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Lymphopenia and neutrophilia at admission predicts severity and mortality in patients with COVID-19: a meta-analysis

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Summary. *Background:* There is a compelling need to identify clinical and laboratory predictors of unfavorable clinical course and death in patients with coronavirus disease (COVID-19). A trend towards low lymphocyte count and high neutrophil counts in patients with poor outcomes has been reported by earlier studies. We aim to synthesize existing data evaluating the relationship between clinical outcomes and abnormal neutrophil and lymphocyte counts at admission in COVID-19 patients. *Methods:* An electronic search was carried out in PubMed, China National Knowledge Infrastructure (CNKI) and Cochrane Central Register of Controlled Trials (CENTRAL) to identify eligible studies reporting frequency data on neutrophilia and lymphopenia at admission in hospitalization in COVID-19 patients. Pooled odds ratios of clinical outcomes for each parameter were calculated using Comprehensive Meta-Analysis. *Results:* A total of 22 studies (4,969 patients) were included in this meta-analysis. Lymphopenia at admission was found to be significantly associated with increased odd of progression to severe disease (odds ratio [OR], 4.20; 95% confidence interval [95CI%], 3.46-5.09) and death (OR, 3.71; 95%CI, 1.63-8.44). Neutrophilia at admission was also found to be significantly associated with increased odd of progression to severe disease (OR, 7.99; 95%CI, 1.77-36.14) and death (OR, 7.87; 95%CI, 1.75-35.35). Subgroup analysis revealed that COVID-19 patients with severe lymphopenia (<0.5 x10×^{9}/L) had 12-fold increased odds of in-hospital mortality. *Conclusion:* Admission lymphopenia and neutrophilia are associated with poor outcomes in patients with COVID-19. Regular monitoring and early and even more aggressive intervention shall hence be advisable in patients with low lymphocyte and high neutrophil counts. These variables may be useful in risk stratification models. (www.actabiomedica.it)

Key words: lymphocytes; neutrophils; SARS-CoV-2; outcomes; laboratory medicine; prognosis

Introduction

In the ongoing coronavirus disease 2019 (COVID-19) pandemic, there is a compelling need to identify clinical and laboratory predictors for early risk stratification of severe, critical and even lethal forms of this infectious disease [1,2]. These predictors may not only enable better risk assessment, but may also be helpful to optimize limited resource allocation, and provide better patient selection for clinical studies.

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Early evidence has indicated that an elevated neutrophil count and a decrease number of circulating lymphocytes could be associated with both severity and mortality in COVID-19 [1].

Lymphopenia has been commonly reported in COVID-19 patients with severe and fatal disease, and it has been suggested that repletion of lymphocyte counts may be essential to COVID-19 recovery [3]. As CD4+ T cells are required for maintaining a balanced and effective immune response, it is not surprising that low lymphocyte counts may enhance hyperinflammation and contribute to morbidity and mortality [4]. Recently it has been shown that the virus responsible for COVID-19, severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2), can directly infect lymphocytes. This virus-induced direct cytopathic damage combined with a deranged cytokine milieu likely contributes to this phenomenon [5]. Interestingly, lymphocyte infection by SARS-CoV-2 is accompanied by a vast array of abnormalities in peripheral blood, such as large granular lymphocytes with round to indented nuclei, condensed chromatin, prominent nucleoli, pale blue cytoplasm and variably sized azurophilic granules. This likely reflects lymphocyte activation combine with cytopathic injury [6].

Recent evidence has also suggested that neutrophils may be strong player in the pathogenesis of COVID-19, since they may promote organ injury and coagulopathy via direct tissue infiltration and formation of neutrophil extracellular traps (NETs) [4,7]. An ample range of cytotoxic abnormalities has also been described for these cells in peripheral blood, such as neutrophils with C-shaped, fetus-like nuclei with aberrant nuclear projections, toxic granulations and vacuolations [6].

To further our understanding of neutrophil and lymphocyte counts at hospital admission as predictors of severe and fatal forms of COVID-19, we performed a meta-analysis. We also performed a meta-regression to evaluate which patient demographic factors, comorbidities, and symptoms may be significantly associated with lymphopenia and neutrophilia.

Methods

Study protocol and registration

This systematic review and meta-analysis were conducted in strict conformity with the Preferred Reporting Items for Systematic Reviews and Meta-Analyses (PRISMA) guidelines [8]. The protocol for this study has been reported in the International Prospective Register of Systematic Reviews (PROSPERO identifier: CRD42020184338).

Literature Search Strategy

A comprehensive and systematic search of literature was conducted from November 1, 2019 to May 6th, 2020 on the electronic databases PubMed, China National Knowledge Infrastructure (CNKI) and Cochrane Central Register of Controlled Trials (CENTRAL) to identify studies eligible for inclusion. The electronic search was carried out using the following strategy: 1) "COVID-19" OR "SARS-CoV- 2" OR "coronavirus disease 2019"; 2) "lymphopenia" OR "lymphocyte count" OR "neutrophilia" OR "neutrophil count" OR "laboratory characteristics"; 3) 1 AND 2. No language restriction was applied. When articles were published by the same study group and an overlap of the search period could be found, only the most recent article was included to avoid data duplication. The PubMed function "related articles" was used to extend our search. We also searched major infectious disease, hematology and general medicine journals reporting articles about COVID-19 infection to identify additional pertinent clinical investigations. We then performed hand-search of the bibliography of included studies, to detect other potentially eligible studies.

Eligibility Criteria

All studies were screened and assessed for eligibility by five independent reviewers (I.C, J.W, V.M, V.K, B.M.H). Search results were screened by title and abstract, with those of potential relevance evaluated by full text. Studies were deemed eligible for inclusion when fulfilling the following criteria: (1) reported extractable lymphopenia and/or neutrophilia frequency

data; (2) laboratory data reported were from admission, or at the earliest point in hospitalization; (3) compared survivors to non-survivors OR severe vs. non-severe COVID-19 in a general cohort; (4) disease severity was monitored over the course of the study; (5) used an appropriate definition of severe disease; and (6) sample size was >10. Severe disease was defined in this analysis as a composite of: (1) Respiratory distress, respiratory rate ≥30 per min; or (2) Oxygen saturation on room air at rest ≤93%; or (3) Partial pressure of oxygen in arterial blood/fraction of inspired oxygen ≤300 mmHg; or (4) Patients requiring mechanical ventilation/vital life support/intensive care unit admission (ICU); or (5) Death. Exclusion criteria were the following: 1) studies reporting laboratory data with unclear collection time points; and 2) studies reporting disease severity only at admission. Any disagreement between reviewers arising during the eligibility assessment was settled through a consensus.

Data Extraction and Quality Assessment

Data extraction was conducted by three independent reviewers (I.C, J.W and B.M.H). For each study, the following information was extracted: the surname of the first author and the year of publication, the geographical region where the study was performed, the type of the study (cohort or case-control), sample size, baseline demographic characteristics, proportion of patients with severe and non-severe COVID-19, proportion of patients with lymphopenia and/or neutrophilia, and mortality from COVID-19. Any variances were resolved by consensus. Quality assessment and analysis of risk of bias of all selected full-text articles was performed using the Newcastle-Ottawa Scale (NOS) for non-randomized studies.

Outcomes of Interest

The primary outcome of interest was the association between lymphopenia/neutrophilia and COVID-19 severity. The secondary outcome was the association between lymphopenia/neutrophilia and COVID-19 mortality. Thus, four different meta-analyses were performed.

Statistical Analysis

The statistical analysis was carried out using the Comprehensive Meta-Analysis software (Version 3.3.070, Biostat, New Jersey, United States). The strength of association between lymphopenia/neutrophilia and COVID-19 severity and mortality was estimated using odds ratio (OR). Based on the amount of heterogeneity, a fixed-effect or a random-effect model was chosen. The magnitude of heterogeneity among the included studies was assessed using the chi-squared test (Chi^2) and I-squared statistic (I^2). For the Chi^2 test, a Cochrane's Q p value of <0.10 was considered significant. The value of the I^2 statistic less than 50% was not considered significant. Subgroups were used to compare results for various cut-offs for lymphopenia and neutrophilia. Publication bias was assessed by a funnel plot analysis. A random effects meta-regression using log OR was performed to evaluate the impact of baseline characteristics (age and sex) on the association of lymphopenia/neutrophilia with disease severity. Additionally, leave-one-out sensitivity analysis was performed to assess the robustness of the results, and to probe the sources of inter-study heterogeneity.

Results

Study Identification

The initial search produced 135 potentially relevant articles. Following the removal of duplicates and primary screening, 53 articles were assessed by full text for eligibility in the meta-analysis. Of these, 31 were excluded because the primary and secondary outcome of the study did not match that of this review. Thus, a total of 22 articles (n=4,969 patients) [9-30] were included in this systematic review and meta-analysis (Figure 1; Table 1, 2 and 3).

Characteristics of the Included Studies and Quality Assessment

From included studies, 18 were from China, 2 were from the United States (US) and 2 were from Italy. In the lymphopenia analysis cohort, 17 stud-

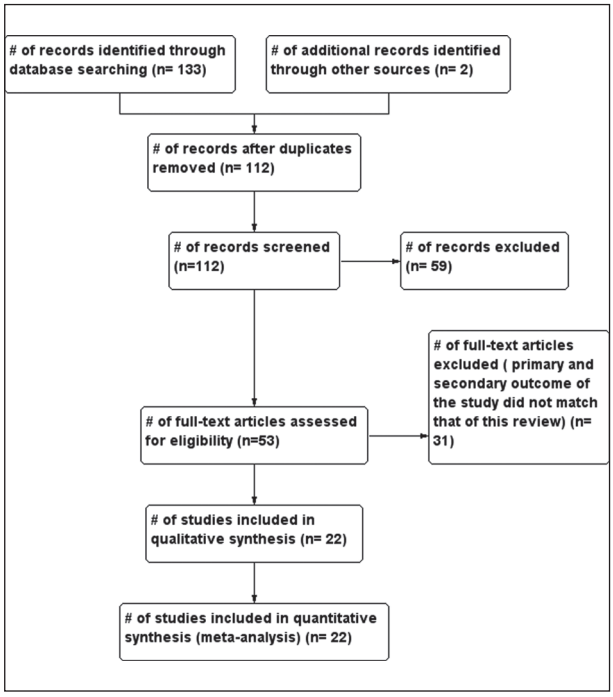

Figure 1. Flow of studies through the meta-analysis

ies reported data in patients with and without severe COVID-19, 4 studies reported data on mortality, and one study reported both. In the neutrophilia analysis cohort, 4 studies reported data in patients with and without severe COVID-19, and 2 studies reported data on mortality in COVID-19 patients. Essential characteristics of the included studies are outlined in tables 1, 2 and 3. Summary of the Newcastle-Ottawa Scale for the included studies is provided in supplementary material.

Admission lymphopenia as a predictor of COVID-19 severity

Twenty-one studies from 17 articles (n=4133 COVID-19 patients, 1140 with severe and 2993 with non-severe illness) reporting lymphopenia rate according to disease severity were included. The cut-off values for lymphopenia ranged from 0.5 to 1.5×10^9/L. Lymphopenia on admission was significantly associated with an over 4-fold increased odds of progression to severe disease (OR, 4.20; 95%CI, 3.46-5.09; p<0.001; I^2=0.0%) (Figure 2). In the subgroup analysis, lymphopenia was significantly associated with increased odds of severe COVID-19 across all cut-offs (Figure 3). Visually inspecting the funnel plot (Figure 4), carrying out the Egger's linear regression test and the Duval' and Tweedie's trim-and-fill test, evidence of publication bias could be detected, with 3 studies trimmed (real estimated OR of 4.14 [95%CI 3.42-5.02]). At the meta-regression, only the mean age was found to be statistically significant (regression coefficient=-0.04, p=0.015) (Supplementary material), whereas the percentage of cancer patients was statistically borderline (regression coefficient=-6.87, p=0.074). All other variables, including lymphopenia cut-off, study setting and study design, were not statistically significant in meta-regression.

Admission lymphopenia as a predictor of COVID-19 in-hospital mortality

Nine studies from five articles (n=1708 COVID-19 patients, 908 survivors and 800 non-survivors) reported the rate of lymphopenia in COVID-19 patients who died or survived. Lymphopenia was found to be associated with a nearly 4-fold increased odds of mortality (OR 3.71; 95%CI, 1.63-8.44; p=0.002; I2=88.4%) (Figure 5). In subgroup analysis by cut-off, COVID-19 patients presenting with severe lymphopenia (<0.5×10^9/L) had 12-fold increased odds of in-hospital mortality (Figure 6). Visually inspecting the funnel plot (Figure 7), carrying out the Egger's linear regression test and the Duval' and Tweedie's trim-and-fill test, no evidence of publication bias could be observed. In a meta-regression, fever (regression coefficient =18.07, p<0.001) was found to be significant associated with log OR for lymphopenia (Supplementary material). All the other variables, including lymphopenia cut-off, study setting and study design, were not found to be statistically significant.

Admission neutrophilia as a predictor of COVID-19 severity

Four studies (n=665 COVID-19 patients, 313 with severe and 352 with non-severe illness) reported neutrophilia rates among COVD-19 patients. The cut-offs ranged from 6 to 7×10^9/L. Neutrophilia was significantly associated with 8-fold enhanced odds of

Table 1. Characteristics of studies included in the lymphopenia severity cohort analysis

Study	Country & City	Sample Size	Cut-off (x10*9/l)	Severe patients				Non-severe patients			
				n (%)	Age (yrs)*	Women (%)	Lymphopenia n (%)	n (%)	Age (yrs)*	Women (%)	Lymphopenia n (%)
Guan et al. [9]	Outside Hubei, China	1099	1.5	153 (17.4%)	52 (40–65)	73 (42%)	147 (96.1%)	726 (81.4%)	45 (34-57)	386 (42%)	584 (80.4%)
Huang et al. [10]	Wuhan, China	41	1	13 (31.7%)	49 (41-61)	2 (15%)	11 (84.6%)	28 (68.3%)	49 (41-57.5)	15 (53.6%)	3 (11%)
Liu et al. [11]	Shenzhen, China	12	0.5	6 (54.5%)	64	3 (50%)	2 (33.3%)	5 (45.5%)	43.3	1 (16%)	0 (0%)
			0.8	6 (54.5%)	64	3 (50%)	3 (50%)	5 (45.5%)	43.3	1 (16%)	0 (0%)
			1.1	6 (54.5%)	64	3 (50%)	5 (83.3%)	5 (45.5%)	43.3	1 (16%)	1 (20%)
Wan et al. [12]	Chongqing, China	135	1.1	40 (29.6%)	56 (52-73)	19 (47.5%)	32 (80%)	95 (70.4%)	44 (33-49)	43 (45.3%)	36 (37.9%)
Zhang J et al. [13]	Wuhan, China	140	1.1	56 (40.6%)	64 (25-87)	25 (43%)	46 (82.1%)	82 (59.4%)	52 (26-78)	44 (54%)	58 (70.3%)
Feng et al., [14]	Shanghai, Wuhan & Anhui, China	476	1	124 (26.1%)	58 (48-67)	43 (34.7%)	89 (71.8%)	352 (73.9%)	51 (37-63)	162 (46%)	136 (38.6%)
Zheng F et al. [15]	Changsha,China	161	0.8	30 (18.6%)	57 (46.5-66)	16 (53.3%)	13 (43.3%)	131 (81.4%)	40 (31-51)	65 (49.6%)	29 (22.1%)
Zhang Guqin et al., [16]	Wuhan, China	221	0.5	55 (24.9%)	62 (52-74)	20 (36.4%)	18 (32.7%)	166 (75.1%)	51 (36-64.3)	93 (56%)	21 (12.65%)
			1.1	55 (24.9%)	62 (52-74)	62 (52-74)	48 (87.3%)	166 (75.1%)	51 (36-64.3)	93 (56%)	115 (69.3%)

Table 1. Characteristics of studies included in the lymphopenia severity cohort analysis

Study	Country & City	Sample Size	Cut-off (x10*9/l)	Severe patients				Non-severe patients			
				n (%)	Age (yrs)*	Women (%)	Lymphopenia n (%)	n (%)	Age (yrs)*	Women (%)	Lymphopenia n (%)
Goyal et al., [17]	New York, USA	393	1.5	130 (33.1%)	64.5 (51.7-73.6%)	38 (29.2%)	124 (95.4%)	263 (66.9%)	61.5 (47-75)	117 (45.5%)	227 (86.3%)
Cai et al., [18]	Shenzen, China	298	1.1	58 (19.8%)	62.5 (56-66)	19 (32.8%)	39 (67.2%)	235 (80.2%)	41 (31-56)	134 (51.3%)	75 (31.9%)
Wang Z et al., [19]	Wuhan, China	69	1.1	14 (20.9%)	70.5 (62-77)	7 (50%)	11 (78.6%)	53 (79.1%)	37 (32-51)	30 (55%)	17 (32.1%)
Ji D et al., [20]	Ji D et al., 2020	208	1	40 (19.2%)	57.7±15.9	14 (30%)	30 (75%)	168 (80.8%)	40.7±14.7	79 (47%)	48 (28.6%)
Colaneri et al., [21]	Pavia, Italy	44	1.5	17 (38.6%)	-	4 (23.5%)	17 (100%)	27 (61.4%)	-	12 (44.4%)	22 (81.5%)
Yao et al., [22]	Dabieshan, China	108	0.8	25 (23.1%)	-	12 (48%)	13 (52%)	83 (76.9%)	50.0 (34.0 56.0)	53 (63.9%)	10 (12.0%)
Li X et al., [23]	Wuhan, China	548	0.5	267 (49.3%)	65 (54-72)	116 (43.1%)	64 (24%)	275 (50.7%)	56 (44-66)	153 (54.8%)	21 (7.6%)
Zheng Y et al., [24]	Taizhou, China	141	1.1	29 (20.6%)	55 (47-63)	13 (44.8%)	24 (82.8%)	112 (79.4%)	45 (37-55)	54 (48.3%)	43 (38.4%)
Aggarwal et al., [25]	Iowa, USA	16	0.8	8 (50%)	67 (38-70)	3 (38%)	3 (37.5%)	8 (50%)	68.5 (41-95)	1 (13%)	2 (25%)
			1.1	8 (50%)	67 (38-70)	3 (38%)	5 (62.5%)	8 (50%)	68.5 (41-95)	1 (13%)	5 (62.5%)

Table 2: Characteristics of studies included in the lymphopenia mortality analysis cohort

Study	Country & City	Sample Size	Cut-off (x10*9/l)	Survivors				Non-survivors			
				n (%)	Age (yrs)*	Women (%)	Lymphopenia n (%)	n (%)	Age (yrs)*	Women (%)	Lymphopenia n (%)
Zhou F et al., [26]	Wuhan, China	191	0.8	137 (71.7%)	52 (45-58)	56 (41%)	36 (26.3%)	54 (28.3%)	69 (63-76)	16 (30%)	41 (75.9%)
Chen T et al., [27]	Wuhan, Chins	274	0.5	161 (58.8%)	51 (37-66)	73 (45%)	8 (5%)	113 (41.2%)	68 (62-77)	30 (27%)	44 (40%)
			0.8	161 (58.8%)	51 (37-66)	73 (45%)	48 (29.8%)	113 (41.2%)	68 (62-77)	30 (27%)	87 (78%)
			1	161 (58.8%)	51 (37-66)	73 (45%)	76 (47.2%)	113 (41.2%)	68 (62-77)	30 (27%)	103 (91.2%)
Fan et al., [28]	Wuhan, China	155	1.1	54 (34.8%)	-	-	47 (87.0%)	101 (65.2%)	65.5±9.74	37 (37%)	94 (93.1%)
Yao et al., [22]	Dabieshan, China	108	0.8	12 (11.1%)	-	-	5 (41.7%)	96 (88.9%)	65.0 (51.0,73.5)	5 (41%)	18 (18.75%)
Bonetti et al., [29]	Valcamonica, Italy	144	0.5	74 (51.4%)	62.1 (53-72.8)	24 (31.1%)	1 (1.4%)	70 (48.6%)	78 (64.2-84)	25 (35.7%)	13 (18.6%)
			0.8	74 (51.4%)	62.1 (53-72.8)	24 (31.1%)	17 (23%)	70 (48.6%)	78 (64.2-84)	25 (35.7%)	28 (40%)
			1.1	74 (51.4%)	62.1 (53-72.8)	24 (31.1%)	22 (29.7%)	70 (48.6%)	78 (64.2-84)	25 (35.7%)	15 (21.4%)

Table 3: Characteristics of studies included in the neutrophilia analysis cohort

Study	Country & City	Sample Size	Cut-off (x10*9/l)	Severe patients				Non-severe patients			
				n (%)	Age (yrs)*	Women (%)	Neutrophilia n (%)	n (%)	Age (yrs)*	Women (%)	Neutrophilia n (%)
Liu et al. [11]	Shenzen, China	12	6	6 (50%)	64	3 (50%)	1 (16.7%)	6 (50%)	43.3	1 (16%)	1 (16.7%)
Zhang Gemin et al. [30]	Wuhan, China	95	7	32 (33.7%)	50.5 (38.3-58.8)	11 (34.4%)	26 (81.3%)	63 (66.3%)	49 (41-57)	31 (49.2%)	4 (6.3%)
Li X et al. [23]	Wuhan, China	548	6.5	267 (48.7%)	65 (54-72)	116 (43.1%)	96 (40%)	275 (50.1%)	56 (44-66)	153 (54.8%)	22 (8%)
Aggarwal et al. [25]	Iowa, US	16	6.3	8 (50%)	67 (38-70)	3 (38%)	4 (50%)	8 (50%)	68.5 (41-95)	1 (13%)	2 (25%)
				Survivors				Non-survivors			
				n (%)	Age (yrs)*	Women (%)	Neutrophilia n (%)	n (%)	Age (yrs)*	Women (%)	Neutrophilia n (%)
Chen T et al., [27]	Wuhan China	274	6.3	161 (58.8%)	51 (37-66)	73 (45%)	17 (10.6%)	113 (41.2%)	68 (62-77)	30 (27%)	75 (66.4%)
Bonetti et al., [29]	Valcamonica, Italy	144	6.3	74 (51.4%)	62.1 (53-72.8)	24 (31.1%)	14 (18.9%)	70 (48.6%)	78 (64.2-84)	25 (35.7%)	32 (45.7%)

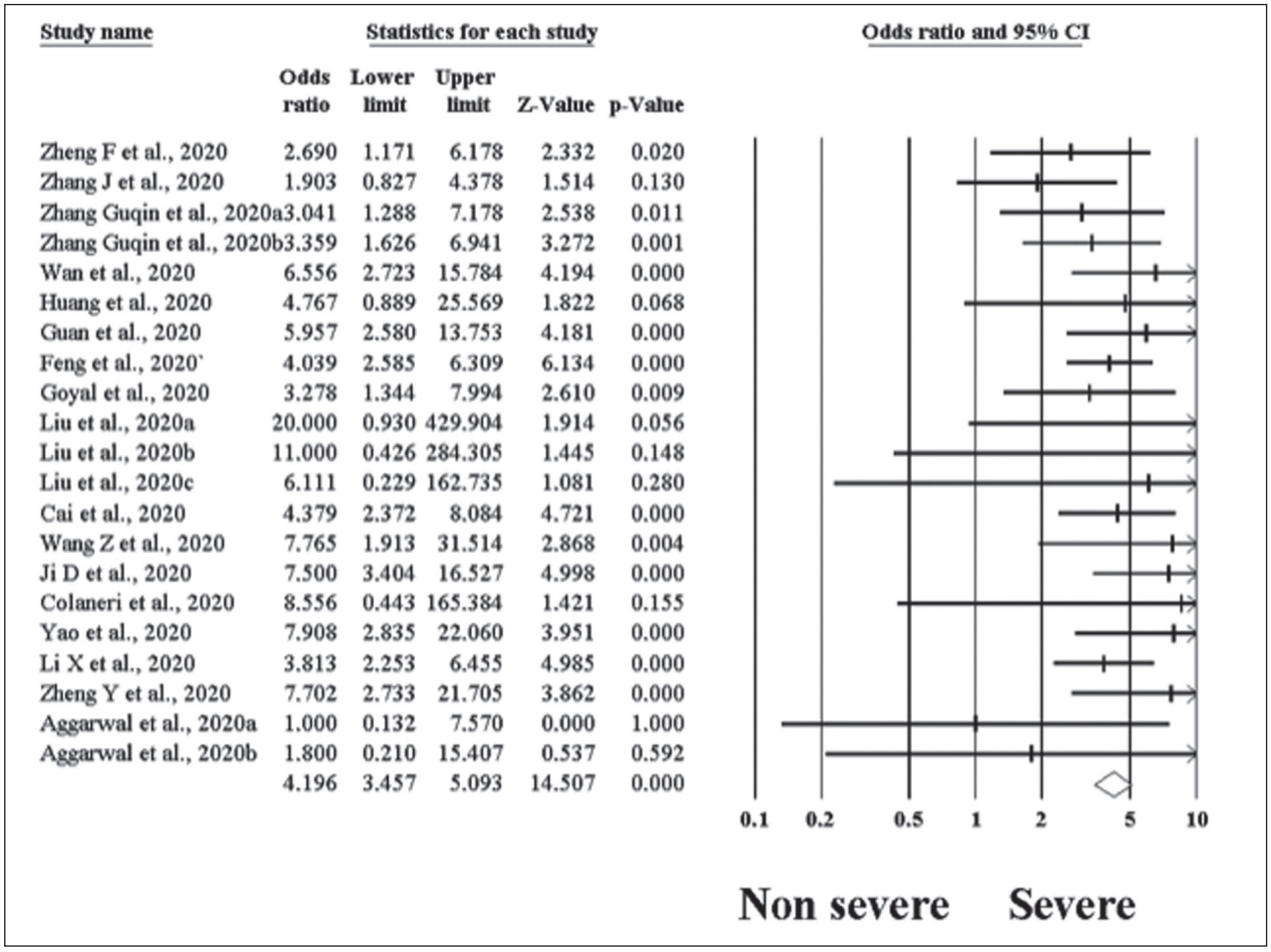

Figure 2. Forest plot of Lymphopenia rate in COVID-19 severe and non-severe patients

progressing to severe disease (OR, 7.99; 95%CI, 1.77-36.14; p=0.007; I^2=75.9) (Figure 8). No evidence of publication bias could be found, and no variable was significant at the meta-regressions.

Admission neutrophilia as a predictor of COVID-19 mortality

Two studies (n=418 COVID-19 patients, 235 survivors and 183 non-survivors) reported neutrophilia rates and mortality. The cut-off used in the studies was 6.3×10^9/L. Neutrophilia was significantly associated with a nearly 8-fold higher odds of mortality (OR, 7.87; 95%CI 1.75-35.35; p=0.007; I^2=89.3) (Figure 9). Due to the limited number of available studies, assessing the publication bias or carrying out meta-regressions were not performed.

Discussion

Identification of COVID-19 patients at enhanced risk of progression towards advanced illnesses, especially those needing sub-intensive or intensive care, should be considered a priority, as early management may be essential to prevent an unfavorable short- and long-term outcome [31]. This systematic review and meta-analysis found that admission lymphopenia and neutrophilia are associated with worse outcomes in patients with COVID-19. Lymphopenia conferred a

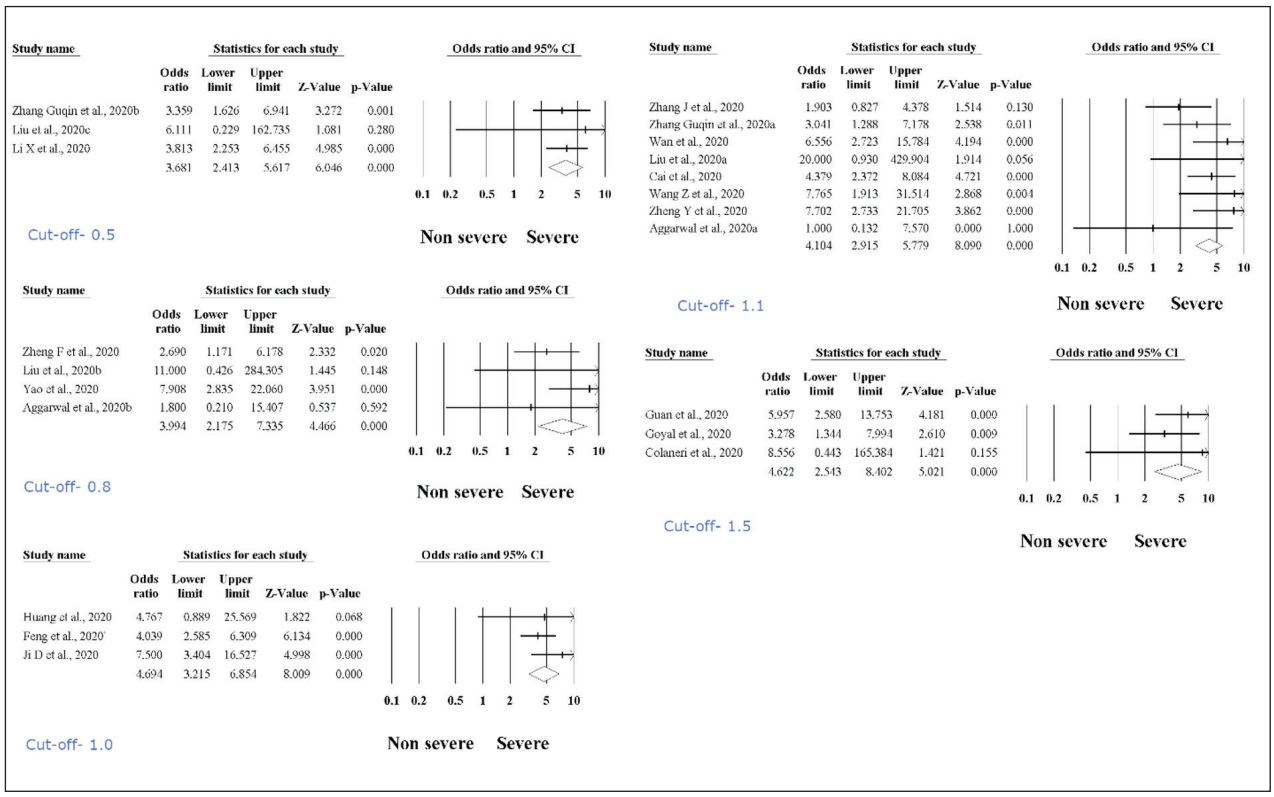

Figure 3. Forest plot for various cut-off values for lymphopenia rate in COVID-19 severe and non-severe patients

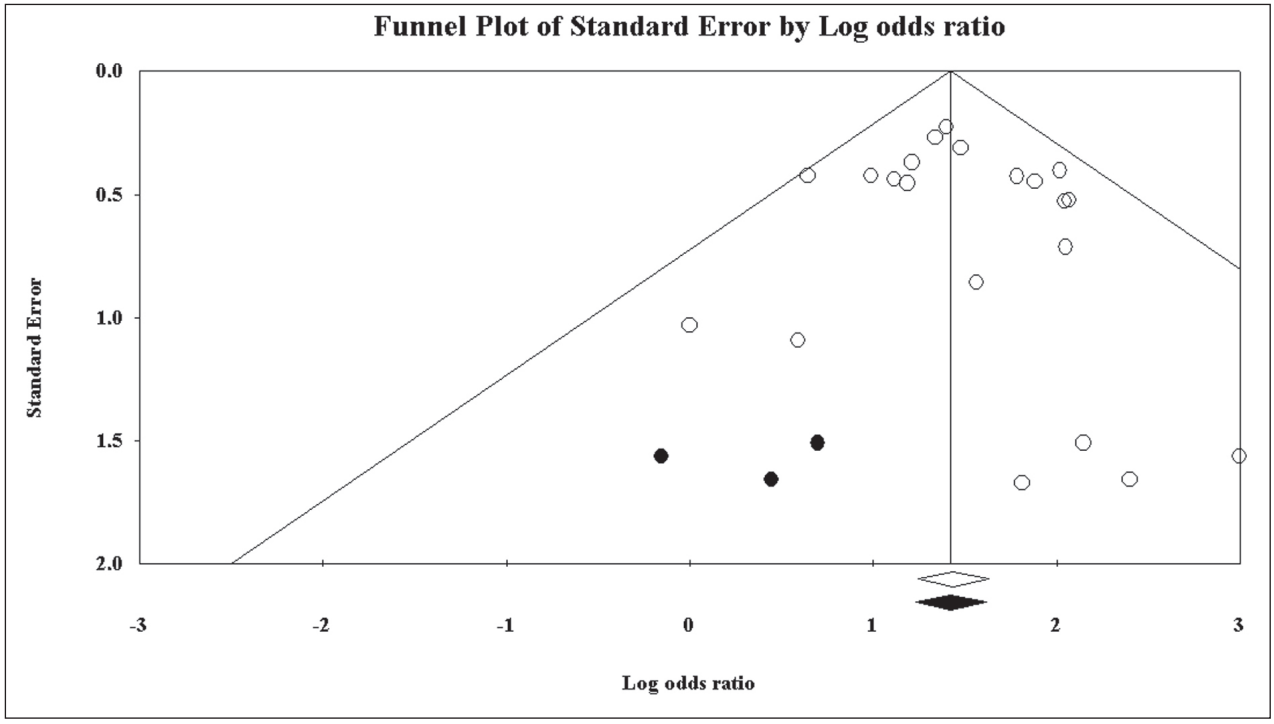

Figure 4. Funnel plot of the Lymphopenia rate in COVD-19 severe and non-severe patients

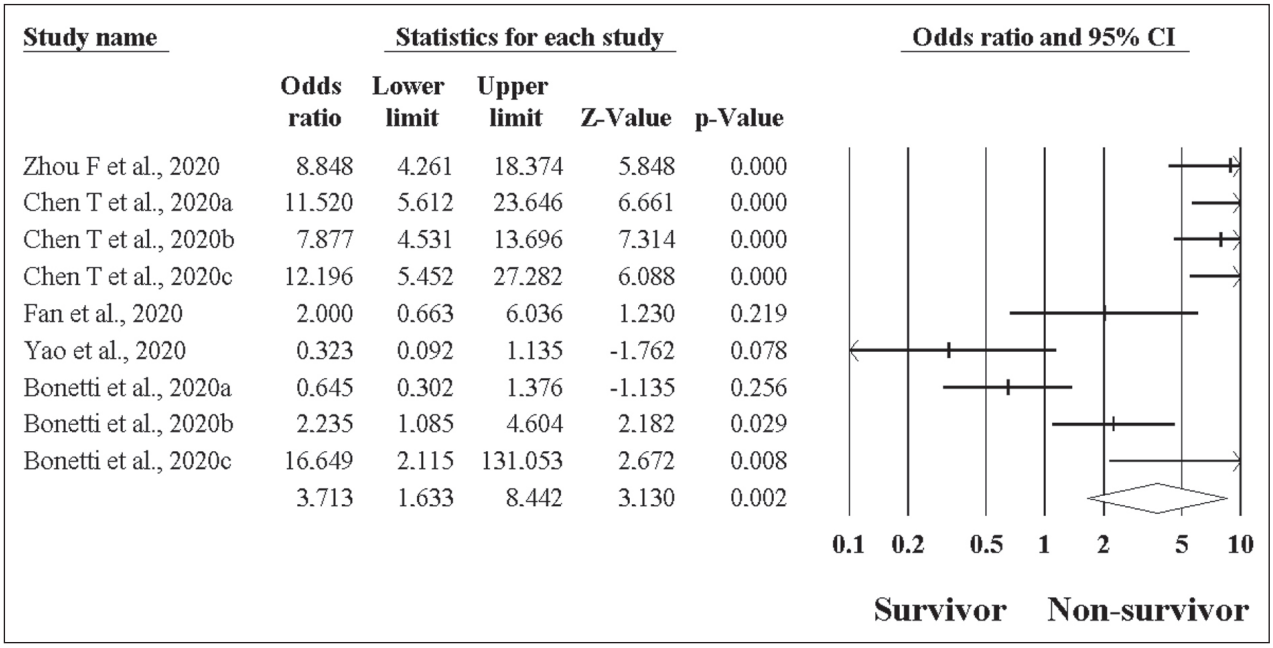

Figure 5. Forest plot of the Lymphopenia rate in COVID-19 survivors and non-survivors

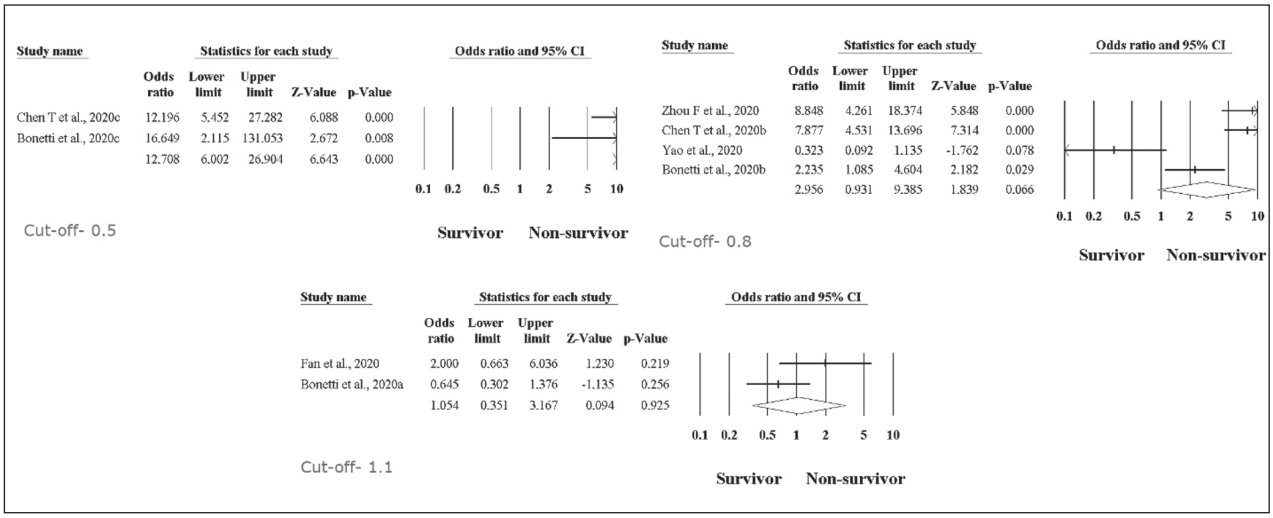

Figure 6. Forest plot of the various lymphopenia rate cut-offs in COVID-19 survivors and non-survivors

more than 3-fold increase in the odds of both severe and fatal COVID-19, while neutrophilia was associated with a more than 7-fold increase in odds for the same outcomes.

Lymphocytes (B-cells and T-cells) constitute the adaptive antiviral response. Activated B-cells (plasma cells) produce neutralizing antibodies which can bind to extracellular virus particles, thus preventing binding to and infection of host cells [32]. Cells infected by the virus can be recognized and eliminated by CD8+ cytotoxic T-cells [33]. CD4+ T-cells exert a multitude of effects, including the stimulation of efficient antiviral B and CD8+ T-cell responses, and regulation of innate and adaptive immunity, limiting detrimental immune-mediated multiple organ damage [33]. Lymphopenia will not only impair the adaptive antiviral

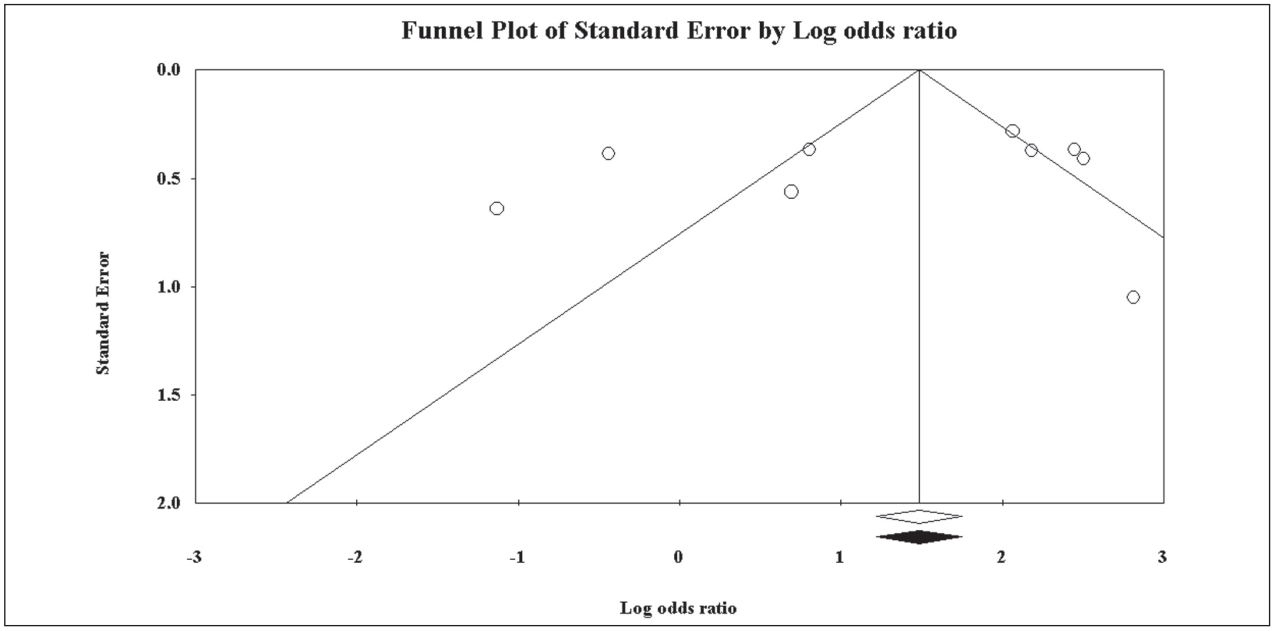

Figure 7. Funnel plot of the Lymphopenia rate in COVD-19 survivors and non survivors

Study name	Odds ratio	Lower limit	Upper limit	Z-Value	p-Value
Liu et al., 2020	1.000	0.048	20.829	0.000	1.000
Zhang G et al., 2020	63.917	16.625	245.729	6.051	0.000
Li X et al., 2020	6.456	3.907	10.669	7.278	0.000
Aggarwal et al., 2020	3.000	0.361	24.919	1.017	0.309
	7.993	1.768	36.143	2.700	0.007

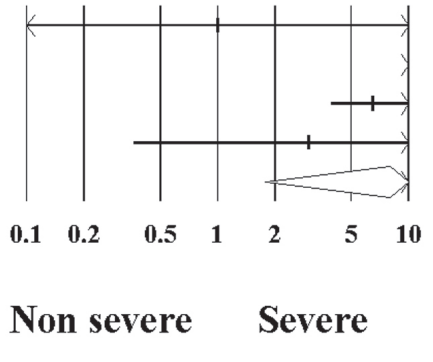

Figure 8. Forest plot of the odds-ratio of neutrophilia in COVID-19 severe and non-severe patients

response, but potentially also render the host susceptible to severe hyperinflammatory immunopathology. This is illustrated by a previous study, showing that CD4+ lymphopenia may be associated with increased immune mediated pneumonitis in a mouse model of severe acute respiratory syndrome (SARS) [34].

The mechanisms underlying the onset of lymphopenia in COVID-19 patients may include direct cytopathic effects and increased apoptosis secondary to a deranged cytokine milieu [35,36]. Neutrophilia in COVID-19 may potentially be caused by deranged immune homeostasis, but could also be the result of secondary bacterial infections and/or glucocorticoid therapy (either endogenous as in a stress response, or

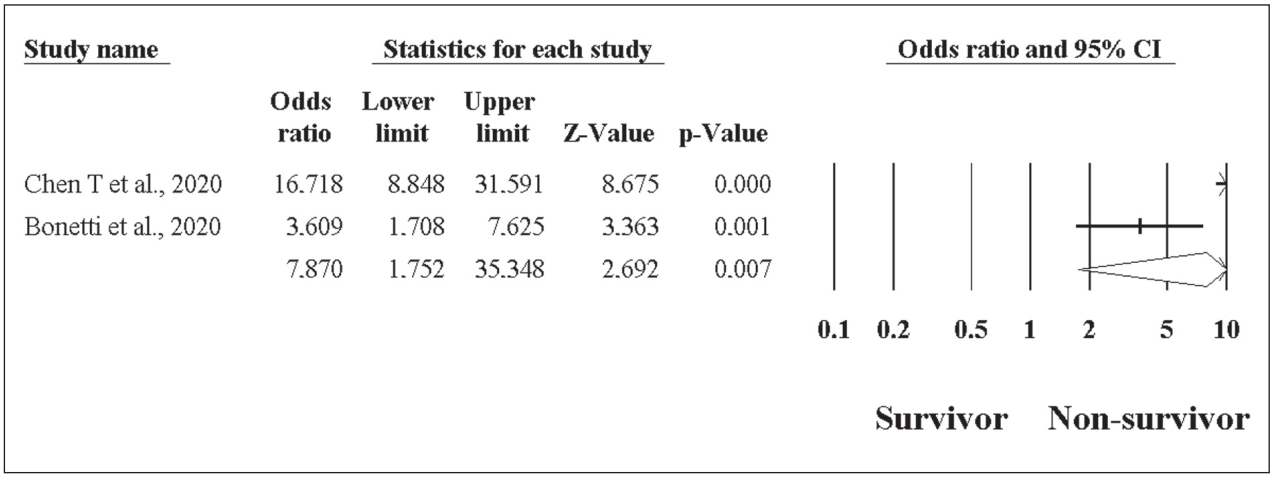

Figure 9. Forest plot of the odds-ratio of neutrophilia in COVID-19 survivors and non-survivors

through exogenous administration), the latter of which may also contribute to lymphopenia.

Neutrophils are integral to the innate defense against extracellular pathogens, such as bacteria and fungi, since they exert several potent antimicrobial effects, including production of reactive oxygen species (ROS) and release of NETs [37]. While activated neutrophils are crucial in the defense against a range of pathogens, an improper hyperactivation can lead to severe collateral damage in form of immune-mediated target organ damage [38]. An animal study of SARS demonstrated that a deregulated initial innate antiviral response (with delayed and persistent type I interferon signaling) resulted in massive pulmonary infiltration of monocytes/macrophages and neutrophils, accompanied by severe lung immunopathology [39]. As we have previously hypothesized, dysregulated neutrophils may also contribute to detrimental and widespread immunothromobosis, leading to thrombotic end-organ damage [4]. Thus, we suspect the observed lymphopenia and neutrophilia seen in this study may be detrimental in COVID-19, resulting in an impaired antiviral response and increased immune mediated and thrombotic organ damage. Therefore, we recommend regular monitoring of these parameters in COVID-19 patients, and potentially earlier and more aggressive intervention in those presenting with either lymphopenia or neutrophilia.

Based on our observations, we encourage the inclusion of lymphopenia and neutrophilia at initial presentation in future risk stratification models. Importantly, as observed in the meta-regression, no patient co-morbidity, including those associated with severe and fatal COVID-19, such as heart disease and chronic obstructive pulmonary disease, were associated with the observed pooled ORs [40,41]. This indicates that these biomarkers appear to be independent of other variables associated with increased risk of poor outcomes. With respect to the value of admission lymphopenia for predicting severe COVID-19 disease, a mild but statistically significant association was noted with age, thus reflecting the fact that this parameter may be a stronger predictor of severe disease in younger patients. Nonetheless, these biomarkers appear rather robust clinical predictors of COVID-19 outcomes, irrespective of other confounding variables.

In meta-regression, no significant influence was observed with respect to cut-off values. Notwithstanding, we performed subgroup analysis to further understand the impact of different cut-off points and inform a recommendation for potential thresholds for defining clinically significant lymphopenia in COVID-19

patients. The OR of lymphopenia was consistent at ~3-4, regardless of the cut-off value. However, alterations in threshold appeared to be more influential for mortality. Cut-offs for lymphocyte count of 1.1 and 0.8×10^9/L resulted in insignificant ORs, while including only those studies applying a cut-off of 0.5×10^9/L yielded a nearly 12-fold increase in the odds of death. Thus, patients presenting with severe lymphopenia should alert the clinical team to high potential for poor outcome. As it has been hypothesized that recovery of lymphocyte count may be predictive and indicative of recovery, serial lymphocyte tracking during hospitalization should be evaluated in future studies to monitor patient prognosis. Unfortunately, limited number of studies and narrow range of cut-offs prohibited subgroup analysis for neutrophilia. Future studies should investigate the most optimal cut-off points of neutrophilia and lymphopenia as clinical predictors.

Some limitations should be noted in this analysis. All the included studies were retrospective in nature, and thus susceptible to bias inherent of this study design. While data from 3 countries were included, the majority of studies were from a single country (China). Although no differences could be observed in meta-regression by country, future investigations should compare laboratory data from different geographical areas. Study quality was also heterogeneous, and some studies displayed limited data on neutrophilia, thus hindering subgroup analyses and meta-regressions, as well as evaluation of publication bias and threshold effects (albeit a narrow range of cut-offs were used). Given the association of neutrophilia and poor outcomes as reported in this study, this parameter requires more attention in future investigation.

Conclusion

Admission lymphopenia and neutrophilia were found to be associated with worse outcomes in patients with COVID-19. Patients presenting with severe lymphopenia (0.5×10^9/L) have an especially high (i.e., ~12-fold increased) odds of mortality. As such, we strongly recommend regular monitoring of these parameters and early and even more aggressive therapeutic management in patients with low lymphocyte count and high neutrophil counts. These variables could be included in future risk stratification models. Future studies should investigate the most optimal cut-off points of neutrophilia and lymphopenia as clinical predictors.

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Conceptualization- BMH
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Software & Formal Analysis- NB
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Writing – Original Draft Preparation- BMH, IC, JV
Writing – Review & Editing- All authors
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