4	3.3/2.5	145/195	117/127	-	-	-
Li and colleagues[42]	China	548	50.9	269/279	60	228/476 (47.9)	-	-	-	-	-	-	-	-
Zheng and colleagues[38]	China	141	52.4	29/112	47	25/98 (25.5)	6.1/4.9	0.7/1.3	5.1/3.2	158/203	135/128	-	-	-
Wang and colleagues[40]	China	45	57	30/15	57.1	-	8.7/5.2	0.5/0.9	7.7/3.8	-	-	-	-	1368/821.1
Medetalibeyoğlu and colleagues[41]	Turkey	68	69.1	11/57	55.8	13/45 (28.8)	5.6/5.7	0.6/1	4.7/4	189.5/186.7	117/134	64.8/36.3	5.829/4.939	818.3/692.1
Di Micco and colleagues[43]	Italy	67	70	24/43	-	-	-	-	-	-	-	-	7.5/5.7	-
Total	-	2459	-	710/1737	-	-	-	-	-	-	-	-	-	-

WBC: White blood cells; AST: Aspartate-aminotransferase

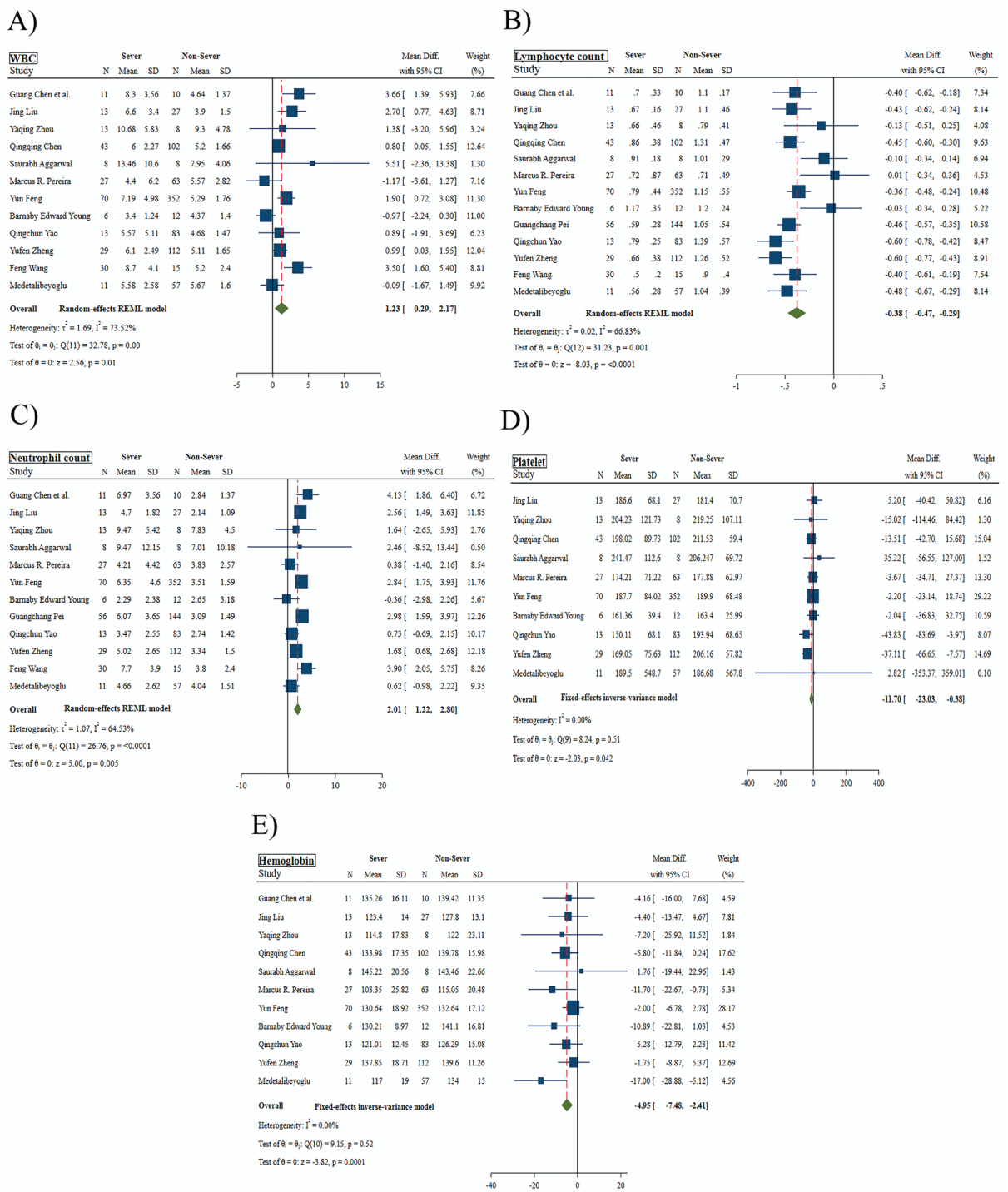

Figure 2: Forest plots show the mean levels of blood cell parameters, including WBC (A), lymphocyte (B), neutrophil (C), platelet (D), and hemoglobin (E) in severe and non-severe COVID-19 patients. The effect size of each study is mean difference with 95% CI and the pooled estimates are shown by red dashed-line.

with non-severe COVID-19, and the difference was significant (MD=1.23 [0.29,2.17], P=0.010) (figure 2A). For lymphocyte, although one of the studies[29] showed that patients with sCOVID-19 had a higher lymphocyte count than non-severe COVID-19 patients (MD=0.01 [-0.34,0.36]), the pooled results of thirteen studies[27, 29-31, 34-41, 44] showed that lymphocyte count in patients with sCOVID-19 was significantly lower than non-severe COVID-19 patients (MD=-0.38 [-0.47,-0.29], P<0.001) (figure 2B). In twelve studies,[27, 29, 31, 34-41, 44] patients with severe conditions had significantly higher neutrophil count than those with non-severe complications (MD=2.01 [1.22,2.80], P=0.005) (figure 2C). The results of the fixed-effects meta-analysis of ten studies[27, 29-31, 34, 36-39, 41] showed that platelet count in patients with sCOVID-19 was significantly

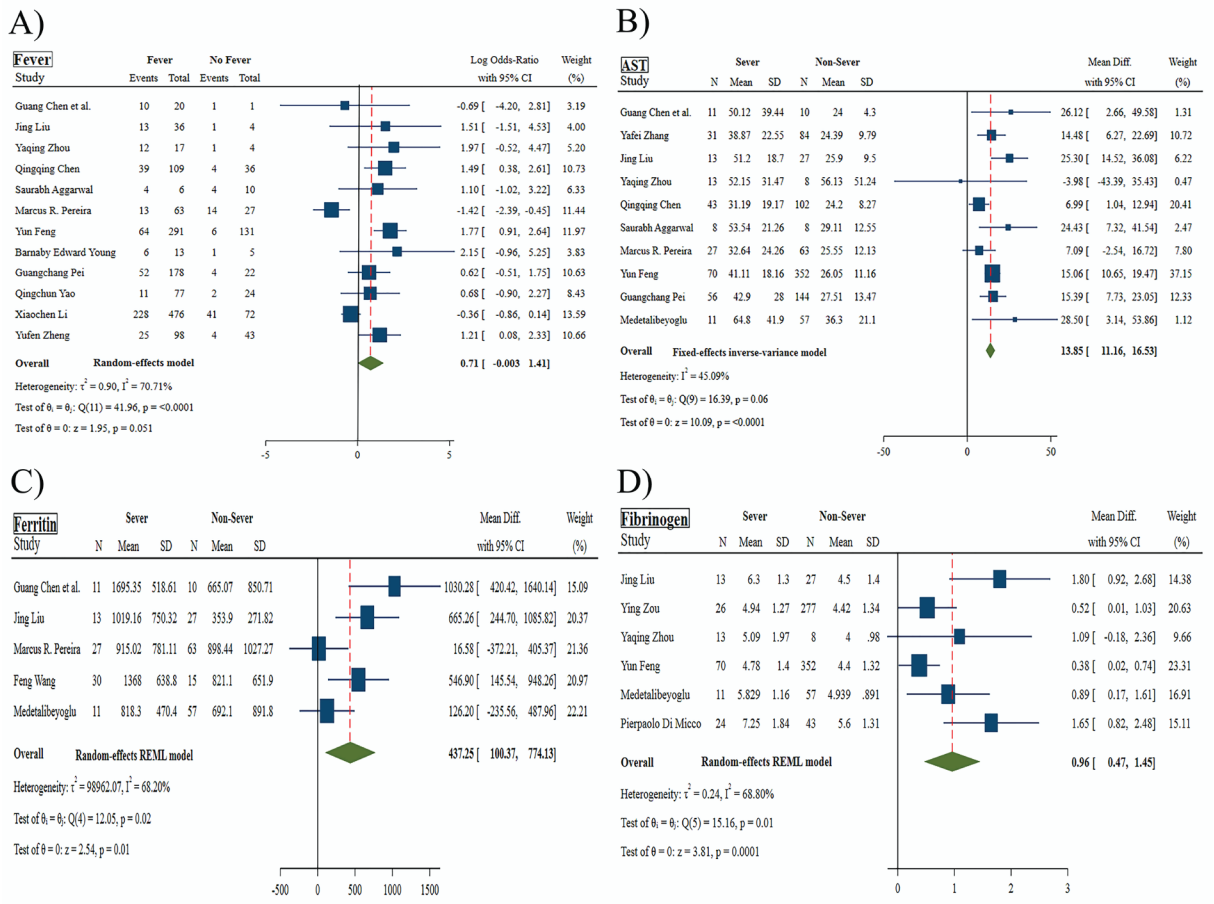

Figure 3: Forest plots show the mean levels of AST (A), ferritin (B), and fibrinogen (D) in severe and non-severe COVID-19 patients. The effect size of each study for AST, ferritin, and fibrinogen is mean difference with 95% CI. The proportion of severe patients in the fever and no fever groups is shown in section (A). The effect size for fever is odd-ratio with 95% CI. The pooled estimates are shown by a red dashed-line.

lower than patients with non-severe COVID-19 (MD=-11.7 [-23.03, -0.38], P=0.042) (figure 2D). Finally, in eleven studies,[27, 29-31, 34, 36-39, 41] those categorized as severe patients had lower hemoglobin than non-severe patients, and the difference was significant (MD=-4.95 [-7.48,-2.41], P<0.001) (figure 2E).

Fever

The results of our random-effects meta-analysis (I^2=70.71%, P<0.001) on twelve studies[27, 29-31, 34-39, 41, 44] showed that fever was nearly associated with twice the increased odds of sCOVID-19 (OR=2.01 [0.99,4.09], P=0.051) (figure 3A).

AST

There was no heterogeneity for AST and a fixed-effects model was employed (I^2=45.09%, P=0.059). The results of our meta-analysis on ten studies[27, 29-31, 33, 35-37, 41, 44] showed that AST was significantly higher in patients with sCOVID-19 than in non-severe patients (MD=13.85 [11.16,16.53], P<0.001) (figure 3B).

Ferritin

There was heterogeneity for ferritin, and a random-effects model was used (I^2=68.20%, P=0.017). The results of our meta-analysis on five studies[29, 36, 40, 41, 44] showed that ferritin was significantly higher in patients with sCOVID-19 than in those with a non-severe form of the disease (MD=437.25 [100.37,774.13], P=0.010) (figure 3C).

Fibrinogen

The results of our random-effects meta-analysis (I^2=68.80%, P=0.009) on six studies[27, 32, 36, 37, 41, 43] showed that fibrinogen was significantly higher in patients with sCOVID-19 than patients with non-severe COVID-19 (MD=0.96 [0.46,1.54], P<0.001) (figure 3D).

Publication Bias

Regression-based Egger test showed small-study effects for fibrinogen and lymphocyte count (P=0.002 and 0.001, respectively) and no small-study effects for AST, hemoglobin, neutrophil count, platelet count, and WBC (P=0.281, 0.163,

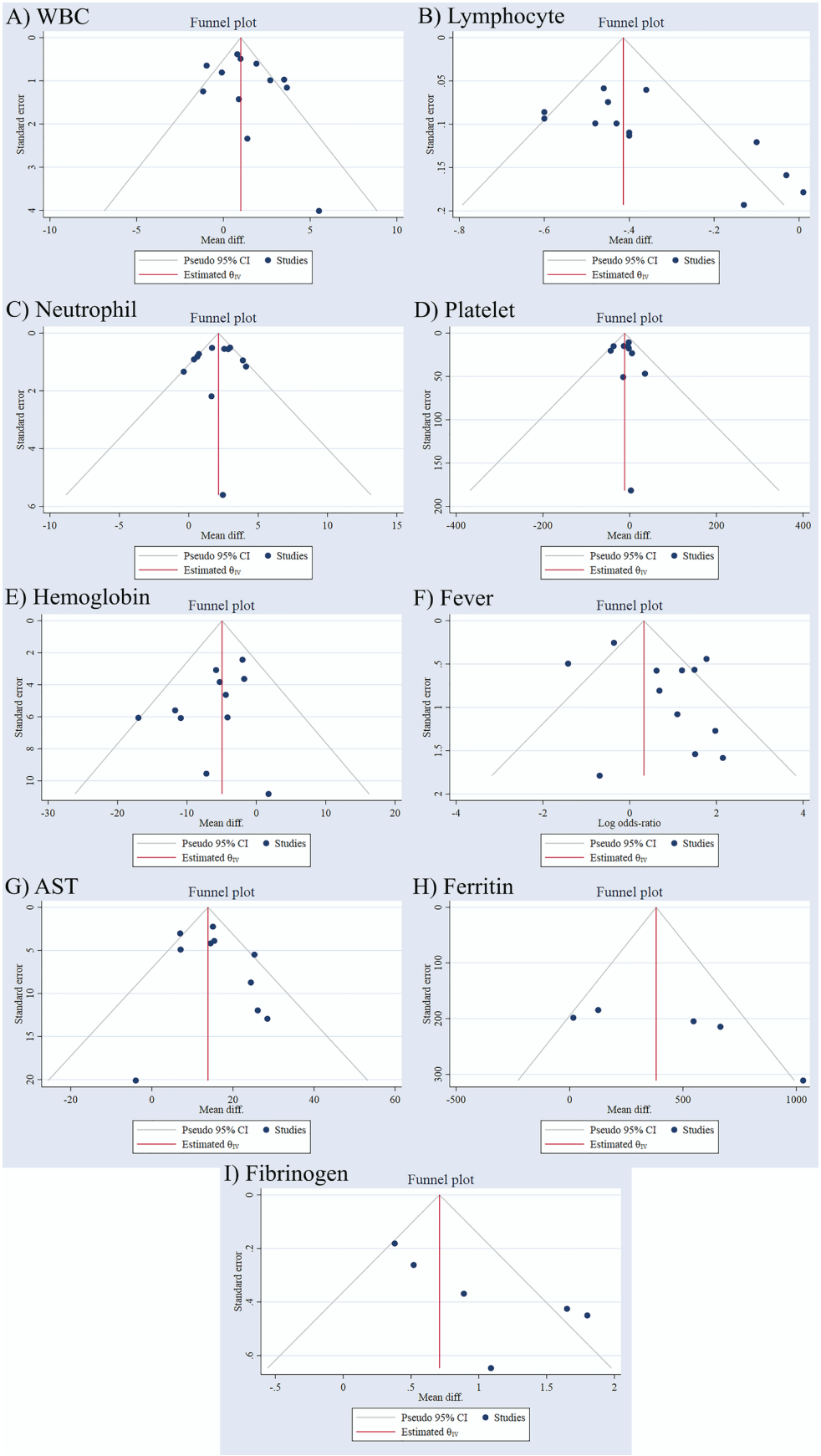

Figure 4: Funnel plots show the publication bias status of the studied biomarkers, including WBC (A), lymphocyte (B), neutrophil (C), platelet (D), hemoglobin (E), fever (F), AST (G), ferritin (H), and fibrinogen (I). The X-axis represents the effect size and the Y-axis shows the standard error. The dots represent the included studies.

0.698, 0.832, and 0.286, respectively). For ferritin, the regression-based Egger test showed that there were small-study effects (P=0.008), but the Begg test showed no small-study effects (P=0.079). The Harbord test showed that there were no small-study effects for fever (P=0.556) (figure 4).

Sensitivity Analysis

For ferritin and fibrinogen parameters, the exclusion of studies,[44, 36] respectively, reduced the heterogeneity almost significantly. However, ferritin and fibrinogen levels were still higher in sCOVID-19 patients than non-severe COVID-19 patients (MDs=328.94 [22.91,634.98] and 0.78 [0.33,1.23], P=0.035 and 0; I^2s=58.9% and 58.32%, P=0.064 and 0.066). For other parameters, the exclusion of individual studies did not significantly reduce heterogeneity.

Meta-regression

The results of exploring the relationship between moderators and the effect size of the study showed that for ferritin, diabetes almost affected the high level of ferritin in sCOVID-19 patients (P=0.068) (figure 5A). Furthermore, the positive approximate association of sCOVID-19 with fever was affected by diabetes (P=0.029) (figure 5B). The association of lymphocyte and neutrophil counts with COVID-19 severity was affected by hypertension (P=0.006 and 0.021) (figures 5C and 5D). Higher WBC counts in sCOVID-19 patients were affected by age, hypertension, and respiratory disorders (P=0.005, 0.016 and 0.046, respectively) (figures 5E, 5F, and 5G).

Subgroup Analysis

According to the results of the meta-regression, the association of WBC with sCOVID-19 was affected by age. Therefore, a subgroup analysis was performed based on the cut-off point of age of 55 years old. The difference in WBC counts between severe and non-severe groups in studies with the mean/median of ≥55 years old (MD=2.07 [0.27,3.87], P=0.018; I^2=64.45%, P=0.008) was greater than those with the mean/median of <55 years old (MD=1.17 [0.21,2.12], P=0.009; I^2=70.84%, P=0.010).

Discussion

The quality score of the included studies based on the AXIS checklist ranged from 12 to 19. There was heterogeneity in most of the variables and the outcome of interest (lymphocyte and neutrophil counts, fever, ferritin, and fibrinogen). Therefore, the random-effects meta-analysis approach was used to obtain a pooled estimate. This approach allows us to address heterogeneity that cannot be easily expressed by other factors. Regression-based Egger and nonparametric Begg tests were employed to assess the publication bias. Only two parameters, fibrinogen and lymphocyte, had publication bias. However, ferritin showed publication bias based on the Egger method, and no publication bias was seen in ferritin, based on the Begg method. When the number of studies is small, the Egger test is weak in detecting bias.[45] The heterogeneity of the studies might be due to numerous other causes, which are not yet well investigated in COVID-19. The various quality levels of the studies might be a cause of heterogeneity. Moreover, the effects of other clinical and laboratory factors were investigated in other meta-analyses.[46-48] Nevertheless, we have evaluated the possible sources of heterogeneity. Meta-regression analyses showed that comorbidities such as diabetes, hypertension, and respiratory disorders could affect the association of some HScore parameters such as fever, ferritin level, WBC, lymphocyte, and neutrophil counts with sCOVID-19. This suggests that comorbidities are important risk factors of sCOVID-19 and should be evaluated in COVID-19 patients. Numerous studies also suggested the undeniable role of comorbidities in the severity and mortality of COVID-19 patients.[49-51]

For ferritin, based on sensitivity analysis with the exclusion of the Chen and colleagues study, heterogeneity was insignificantly reduced to 58.9%.[44] According to the ferritin forest plot, the confidence interval for the effect size of the Chen and colleagues study had a very poor overlap with the confidence intervals for the results of other studies.[44] When the number of studies is small, the power of the Chi-square test is decreased. Therefore, it is better to assume a significance level of 0.1.[52] However, the heterogeneity was still significant for ferritin at the significance level of 0.1. The heterogeneity results of fibrinogen, based on the sensitivity analysis, resemble those of ferritin. According to the meta-regression results for the fibrinogen parameter, there was no relationship between the moderators and the pooled effect size. The results of the meta-regression bubble plot for fever showed that the proportion of diabetic patients in the Pereira and colleagues study was far from the range of diabetes proportions in the other studies.[29] Sensitivity analysis of fever showed that with the exclusion of the Pereira and colleagues study, the I^2 index was not reduced enough to override the heterogeneity

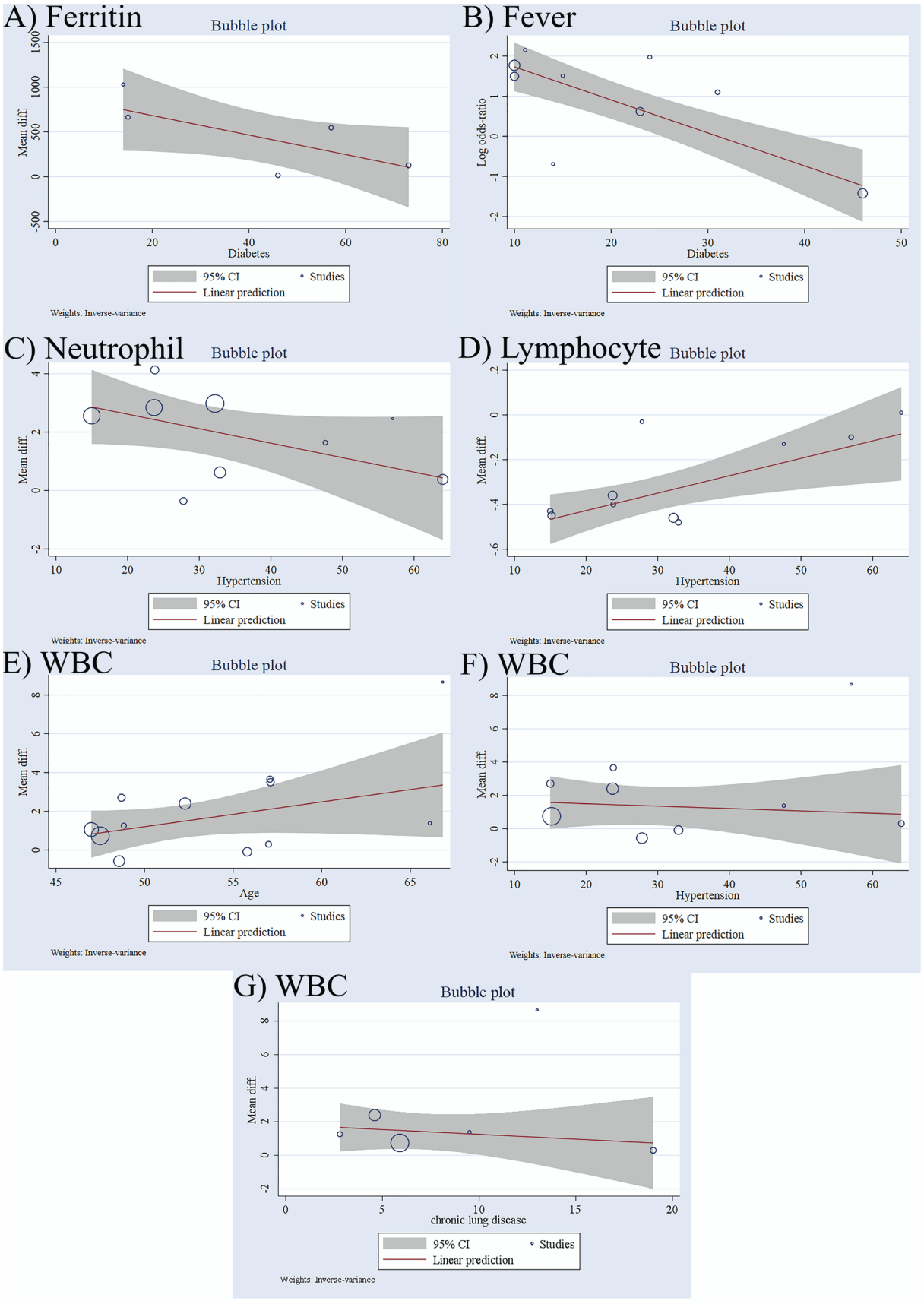

Figure 5: Meta-regression bubble plots show the correlation between effect sizes of parameters and moderators. Diabetes for ferritin (A) and fever (B), and hypertension for neutrophil (C) and lymphocyte (D) are moderators. For WBC, the moderators are age (E), hypertension (F), and respiratory disorders (G). Each bubble in each plot indicates studies. The size of the bubbles represents the precision of the studies.

(P=0.001, 54.38%).[29] Interestingly, the exclusion of the Pereira and colleagues study made the association of fever with sCOVID-19 completely significant (OR=2.53 [1.36,4.71], P=0.003).[29] According to the meta-regression results for WBC, the center of the WBC bubble plot is approximately 47 to 57 years old. Hence, we selected 55 as the cut-off point, so that an equal number of studies fell on the left and right of this cut-off point. We found that the association between WBC and sCOVID-19 was stronger in older patients than younger patients. In line with our finding, it has been reported that older sCOVID-19 patients have a significantly higher WBC count than younger sCOVID-19 patients. This may suggest that the WBC count is a critical parameter in elderly sCOVID-19 patients.[53, 54]

Our results indicated that sCOVID-19 patients had leukocytosis and neutrophilia, as well as lymphopenia and thrombocytopenia. Lymphopenia and thrombocytopenia are in common with sHLH and also were reported previously to have an association with severity in COVID-19.[55, 56] Cytopenia is an important HScore parameter in which two or three lineages of cytopenias have different scores.[11] Reduced counts of lymphocytes and platelets are two-lineage cytopenia that are reported to have an association with severity in COVID-19.[57, 58] In several studies, platelet and lymphocyte counts were significantly lower in sCOVID-19 patients than the mild cases. Lymphopenia and thrombocytopenia are also associated with a higher mortality rate in COVID-19.[57, 59] Moreover, lower hemoglobin is found in both sCOVID-19 and sHLH.[9] Besides, anemia, which is a type of cytopenia, hypohemoglobulinemia could also be an indicator of anemia.[60] Therefore, although anemia was not investigated in the included studies, hypohemoglobolinemia might be indicative of anemia. Furthermore, several reports accounted for low hemoglobin as an HScore item.[61, 62]

The other parameters found in our study that are parallel to sHLH are the high fever and high levels of AST and ferritin. Fever is one of the primary symptoms of COVID-19 and is associated with disease severity in patients.[63] Ferritin is an acute-phase protein that is released in hyper-inflammatory conditions.[64] Hence, it is no surprise to see hyperferritinemia in both sCOVID-19 and sHLH. Higher levels of ferritin is found to be associated with severity and mortality in COVID-19.[65] In inflammatory conditions, pro-inflammatory cytokines induce the liver to release acute phase proteins and liver enzymes.[66, 67] Moreover, liver injury and high levels of AST and ALT are also found to have associations with severity in COVID-19.[68] However, we did not investigate the association of ALT levels with severity in COVID-19 due to its absence in the HScore items.

Interestingly, leukocytosis, neutrophilia, and hyperfibrinogenemia that are observed in sCOVID-19 are in contrast with HScore.[9, 11, 48] Elevated WBC and neutrophil counts are reported to have an association with severity in COVID-19.[69] Contrarily, reduced blood cell counts (cytopenia) is considered in HScore as a risk factor of HLH.[11] Moreover, hyperfibrinogenemia is associated with sCOVID-19, while hypofibrinogenemia is a sHLH biomarker.[9, 11] Fibrinogen is also an acute-phase protein and is elevated in inflammatory conditions.[67] Coagulation disorders such as longer PT, shorter APTT, and high levels of fibrinogen and D-dimer are associated with sCOVID-19.[70] Therefore, both the inflammatory condition and the coagulation disorders cause hyperfibrinogenemia in sCOVID-19.

Fatal multi-organ failure, hyper-inflammation, cytokine storm, and coagulative disorders suggest that COVID-19 is not only an airways disease but also an air-borne systemic complication.[2] The daily increase in the trend of the disease and the mortality statistics along with the growing evidence of severe infection in youngsters and even children emphasize the necessity of developing preventive and curative approaches. The interesting hypothesis of using HScore in the treatment of COVID-19 patients stems from sHLH manifestation in sCOVID-19 patients, including hemophagocytosis in BM aspirates, hyperferritinemia, and cytopenia.[9] Although some evidence suggested that sCOVID-19 could cause sHLH, it may be too soon to associate sCOVID-19 with sHLH.[12, 13, 20] However, hyper-inflammatory conditions such as the release of a massive amount of cytokines/chemokines, including IL-6, IL-1β, TNF-α, IL-7, IL-8, granulocyte-macrophage colony-stimulating factor (GM-CSF), granulocyte colony-stimulating factor (G-CSF), interferon gamma-induced protein (IP-10), monocyte chemoattractant protein-1 (MCP-1), and macrophage inflammatory protein 1(MIP-1α) are seen in both sHLH and sCOVID-19. Thus, given the promising results of using immunosuppressive agents in both diseases, it can be concluded that the current therapeutic approaches for sHLH could be beneficial for sCOVID-19.[2] Hence, it has been proposed that the HScore table might be applicable to determine the subgroup of COVID-19 patients for whom immunomodulators and immunosuppressants are propitious.[9, 20] Although recent comments have questioned the application of HScore in the COVID-19 due to its limitation and low sensitivity,[6, 21, 22] new results suggest an

overlap between the HScore clinical/laboratory parameters and those found in sCOVID-19 patients.[2, 9, 12, 13] Therefore, the evaluation of HScore parameters as biomarkers for severity in COVID-19 will facilitate future decisions on COVID-19 management. To the best of our knowledge, no meta-analysis has yet explicitly focused on the relationship between HScore parameters and severity in COVID-19 patients.

Two recent studies calculated the score of sCOVID-19 patients based on the HScore to investigate the use of HScore in sCOVID-19.[6, 22] They found that the levels of most HScore parameters, such as ferritin, AST, hemoglobin, fibrinogen, lymphocyte, white blood cells, neutrophil, and platelet, are significantly different in sCOVID-19 patients than the mild cases.[6, 22] However, HScore calculation in sCOVID-19 was not beneficial enough to identify the patients for whom immunosuppressive drugs are needed. Actually, they stated that although the levels of HScore parameters are significantly different between severe and mild COVID-19 patients, HScore calculation is not indicated in sCOVID-19.[6, 22] We assumed that the insufficient capability of HScore in identifying sCOVID-19 may be due to the contrasting role of some parameters, such as fibrinogen, neutrophil, and leukocyte, between HLH and sCOVID-19. Our results indicated that hyperfibrinogenemia, neutrophilia, and leukocytosis are associated with sCOVID-19 while in HScore, hypofibrinogenemia, neutropenia, and leukopenia are considered as sHLH biomarkers.[11] Wood and colleagues also pointed to this contradiction between HScore and sCOVID-19.[22] Such contradictions have limited the HScore application in sCOVID-19, and suggest the need for a modification of the HScore or a new interpretation of the HScore in sCOVID-19.

Moreover, there are some points that should be considered: The HScore is based on some criteria with specific cut-offs and scores. The sum of these scores leads us to HLH diagnosis. Due to the lack of data on COVID-19, our study is not based on the cut-off defined in the HScore. Further investigations are required to precisely calculate the HScore in COVID-19 patients. Some HScore parameters, such as ferritin, should be at a very high level in order to be associated with HLH, while this high level is not common in COVID-19. According to the high heterogeneity of included studies, the results should be interpreted cautiously. We have searched all keywords related to the HScore parameters in COVID-19 patients and retrieved the records. Evidence on some of the parameters, such as triglyceride, organomegaly, history of immunosuppressive therapy, or immunocompromised conditions, as well as monocyte-, basophil-, eosinophil-, and red blood cell (RBC)-related cytopenia, in COVID-19 is limited. Hence, they were not included in the meta-analysis. With the progress of studies on these parameters in COVID-19, future meta-analyses could comprise all HScore parameters to elucidate the possible relationship with severity in COVID-19.

Conclusion

In conclusion, our study indicated that the majority of HScore parameters are associated with severity in COVID-19, some of which, such as lymphopenia, thrombocytopenia, low hemoglobin level, fever, and high levels of AST and ferritin, are associated with sCOVID-19 and HLH. However, leukocytosis, neutrophilia, and hyperfibrinogenemia, which are observed in sCOVID-19, had contradictory effects on HLH. HScore parameters could be risk factors for the severity of COVID-19. However, roles of some parameters are contradictory, suggesting the need for further investigation and a new way of HScore interpretation in sCOVID-19 patients.

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Conflict of Interest: None declared.

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