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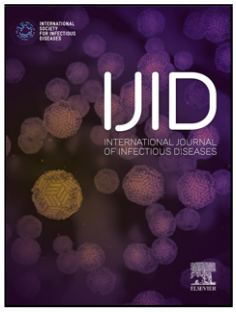

Serum amyloid A concentrations, COVID-19 severity and mortality: an updated systematic review and meta-analysis

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Serum amyloid A concentrations, COVID-19 severity and mortality: an updated systematic review and meta-analysis

Running title: Serum amyloid A and COVID-19

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Highlights

- Specific acute phase reactants might be useful for risk stratification in patients with COVID-19.
- We conducted a systematic review and meta-analysis of studies of serum amyloid A (SAA) in patients with COVID-19.
- SAA concentrations were significantly higher in COVID-19 patients with severe disease and non-survivors.
- SAA might assist with risk stratification and monitoring in this group.

Abstract

Background and objectives: An excessive inflammatory response in patients with coronavirus disease 2019 (COVID-19) is associated with high disease severity and mortality. Specific acute phase reactants might be useful for risk stratification. We conducted a systematic review and meta-analysis of studies of serum amyloid A (SAA) in patients with COVID-19.

Methods: We searched PubMed, Web of Science and Scopus, between January 2020 and December 2020, for studies reporting SAA concentrations, COVID-19 severity and survival status.

Results: Nineteen studies in 5,617 COVID-19 patients were included in the meta-analysis. Pooled results showed that SAA concentrations were significantly higher in patients with severe disease and non-survivors (standard mean difference, SMD, 1.20, 95% CI 0.91-1.49, P<0.001). Extreme between-study heterogeneity was observed (I^2=92.4%, P<0.001). In sensitivity analysis, the effect size was not significantly affected when each study was in turn removed (range between 1.10-1.29). The Begg's (P=0.030), but not the Egger's (P=0.385), test revealed the presence of publication bias. Pooled SMD values were significantly and positively associated with gender (t=2.20, P=0.047) and aspartate aminotransferase (t=3.44, P=0.014).

Conclusions: SAA concentrations were significantly and positively associated with higher COVID-19 severity and mortality. This acute phase reactant might assist with risk stratification and monitoring in this group.

Key words: serum amyloid A, COVID-19, disease severity, mortality.

Introduction

A state of excessive local and systemic inflammation and immune activation are strongly associated with oxidative stress, coagulation abnormalities, and multi-organ dysfunction in patients with

coronavirus disease 2019 (COVID-19) (Fajgenbaum and June, 2020, Hojyo et al., 2020). While safe and effective vaccines have been developed and are currently being rolled out effective therapies, e.g., repurposed antiviral and immunosuppressant agents, to mitigate the clinical manifestations of COVID-19 remain limited (Siemieniuk et al., 2020). In this context, the use of biomarkers of disease severity and clinical progression would facilitate the early identification of patients requiring aggressive management and monitoring and assist with the judicious use of health care resources. Given the key pathophysiological role of inflammation and immunity in the clinical progress of COVID-19, markers that reflect the activation of these pathways might be particularly useful for risk stratification and effective management. Serum amyloid A (SAA) genes and proteins are significantly activated during the acute phase response, which comprises a number of phenomena, e.g., increased temperature and hormonal and metabolic alterations, that occur in the presence of inflammation and infection (Yoo and Desiderio, 2003). Circulating SAA concentrations, typically low under physiological circumstances (20-50 mg/L), can increase up to 1,000-fold within the first 24-48 hours of an acute phase response. This is the consequence of an increase synthesis in the liver that is triggered by several stimuli, including tumor necrosis factor (TNF), interleukin-1β (IL-1β), interleukin-6 (IL-6) and interferon-γ (Morrow et al., 1981, Uhlar and Whitehead, 1999). SAA, in turn, can activate the complement system and the nucleotide-binding domain leucine-rich repeat-containing family pyrin-domain containing 3 (NLRP3) inflammasome, further increase the synthesis of TNF, IL-1β, and IL-6, and activate other pro-inflammatory cytokines such as interleukin-1α and IL-23 (Ather et al., 2011, De Buck et al., 2016, Yuste et al., 2007). Notably, these mediators have been shown to significantly contribute to the onset of the cytokine storm and its adverse clinical consequences in COVID-19 (Fajgenbaum and June, 2020). Therefore, it is plausible that the acute increase in SAA concentrations in patients with COVID-19 might not only reflect the presence of an acute phase response but also herald the development of a cytokine storm and, consequently, multi-organ failure and an increased risk of adverse outcomes. Two systematic reviews and meta-analyses on a relatively limited number of studies, three and five,

respectively, have reported a significant and positive association between SAA concentrations and COVID-19 severity (Akbari et al., 2020, Zeng F. et al., 2020). Following the publication of several additional studies, we conducted an updated systematic review and meta-analysis of the available evidence on the clinical implications of SAA concentrations in patients with COVID-19.

Materials and methods

Search strategy, eligibility criteria and study selection

We conducted a systematic search, using the terms "serum amyloid A" or "SAA" and "coronavirus disease 19" or "COVID-19", in the electronic databases PubMed, Web of Science, and Scopus, between January 2020 and December 2020, for studies investigating SAA concentrations in COVID-19 patients according to disease severity or survival status. The references of the retrieved articles were also searched to identify additional studies. Inclusion criteria were the following: (a) studies reporting continuous data on SAA concentrations in COVID-19 patients, (b) articles investigating COVID-19 patients with different disease severity or survival status, (c) adult patients, (d) **≥10 participants, (e)** English language, and (**f**) full-text available. Two investigators independently screened the abstracts. If relevant, the two investigators independently reviewed the full articles. We used the Newcastle-Ottawa scale to assess the quality of each study, with a score ≥6 indicating high quality (Wells et al., 2013).

Statistical analysis

Standardized mean differences (SMD) were calculated to build forest plots of continuous data and to evaluate differences in SAA concentrations between COVID-19 patients with low vs. high disease severity or survivors vs. non-survivors during follow up. A P-value of less than 0.05 was considered statistically significant, and 95% confidence intervals (CIs) were reported. When studies reported concentrations as median and interquartile range (IQR) the mean and standard deviation

were estimated as previously described (Wan et al., 2014). The Q-statistic was used to test heterogeneity of SMD across studies (the significance level was set at $P<0.10$). Inconsistency across studies was evaluated using the I^2 statistic where $I^2<25\%$ indicated no heterogeneity, I^2 between 25-50% moderate heterogeneity, I^2 between 50-75% large heterogeneity, and $I^2>75\%$ extreme heterogeneity (Bowden et al., 2011, Higgins and Thompson, 2002). A random-effects model was used to calculate the pooled SMD and the corresponding 95% CIs in presence of significant heterogeneity. Sensitivity analyses were conducted to assess the influence of individual studies on the overall effect size using the leave-one-out method (Tobias, 1999). The presence of publication bias was assessed using the of Begg's adjusted rank correlation test and the Egger's regression asymmetry test at the $P<0.05$ level of significance (Begg and Mazumdar, 1994, Sterne and Egger, 2001). The Duval and Tweedie "trim and fill" procedure was also used to investigate the effect of publication bias. This method recalculates a pooled SMD by incorporating the hypothetical missing studies as though they actually existed, to augment the observed data so that the funnel plot is more symmetric (Duval and Tweedie, 2000). Statistical analyses were performed using Stata 14 (STATA Corp., College Station, TX, USA). The study was fully compliant with the PRISMA statement regarding the reporting of systematic reviews and meta-analyses (Liberati et al., 2009).

Results

Literature search and study selection

A flow chart describing the screening process is presented in Figure 1. We initially identified 256 studies. A total of 230 studies were excluded because they were either duplicates or irrelevant. After a full-text revision of the remaining 26 articles, seven were excluded because they did not meet the inclusion criteria. Thus, nineteen studies, all conducted in China, were included in the meta-analysis (Table 1) (Chen M. et al., 2020, Chen R. et al., 2020, Cheng et al., 2020, Dong et al., 2020, Fu et al., 2020, Li et al., 2020, Li and Chen, 2020, Liu J. et al., 2020, Liu Q. et al., 2020, Liu S. L. et al.,

2020, Mo et al., 2020, Wang et al., 2020, Xu et al., 2020, Yang et al., 2020, Yu et al., 2020, Zeng Z. et al., 2020, Zhang J. J. et al., 2020, Zhang Q. et al., 2020, Zhao et al., 2020). A total of 5,617 COVID-19 patients were studied, 3,723 (49% males, mean age 53 years) with low disease severity or alive during follow-up and 1,894 (63% males, mean age 64 years) with high severity or not surviving during follow up. Fifteen studies were retrospective (Chen R. et al., 2020, Cheng et al., 2020, Dong et al., 2020, Fu et al., 2020, Li et al., 2020, Liu J. et al., 2020, Liu Q. et al., 2020, Liu S. L. et al., 2020, Wang et al., 2020, Xu et al., 2020, Yang et al., 2020, Yu et al., 2020, Zeng Z. et al., 2020, Zhang Q. et al., 2020, Zhao et al., 2020), one was prospective (Li and Chen, 2020), whilst no information was available in the remaining three (Chen M. et al., 2020, Mo et al., 2020, Zhang J. J. et al., 2020). Endpoints included disease severity, based on current clinical guidelines (16 studies) (Dong et al., 2020, Fu et al., 2020, Li et al., 2020, Li and Chen, 2020, Liu J. et al., 2020, Liu Q. et al., 2020, Liu S. L. et al., 2020, Mo et al., 2020, Wang et al., 2020, Xu et al., 2020, Yang et al., 2020, Yu et al., 2020, Zeng Z. et al., 2020, Zhang J. J. et al., 2020, Zhang Q. et al., 2020, Zhao et al., 2020) or the occurrence of acute respiratory distress syndrome (ARDS, one study) (Chen M. et al., 2020), and survival status (two studies) (Chen R. et al., 2020, Cheng et al., 2020).

Meta-analysis

The overall SMD in SAA concentrations between COVID-19 patients with low vs. high severity or survivors vs. non-survivors is shown in Figure 2. In 18 studies, patients with high severity or non-surviving status had higher SAA concentrations when compared to those with low severity or alive during follow up (mean difference range, 0.08 to 3.40) (Chen M. et al., 2020, Chen R. et al., 2020, Dong et al., 2020, Fu et al., 2020, Li et al., 2020, Li and Chen, 2020, Liu J. et al., 2020, Liu Q. et al., 2020, Liu S. L. et al., 2020, Mo et al., 2020, Wang et al., 2020, Xu et al., 2020, Yang et al., 2020, Yu et al., 2020, Zeng Z. et al., 2020, Zhang J. J. et al., 2020, Zhang Q. et al., 2020, Zhao et al., 2020), although the difference was non statistically significant in two studies (Zeng Z. et al., 2020, Zhang J. J. et al., 2020). By contrast, in the remaining study, SAA concentrations were

slightly higher in patients with low severity or alive during follow up (mean difference -0.33) (Cheng et al., 2020). The pooled results confirmed that SAA concentrations were significantly higher in patients with high severity or non-surviving status (SMD 1.20, 95% CI 0.91 to 1.49, P<0.001) (Figure 2). Extreme heterogeneity between studies was observed (I^2=92.4%, P<0.001). SAA concentrations remained significantly higher (SMD 1.25, 95% CI 0.87 to 1.63, P=0.001; I^2=92.7%, P<0.001) in patients with high severity or non-survival status after excluding the large study by Yu et al (Yu et al., 2020), which accounted for nearly 58% of the overall sample size.

Sensitivity analysis, performed by removing each study in turn and re-assessing the pooled estimates, showed that the magnitude and direction of the effect size were not affected (effect size ranged between 1.10 and 1.29) (Figure 3). The Begg's (P=0.03), but not the Egger's (P=0.385), test revealed the presence of publication bias. Accordingly, the trim-and-fill method identified four potential missing studies to add on the left side of the funnel plot to ensure symmetry (Figure 4). The adjusted SMD was attenuated but remained significant (SMD 0.89, 95% CI 0.59 to 1.20, P=0.001).

To explore possible contributors to the between-study variance, we investigated the effects of age, gender, end point studied, study design (retrospective or prospective), aspartate aminotransferase (AST), alanine aminotransferase (ALT), D-Dimer (DD), serum creatinine (Cr), pro-thrombin time (PT), and the inflammation biomarkers C-reactive protein (CRP) and white blood cell (WBC) count on SMD by univariate meta-regression analysis. Both gender (t=2.20, P=0.047) and AST values (t=3.44, P=0.014) were significantly and positively associated with the pooled SMD (Figure 5). By contrast, no significant correlations were observed between SMD and age (t=0.58, P=0.57), end point (t=-1.73, P=0.10), study design (t=0.87, P=0.40), ALT (t=-0.15, P=0.88), DD (t=-0.45, P=0.47), Cr (t=-0.31, P=0.76), PT (t=0.46, P=0.67), CRP (t=0.86, P=0.40) and WBC (t=-0.53, P=0.61).

Sub-group analysis showed that, in the 16 studies in which patients were characterized according to disease severity, the SMD was significantly higher in severe vs. mild disease patients (SMD 1.39, 95% CI 1.09 to 1.69, P=0.001), albeit with extreme between-study variance (I^2=90.3%, P<0.001). Similar results were obtained when considering the 15 retrospective studies (SMD 1.10, 95% CI 0.81 to 1.39, P=0.001), again in the presence of extreme heterogeneity (I^2=90.9%; P<0.001).

Discussion

In our systematic review and meta-analysis, SAA concentrations were significantly higher in COVID-19 patients with more severe disease, assessed on clinical grounds or based on the presence of ARDS, and in those who did not survive during follow up when compared to patients with milder forms of the disease or those who survived during follow up. The observed SMD, 1.20, suggest an effect size that is both biologically and clinically relevant (Cohen, 1988). There was extreme between-study heterogeneity however in sensitivity analysis the overall effect size was not significantly influenced when individual studies were in turn removed. Our analyses, based on the Begg's, but not the Egger's, test revealed the presence of publication bias. Although the trim-and-fill method identified four potential missing studies to add on the left side of the funnel plot to ensure symmetry the adjusted SMD was attenuated but remained significant. The SMD was significantly and positively associated with gender and AST but not with age, end point studied (disease severity, ARDS, or survival status), study design (retrospective or prospective), ALT, DD, Cr, PT, CRP and WBC.

Two previously published systematic reviews and meta-analyses identified a relatively low number of studies investigating SSA in COVID-19 patients. The first, in three studies, reported a weighted mean difference (WMD) between severe and non-severe patients of 43.35 (95% CI 5.85 to 80.85, P=0.020; I^2=66.7%, P=0.050) (Zeng F. et al., 2020). The second, in five studies, reported a WMD of 90.45 (95% CI 28.69 to 152.21; I^2=90.6%, P<0.0001) between severe and non-severe patients

(Akbari et al., 2020). In both meta-analyses, the small number of studies prevented the conduct of meta-regression analysis to identify clinical and demographic factors potentially accounting for the between-study variance. It is important to highlight that, in contrast with these meta-analyses, we evaluated the effect size by using the SMD instead of the WMD. As previously highlighted by other authors, the SMD is particularly appropriate when different studies investigate separate end points, in our case disease severity and mortality (Faraone, 2008).

Increases in SAA concentrations can be useful to diagnose inflammatory processes and monitor the response to therapeutic interventions (Zhang et al., 2019). The magnitude and the speed of the increase in SAA concentrations during the acute phase response is greater than that observed with other inflammatory markers, particularly the CRP. Moreover, SAA concentrations generally return to baseline levels more quickly than the CRP in virtue of the shorter half-life of the former. These characteristics suggest a specific pathophysiological role of SAA, which might complement that provided by other biomarkers, e.g., CRP, in clinical practice (Maury, 1985, Nakayama et al., 1993, Takata et al., 2011). This proposition is further confirmed by the lack of significant associations between the SMD and CRP concentrations in our meta-regression, indicating the potential complementary role of SAA and CRP in informing clinical decisions. An increase in SAA concentrations has been observed in several pro-inflammatory conditions, including infections, particularly viral (Kajiya et al., 2008), liver disease (Siegmund et al., 2016), autoimmune disease (O'Hara et al., 2000), diabetes (Kumon et al., 1994), obesity (O'Brien et al., 2005), atherosclerotic cardiovascular disease (King et al., 2011), amyloidosis (Real de Asua et al., 2014), and cancer (Biran et al., 1986). Notably, most of these conditions, particularly obesity, diabetes, cardiovascular disease, liver disease, and cancer, have also been independently associated with significantly worse outcomes in patients with COVID-19 (Zhou et al., 2020). In addition to its potential role in the pathogenesis of the cytokine storm, it has been recently reported that SAA might also exert pro-coagulant effects that are mediated by an increase in fibrinogen and a concomitant platelet activation to generate a pro-thrombotic state (Page et al., 2019). Therefore, pending further

research, acute increases in SAA concentrations might represent an important factor linking pro-inflammatory and pro-thrombotic pathways. The interplay between inflammation and thrombosis has also been observed in COVID-19, a condition that is often characterized, particularly in patients with the more severe form of the disease, by significant alterations in coagulation and a pro-thrombotic state (Al-Samkari et al., 2020).

The extreme between-study heterogeneity and the presence of publication bias represent potential limitations in our study. However, the overall effect size was not significantly influenced in sensitivity analyses. The lack of significant associations between clinical and demographic characteristics, barring gender and AST, and SMD and the persistently high heterogeneity observed in sub-group analyses suggest that other unreported factors might contribute to heterogeneity. Few studies have investigated the association between SAA concentrations and gender. No significant differences between males and females have been reported in healthy populations (Carbone et al., 2020). In another study, the percent fat mass and the waist-to-hip ratio explained the highest percentage of the variability in SAA concentrations in females and males, respectively (Thorand et al., 2006). While no studies have specifically investigated the associations between SAA concentrations and AST this relationship might be explained, at least in part, by the involvement of this acute phase reactant in several conditions associated with liver injury (Yuan et al., 2019). Potential, unreported, methodological factors contributing to the observed between-study heterogeneity involve the use of different SAA detection methods, based on immuno-based assays, different antibodies against various SAA components, and different calibrators in individual studies (Zhang et al., 2019). While some of these methodological inconsistencies might have been mitigated by the fact that all the identified studies were conducted in the same country this issue should be addressed when planning future multicenter studies on the clinical use of SAA in patients with COVID-19 or other disease states. **Another limitation is represented by the paucity of data in the selected studies on the dynamic changes in SAA concentrations in hospitalized COVID-19 patients, and their associations with disease severity and/or mortality. Notably, in the five**

studies that addressed this issue, serial SAA concentrations remained high, or increased during hospitalization, in COVID-19 patients that experienced adverse clinical outcomes (Chen R. et al., 2020, Cheng et al., 2020, Fu et al., 2020, Liu S. L. et al., 2020, Yu et al., 2020). However, the different time-points for SAA measurement used in individual studies precluded their meta-analysis. Further research is warranted to better characterize and justify the routine clinical use of serial SAA assessments in COVID-19.

In conclusion, our updated systematic review and meta-analysis has shown that the presence of relatively high SAA concentrations is significantly associated more severe disease, based on clinical assessment or presence of ARDS, and increased risk of mortality in patients with COVID-19. The measurement of this acute phase reactant, singly or in combination with other clinical and demographic parameters, might be useful for risk stratification and clinical monitoring in this group.

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Ethical approval

This study did not require ethical approval as it was a systematic review and meta-analysis of published studies.

Conflict of interest

The authors declare no conflict of interest.

Declaration of interests

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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NA

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Figure 1. Flow chart of study selection.

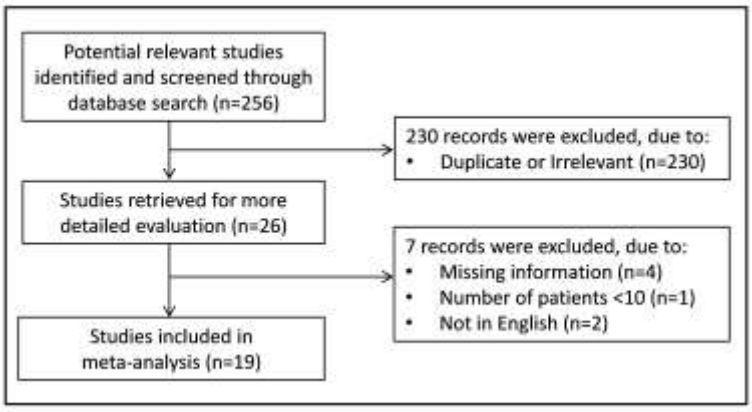

Figure 2. Forest plot of studies examining serum amyloid A concentrations in patients with COVID-19.

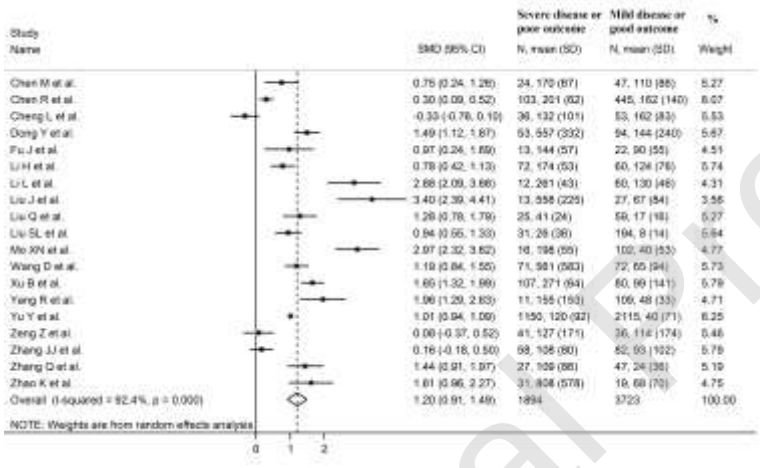

Figure 3. Sensitivity analysis of the association between serum amyloid A concentrations and COVID-19 disease. The influence of individual studies on the overall standardized mean difference (SMD) is shown. The middle vertical axis indicates the overall SMD and the two vertical axes indicate the 95% confidence intervals (CI). The hollow circles represent the pooled SMD when the remaining study is omitted from the meta-analysis. The two ends of each broken line represent the 95% confidence intervals.

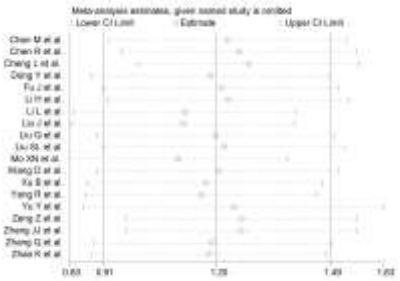

Figure 4. Funnel plot of studies investigating low vs. high severity or survivor vs. non-survivor status after trimming and filling. Dummy studies and genuine studies are represented by enclosed circles and free circles, respectively.

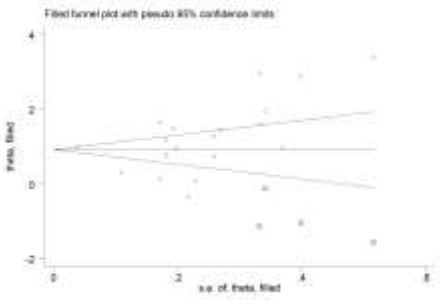

Figure 5. Univariate meta-regression analysis between gender, aspartate aminotransferase, and effect size.

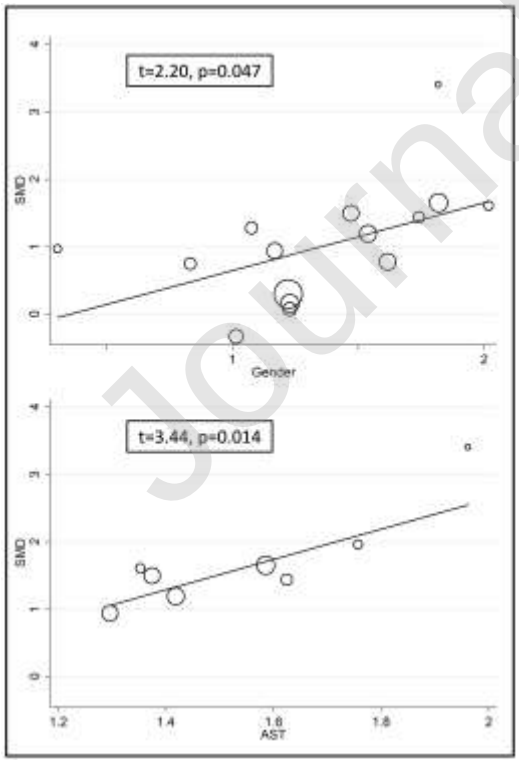

Table 1. Summary of the studies included in the meta-analysis

				Mild disease or survivor				Severe disease or non-survivor			
First Author	**Study design**	**Endpoint**	**NOS** (stars)	**n**	**Age** Years (Mean)	**Gender** (M/F)	**SAA** mg/L (Mean ±SD)	**n**	**Age** Years (Mean)	**Gender** (M/F)	**SAA** mg/L (Mean ±SD)
Chen M et al. (Chen M. et al., 2020)	NR	ARDS Non-ARDS	6	47	42	24/23	110±86	24	57	20/4	170±67
Chen R et al. (Chen R. et al., 2020)	R	Survivor Non-survivor	7	445	64	244/201	162±140	103	65	69/34	201±62
Cheng L et al. (Cheng et al., 2020)	R	Survivor Non-survivor	6	53	54	29/24	162±83	36	69	20/16	132±101
Dong Y et al. (Dong et al., 2020)	R	Severe Non-severe	7	94	40	34/60	144±240	53	60	29/24	554±332
Fu J et al. (Fu et al., 2020)	R	Severe Non-severe	7	22	41	11/11	90±55	13	60	2/11	144±57
Li H et al. (Li et al., 2020)	R	Severe Non-severe	7	60	57	28/32	124±76	72	66	47/25	174±53
Li L et al. (Li and	P	Severe Non-severe	5	60	52	NR	130±46	12	45	NR	261±43

Chen, 2020)											
Liu J et al. (Liu J. et al., 2020)	R	Severe Non-severe	7	27	43	8/19	67±84	13	60	7/6	558±225
Liu Q et al. (Liu Q. et al., 2020)	R	Severe Non-severe	6	59	49	31/28	17±16	25	52	14/11	41±24
Liu SL et al. (Liu S. L. et al., 2020)	R	Severe Non-severe	6	194	43	103/91	8±14	31	64	14/17	26±38
Mo XN et al. (Mo et al., 2020)	NR	Severe Non-severe	5	102	NR	NR	40±53	16	NR	NR	198±55
Wang D et al. (Wang et al., 2020)	R	Severe Non-severe	6	72	44	29/43	65±94	71	65	44/27	561±583
Xu B et al. (Xu et al., 2020)	R	Severe Non-severe	7	80	56	30/50	99±141	107	66	73/34	271±64
Yang R et al. (Yang et al., 2020)	R	Severe Non-severe	6	109	NR	NR	48±33	11	NR	NR	155±153
Yu Y et al. (Yu et al., 2020)	R	Severe Non-severe	7	2,115	NR	NR	40±71	1,150	NR	NR	120±92
Zeng Z et al.	R	Severe Non-severe	6	36	54	20/16	114±174	41	62	28/13	127±171

(Zeng Z. et al., 2020)											
Zhang JJ et al. (Zhang J. J. et al., 2020)	NR	Severe Non-severe	6	82	52	38/44	93±102	58	64	33/25	108±80
Zhang Q et al. (Zhang Q. et al., 2020)	R	Severe Non-severe	7	47	61	18/29	24±36	27	72	18/9	109±86
Zhao K et al. (Zhao et al., 2020)	R	Severe Non-severe	6	19	49	7/12	68±70	31	60	23/8	808±578

ARDS: acute respiratory distress syndrome; Non-severe: patients with mild or moderate disease; NOS: Newcastle-Ottawa quality assessment scale for case-control studies; NR: Not reported; P: prospective; R: retrospective; Severe: patients with severe or critical disease.