Is neutrophilia associated with mortality of COVID-19? A meta-analysis and meta-regression

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To the Editor,

Coronavirus disease 2019 (COVID-19) is spreading rapidly around the world. There are plenty of emerging researches on the risk factors of severe and mortal COVID-19 patients. Huang et al reported that the elevated leukocyte counts and decreased lymphocyte counts were significantly associated with the severity of COVID-19. Although neutrophil counts were not uniformly reported in that study, they thought that neutrophilia was more specific to severe patients than leukocytosis[1]. To our knowledge, a number of studies have investigated the association of neutrophil counts with the mortality of COVID-19, however, the conclusions among studies are contradictive[2-6]. On this basis, we explored the relationship between neutrophil counts and mortality of COVID-19 by quantitative meta-analysis and meta-regression.

We completed our meta-analysis by strictly following the Preferred Reporting Items for Systematic Reviews and Meta-Analyses (PRISMA) guidelines. We conducted an electronic search of PubMed, Web of Science and Chinese National Knowledge Infrastructure to identify potential studies published between January 1, 2020 and May 22, 2020 using the following terms: ("clinical" OR "laboratory" OR "neutrophil") AND ("coronavirus" OR "2019-nCoV" OR "SARS-CoV-2" OR "COVID-19") AND ("outcome" OR "mortality"). In addition, the references of eligible studies were also reviewed by two researchers (Li Shi and Ying Wang), respectively. Extracted data included: authors, locations, number of cases per study, percentages of male, the median or mean of age, and neutrophil counts and corresponding units in the non-survival and survival groups.

The inclusion criteria involved (1) studies presented in English; (2) patients with laboratory-confirmed and clinically-diagnosed COVID-19 pneumonia; (3) clear report about neutrophil counts in the non-survival and survival groups. Case reports, meta-analysis, review, and studies with overlapping data were excluded.

Considering the inherent differences among studies, we calculated the pooled standardized mean difference (SMD) and corresponding 95% confidence interval (CI) for continuous variables by using random-effects model to evaluate the relationship between changes in neutrophil counts and mortality of COVID-19 patients. When the mean and standard deviation could not be extracted directly from studies, we estimated them according to Wan et al's[7] method by utilizing sample size, median and interquartile range (IQR) or median and range. TheI^2 statistic and Cochran's Q statistic were used to quantify the heterogeneity across studies. For the Cochran's Q statistic, significant heterogeneity across studies was deemed as aP value < 0.10. For the I^2statistic, significant heterogeneity across studies was regarded as$I^2 > 50\%$. We used age and gender as covariates to conduct a restricted-maximum likelihood random effects meta-regression. Sensitivity analysis was used not only to identify sources of heterogeneity but also to assess the robustness of the results. For assessing small-study effects, we chose Begg's test and regression-based Egger's test. All calculations were performed in STATA 16.0 (StataCorp, College Station, TX, USA). Two-tailed P values < 0.05 were considered statistically significant.

At the beginning, there were 648 records in the search results, 100 duplicates were deleted, and the remaining 548 studies were screened. Finally, a total of 10 studies[2-6,8-12] having 1,473 COVID-19 cases were included through careful screening of titles, abstracts and full texts (Table 1).

The combined results revealed that higher neutrophil counts were detected in the non-survival COVID-19 patients compared with the survival COVID-19 patients (SMD = 0.93, 95% CI = 0.63-1.24,I^2 = 76.3%, Q = 42.12, $P < 0.001$) (Fig. 1A). The results of sensitivity analysis suggested that removing any individual study of the included studies had no significant effect on the association between changes in neutrophil counts and mortality of COVID-19 infected patients (Fig. 1B). Due to the limitations of the data reported in the included studies, we only used age and gender as covariates for meta-regression. The results of meta-regression analysis indicated that the relationship between changes in neutrophil counts and increased risk of mortality in COVID-19 infected patients was not obviously affected by age ($P = 0.628$) (Fig. 1C) and gender ($P = 0.222$) (Fig. 1D). Begg's test ($P = 1.839$) and regression-based Egger's test ($P = 0.058$) demonstrated no small-study effects for the relationship between neutrophil counts and increased risk of mortality in COVID-19 patients.

Cytokine storms, characterized by aberrant activation of immune cells and excessive release of inflammatory cytokines (e.g., IL1β, IFNγ, MCP1, and so on), were common in patients with severe COVID-19 infection, leading to severe lung injury, acute respiratory distress syndrome (ARDS), and even death[13,14]. Neutrophils are important immune cells and the first line of defense for the body's immune barrier, effecting against foreign invaders[15]. Neutrophils participate in the immune response to pathogen infection by phagocytosis and degranulation. Furthermore, a new mechanism through which neutrophils could kill pathogens reported by Brinkmann et al in 2004[16]. Neutrophils, stimulated by phorbol myristate acetate, lipopolysaccharide or interleukin 8, undergo programmed death and then eventually release neutrophil extracellular traps (NETs), a process called NETosis[16]. NETosis is a double-edged sword. Although NETs can protect the host from pathogens, excessive NET formation can also cause a hyperinflammatory condition, which lead to organ

damage, including lung, kidney, and so on[17,18]. Multiple organ injuries are common in severe and fatal COVID-19 infected patients[3,9]. The aberrant activation of neutrophil—the ability to form excessive NETs—was considered to be the core of the dysregulated host response[19]. Our current study demonstrated that the elevated neutrophil counts was significantly correlated to the mortality of COVID-19 patients. However, the meta-analysis was based on ten published studies with 1,473 COVID-19 cases. Therefore, future studies with larger sample size and no repeated data are needed to support our results. In conclusion, neutrophilia is a risk factor for mortality of COVID-19 patients, and treatment for neutrophilia to reduce the clinical severity of COVID-19 is worth considering, for example targeting NETs if appropriate.

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Table 1. Characteristics of the included studies

Author	Location	Case	Male (%)	Age, years	Non-survival	Non-survival	Survival	S
					n	Neutrophils, $\times 10^9$/L	n	N
Chen T et al.	China	274	171 (62.4)	62 (median)	113	9.0 (5.4-12.7)	161	3
Chen Tielong et al.	China	55	34 (61.8)	74 (median)	19	5.5 (2-21)	36	4
Du R et al.	China	179	97 (54.2)	57.6 (mean)	21	7.7 (3.0-11.5)	158	3
He W et al.	China	13	7 (53.8)	35 (median)	8	0.7 (0.2-6.5)	5	2
Wang D et al.	China	107	57 (53.3)	51 (median)	19	5.4 (3.2-8.5)	88	2
Wang K et al.	China	296	140 (47.3)	47.32 (mean)	19	6.4 (3.2-10.0)	277	3
		44	24 (54.5)	55.2 (mean)	14	5.8 (5.0-8.4)	30	3
Wang L et al.	China	339	166 (49.0)	69 (median)	65	7.65 (4.35-11.74)	274	4
Wu C et al.	China	84	60 (71.4)	58.5 (median)	44	7.43 (5.15-10.60)$^{+}$	40	5
Yan Y et al.	China	48	33 (68.8)	69.4 (mean)	39	8.04 (5.36-12.49)	9	3
Martín-Moro F et al.	Spain	34	19 (55.9)	72.5 (median)	11	7.4 (0-64.2)$^{++}$	23	4

All values are n (%), or median (IQR). $^{+}$, the unit of neutrophils is $\times 10^9$/mL; $^{++}$, the values are median (range).

Figure legend

Fig.1. The pooled standardized mean difference (SMD) and corresponding 95% confidence interval (CI) (A), sensitivity analysis (B), meta-regression for age (C) and gender (D) to evaluating the association between changes in neutrophil counts and mortality of COVID-19 infected patients.