ORIGINAL ARTICLE

Coagulation and Cytokine Profiles as Predictive Biomarkers in Comorbidities Associated With Severe Covid-19

Nazanin Talebabadi[1], Eusni Rahayu Mohd. Tohit[1], Maha Abdullah[1], Syafinaz Amin Nordin[2], Irmi Zarina Ismail[3], and Masriana Hassan[1]

[1] Department of Pathology, Faculty of Medicine and Health Science, Universiti Putra Malaysia, 43400 Serdang, Selangor, Malaysia.
[2] Department of Medical Microbiology, Faculty of Medicine and Health Science, Universiti Putra Malaysia, 43400 Serdang, Selangor, Malaysia.
[3] Department of Family Medicine, Faculty of Medicine and Health Science, Universiti Putra Malaysia, 43400 Serdang, Selangor, Malaysia.

ABSTRACT

Background: COVID-19 patients with comorbidities are more susceptible to severe progression and high mortality, often due to coagulopathy and cytokine storm leading to complications like thrombosis, pulmonary embolism, and disseminated intravascular coagulation. This study aims to identify predictive factors for severe COVID-19 in Malaysian patients with comorbidities by evaluating coagulation and cytokine biomarkers. **Methods:** In this retrospective case-control study, blood samples from COVID-19 patients with (high-risk) and without (low-risk) comorbidities (e.g. diabetes, hypertension, and obesity) were evaluated for coagulation and cytokine levels. Non-COVID-19 subjects as the control group were low-risk (age between 18 to 40 without comorbidities) and high-risk (age ≥65 with comorbidities) individuals. **Results:** Of 66 COVID-19 patients in this study, 79% (n=52) were classified as severe or critical stages, and 59% (n=39) were identified as high-risk individuals. Fibrinogen, D-dimer, tissue plasminogen activator (TPA), and CXCL10, which elevated in the high-risk non-COVID-19 subgroup were found to be further increased in high-risk COVID-19 patients. Significant increases in D-dimer levels and cytokines including IL-1β, IL-6, IL-7, IL-10, G-CSF, TNF-α, CCL2, CCL3, and CXCL10 were observed in the high-risk subgroup of COVID-19 patients compared to the low-risk subgroup. Moreover, regression analysis identified several predictors of severe COVID-19 outcomes, including the presence of comorbidities (β=1.908, CI=1.430-031.773, p=0.016) as well as elevated IL-2 (β=2.515, CI=0.249-0.390, p=0.018), TNF-α (β=2.829, CI=0.000-0.046, p=0.049) and IL-1β (β=1.629, CI=-0.049-0.012, p=0.019), suggesting their potential as predictive biomarkers for severe COVID-19. **Conclusion:** Alteration of coagulation and cytokine levels in COVID-19 patients with comorbidities predispose them to developing severe COVID-19 outcomes.
Malaysian Journal of Medicine and Health Sciences (2025) 21(SUPP12): 3-10.doi:10.47836/mjmhs.21.s12.2

Keywords: Coagulation, Comorbidities, COVID-19, Cytokine, Inflammation.

Corresponding Author:
Masriana Hassan, PhD
Email: masriana@upm.edu.my
Tel: +603-97692381

Introduction

Severe coronavirus disease 2019 (COVID-19) is often associated with significant coagulation abnormalities which increase the risk for deep vein thrombosis (DVT) and pulmonary embolism (PE) (1). The mechanism by which COVID-19 leads to coagulopathy is via infection of severe acute respiratory syndrome *coronavirus* 2 (SARS-CoV-2) to endothelial cells that increase vascular permeability and activation of the coagulation cascade (2). The virus also induces a robust proinflammatory cytokine production, which exacerbates coagulopathy through increased expression of tissue factor and release of microparticles that enhance clotting, and eventually lead to severe disease complications including acute respiratory distress syndrome (ARDS) and organ dysfunction (e.g. renal failure) (3).

Elderly patients (aged ≥65 years) and those with underlying comorbidities such as cardiovascular diseases, diabetes, cancer, obesity (body mass index [BMI] ≥30), and chronic lung disease are significant risk factors for developing severe complications of COVID-19 (4). The rapid progression to severe COVID-19 in patients with comorbidities could be due to pre-existing low-grade inflammation, which is seen in obese, diabetic, and hypertensive patients (5) . High-level secretion of proinflammatory cytokines in these patients could also exacerbate the disease complication leading to severe infection accompanied by coagulopathy and cytokine storm (6,7).

Evaluating coagulation and cytokine biomarkers associated with severe COVID-19 may contribute to

early detection and prediction of disease complications. In the current study, we aimed to investigate the predictive coagulation and cytokine markers through the evaluation of coagulation and cytokine profiles in Malaysian COVID-19 and non-COVID-19 patients in association with comorbidities and severe outcomes of COVID-19.

Materials and Methods

Study design and participant recruitment

This retrospective case-control study was carried out in accordance with the Declaration of Helsinki and approved by the Ethics Committee of Universiti Putra Malaysia (UPM) (JKEUPM-2021-859). The archived COVID-19 samples (N=66) from hospitalized patients between June and August 2021 were obtained from the Laboratory of Microbiology, Hospital Sultan Abdul Aziz Shah (HSAAS). A qualitative reverse transcriptase polymerase chain reaction (qRT-PCR) test was performed upon hospital admission to confirm COVID-19 positivity (data not shown). Disease severity classification was based on COVID-19 Management Guidelines in Malaysia No. 5/2020 (Supplementary Table 1) (6). Only clinical stage three to five COVID-19 patient's samples were collected. The COVID-19 patient's information and medical records (i.e. age, gender, underlying, health condition, COVID-19 clinical stage, length of ward or ICU stay) were obtained from the electronic database of the hospital management information system.

Table I: Comparison of demographic and clinical data of COVID-19 and non-COVID-19 subjects

	Non-COVID-19			COVID-19		
	Low-Risk	High-Risk	p-value	Low-Risk	High-Risk	p-value
Gender, n (%)						
Male	41 (50)	41 (50)	ns	20 (83.3)	28 (66.7)	0.024*
Female	41 (50)	41 (50)	ns	4 (16.7)	14 (33.3)	0.049*
Age	23.34±5.24	67.15±5.46	0.0001*	52.66±13.32	51.72±15.59	0.382
BMI	21.71±2.98	26.91±3.53	0.0001*	26.95±3.05	34.73±7.63	0.003*
Comorbidities, n (%)						
Diabetes	-	82 (100)	-	-	24 (57.14)	-
Hypertension	-	46 (56.09)	-	-	24 (57.14)	-
Obesity	-	8 (9.75)	-	-	11 (16.66)	-
COVID-19 Clinical stage, n (%)						
Three	-	-	-	11 (45.8)	3 (7.14)	0.028*
Four	-	-	-	12 (50.0)	28 (66.6)	0.046*
Five	-	-	-	1 (4.16)	11 (26.19)	0.005*
Hospitalization (days)	-	-	-	17.58±12.47	17.58±8.82	0.524
Admission, n (%)						
ICU	-	-	-	9 (37.5)	14 (33.3)	0.113
Ward	-	-	-	15 (62.5)	28 (66.7)	0.113

Non-COVID-19 low-risk (age 18-40 years old with no comorbidities) and high-risk (age >60 years old with comorbidities i.e. diabetes, hypertension or obesity). COVID-19 low-risk (no comorbidities) and high-risk (with comorbidities). *p<0.05 indicates a significant difference; ns: not significant analysed by Chi-square (χ^2) test, BMI: body mass index, ICU: intensive care unit.

The non-COVID-19 subjects (N=164) were divided further into low-risk (without comorbidities) and high-risk (with comorbidities) groups. Both male and female low-risk non-COVID-19 subjects were selected among students and workers (aged between 18 to 40) at the Faculty of Medicine and Health Sciences, UPM while high-risk non-COVID-19 subjects aged ≥65 years old with underlying comorbidities (e.g. type 2 diabetes mellitus [T2DM], hypertension and/or obesity) were recruited from those attending the Family Medicine Clinic (FMC), HSAAS. The demographic and clinical information of each respondent including gender, BMI, age, and underlying condition were also recorded. Sample size n was calculated according to the previously described formula (8),

$$\text{Sample size (n)} = \frac{r+1}{r} \frac{(p^*)(1-p^*)(z_\beta + z_{\alpha/2})^2}{(p_1 - p_2)^2}$$

where r is the ratio of control to case, p* is the estimated proportion of severe COVID-19 = 0.4, Zβ is the standard normal deviate corresponding to 80% power = 0.84, Z/2 is the standard normal deviation 95% confidence interval (CI) = 1.96, p1 is the proportion in severe COVID-19 =0.6, and p2 is the proportion in control = 0.2.

Exclusion criteria

COVID-19 patients in clinical stage of one and two were excluded. Non-COVID-19 individuals that pregnant, had any infection within two weeks prior to blood collection, and taking medication including warfarin or direct oral anticoagulants (DOACS) - dabigatran

(Pradaxa), rivaroxaban (Xarelto), apixaban (Eliquis), edoxaban (Savaysa), and betrixaban (Bevyxxja) were excluded from the study. In addition, non-COVID-19 low-risk individuals with any underlying condition were also excluded from the study.

Sample collection

Whole blood (~ 12 ml) of non-COVID-19 was collected in 3.2% sodium citrate and plain tubes. For serum preparation, the blood in the plain tube was allowed to clot followed by centrifugation at 2500 rpm for 10 min. All serum samples were transferred into micro tubes and stored at -80°C until further use. On the other hand, the blood samples in the 3.2% sodium citrate tubes were immediately centrifuged at 2800 rpm for 10 min to obtain plasma.

Coagulation marker measurements

The freshly isolated plasma of non-COVID-19 samples were immediately acquired on an automated STA Compact (Stago, France) coagulometer for prothrombin time (PT), activated partial thromboplastin time (aPTT), fibrinogen and D-dimer using a Stago-STA kit reagents (Stago, France). Results of PT, aPTT, PAI-1, fibrinogen and D-dimer of COVID-19 patients were obtained from the electronic database of the hospital management information system. On the other hand, the levels of plasminogen activator inhibitor 1 (PAI 1), tissue plasminogen activator (TPA), thrombin activatable fibrinolysis inhibitor (TAFI) in the serum samples of both COVID-19 and non-COVID-19 were assessed by enzyme-linked immunosorbent assay (ELISA) using a fully automated Elisys Quattro (Germany) with commercial kits (Abcam, UK) following manufacturer's instructions.

Cytokine quantification

A LEGENDplex™ Human Inflammation Panel 1 kit (BioLegend, USA) was used for the quantification of IL-7, CXCL10, IL-2, CCL2, IL-10, TNF-, IL-6, CCL3, IL-1β, and G-CSF in serum samples and acquired on a BD FACSCanto II flow cytometer (Becton Dickinson, USA). The analysis was performed by LEGENDplex data analysis software.

Statistical analysis

The SPSS software V.26 was used for data analysis. Results were expressed as the mean ± standard deviation (SD) for continuous variables and categorical variables stated as number (n) and percentage (%). Shapiro-Wilk test was used to examine the normality of data. The chi-square or Fisher's exact test was used for categorical variables to compare the significant differences among groups. Continuous data with normal distribution was analysed by one-way analysis of variance (ANOVA) and an unpaired T-test, and the Kruskal-Wallis was used to analyse data with a non-normal distribution. Linear and logistic regression analyses were used to determine the factors that predict severe COVID-19 infection.

RESULTS

Demographic and clinical data

The demographic and clinical data are reported in Table 1. The low-risk and high-risk subjects of non-COVID-19 groups comprised an equal number of males and females (n=41 per group). The mean age of low-risk and high-risk COVID-19 patients, however, was not significantly different (52.66±13.32 and 51.72±15.59, p=0.382). The non-COVID-19 high-risk individuals also showed significantly higher BMI levels than the low-risk group (p=0.0001). As pre-selected, all non-COVID-19 high-risk individuals had diabetes (100%), and some of them also had other underlying conditions such as hypertension (56.09%) and obesity (9.75%).

Among the 66 COVID-19 patients, males constituted 83.3% (p = 0.002) of the low-risk group and 66.7% (p = 0.008) of the high-risk group, both significantly higher than the corresponding female proportions. In contrast, females comprised 16.7% of the low-risk group and 33.3% of the high-risk group (p = 0.049). Notably, the proportion of high-risk males (66.7%) was significantly lower (p = 0.024) than that of low-risk males (83.3%). However, these findings should be interpreted with caution due to the small number of female COVID-19 patients. The BMI of COVID-19 patients was significantly higher (p=0.003) in the high-risk (34.73±7.63) than in the low-risk (26.95±3.05) group. The majority of high-risk COVID-19 patients suffer from comorbidities including diabetes (57.14%), hypertension (57.14%), and/or obesity (16.66%). Importantly, the majority of these COVID-19 patients suffered from severe (clinical stage four) and critical (clinical stage five) stages of the disease compared to the low-risk COVID-19 patients. On the other hand, there was no significant difference in hospitalisation days (p=0.525) and admission to the ward (62.5% of low-risk and 66.7% of high-risk; p=0.113) or intensive care unit (ICU) (37.5% of low-risk and 33.3% of high-risk; p=0.113) between low- and high-risk COVID-19 patients.

Comparison of coagulation and cytokine profiles between low- and high-risk of both non-COVID-19 and COVID-19 patients

The coagulation and cytokine profiles between non-COVID-19 and COVID-19 patients are presented in Fig. 1. Within the non-COVID-19 groups, coagulation biomarkers, fibrinogen, D-dimer, TAFI, and TPA were significantly higher in the high-risk subgroup compared to the low-risk subgroup (Fig. 1A). In contrast, within the COVID-19 groups, only D-dimer levels were significantly higher in high-risk subgroup than in low-risk subgroup. Cross-group comparisons revealed that Fibrinogen, D-dimer, PT, PAI-1, and TPA levels were significantly elevated in high-risk COVID-19 patients compared to non-COVID-19 high-risk subgroup. Additionally, fibrinogen, D-dimer, PT, and PAI-1 were

significantly higher in low-risk COVID-19 patients compared to low-risk non-COVID-19 subgroup.

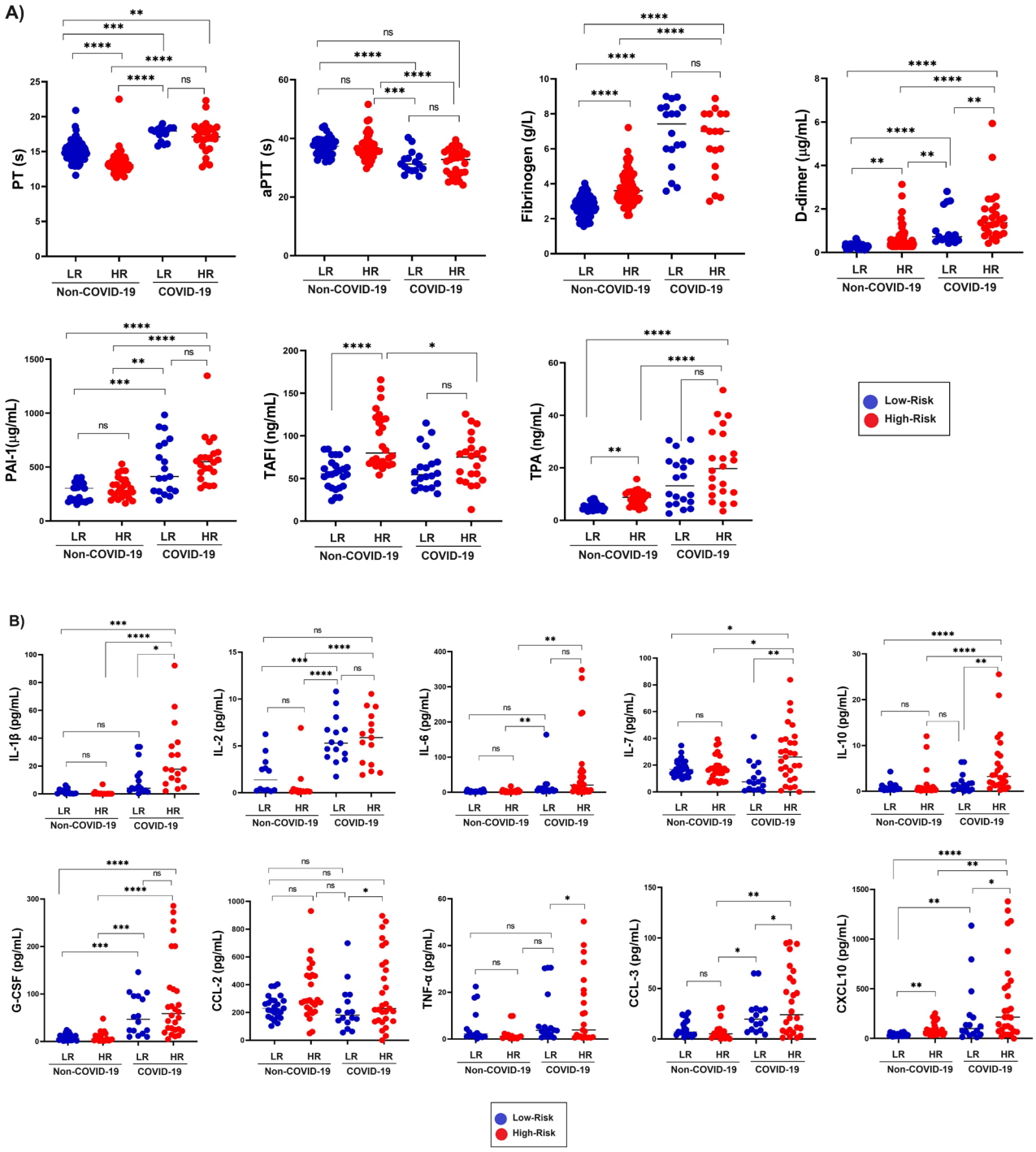

Fig 1: Coagulation (A) and cytokine (B) profiles in low-risk (LR; without comorbidities) and high-risk (HR; with comorbidities) of both non-COVID-19 and COVID-19 patients. *p<0.05, **p<0.01, ***p<0.001, and ****p<0.0001 indicate a significant difference and ns indicates non-significant.

The cytokine profiles (Fig. 1B) showed a significant increase in CXCL10 in high-risk compared to low-risk individuals within the non-COVID-19 group. In the COVID-19 group, levels of IL-1β, IL-7, IL-10, TNF-, CCL-2, CCL-3, and CXCL10 were all significantly higher in high-risk subgroup than in the low-risk subgroup. Cross-group analysis further demonstrated that IL-1β, IL-2, IL-7, IL-10, G-CSF, CCL-3, and CXCL10 were significantly elevated in high-risk COVID-19 patients compared to the high-risk subgroup of COVID-19. In addition, IL-2, G-CSF, and CXCL10 were significantly higher in low-risk COVID-19 patients compared to low-risk non-COVID-19 subgroup.

Association of comorbidities with severe COVID-19

The binary logistic regression analysis showed that patients with underlying comorbidities (β=1.908, CI=1.430-031.773, p=0.016) potentially developed severe COVID-19 compared to low-risk patients (Table 2).

Table II. Logistic regression analysis for the determination of the predictive value of comorbidities in severe COVID-19

		β	S.E.	Wald	df	95% CI for Exp(B)	e^{β}	p-value
Step 1[a]	Comorbidities (1)	1.908	0.791	5.819	1	1.430, 31.773	6.741	0.016*
	Constant	0.251	0.504	0.249	1		1.286	0.618

*p<0.05 indicates a significant difference

Predictive coagulation and cytokine markers for severe COVID-19

Multiple linear regression analysis revealed that IL-2 (β=2.515, CI=0.249-0.390, p=0.018), TNF- (β=2.829, CI=0.000-0.046, p=0.049) and IL-1β (β=1.629, CI=-0.049-0.012, p=0.019) had a significant association with severe outcomes of COVID-19, indicated as severe (clinical stage four) and critical (clinical stage five) conditions (Table 3).

Table III: Multiple linear regression analysis for the determination of predictive coagulation and cytokine biomarkers in the severe COVID-19

Marker	Standardized Coefficients β	95.0% CI	p-value
(Constant)		-1.232,15.599	0.082
PT	-0.322	-0.549, 0.290	0.479
aPTT	-0.092	-0.160, 0.132	0.822
TPA	0.187	-0.050, 0.069	0.712
PAI-1	-0.467	-0.005, 0.002	0.422
TAFI	0.099	0.000, 0.000	0.835
D-dimer	0.481	-0.273, 0.665	0.346
IL-1β	1.629	-0.049, 0.012	0.019*
IL-2	2.515	0.249, 0.390	0.018*
IL-6	-0.005	-0.007, 0.006	0.984
IL-7	-.0359	-0.020, 0.009	0.379
IL-10	0.625	-0.021, 0.144	0.118
CCL2	0.080	-0.001, 0.001	0.772
CCL3	-0.822	-0.050, 0.014	0.209
CXCL10	-0.185	-0.002, 0.002	0.751
G-CSF	0.186	-0.006, 0.010	0.640
TNF-α	2.829	0.000, 0.046	0.049*

*p<0.05 indicates a significant difference

Predictive coagulation and cytokine markers for severe COVID-19 admitted to ICU

There was previously shown that 9 (37.5%) out of 24 low-risk and 14 (33.3%) out of 42 high-risk COVID-19 were admitted to ICU. In multiple linear regression analysis, IL-2 (β=2.375, CI=0.138-0.227, p=0.011) and TNF- (β=2.080, CI=0.003-0.022, p=0.016) had a statistically significant association with admission of COVID-19 patients to ICU (Table 4).

Table IV: Multiple linear regression analysis for the determination of the predictive coagulation and cytokine biomarkers for the admission of COVID-19 patients to ICU

Marker	Standardized Coefficients β	95.0% CI	p-value
(Constant)		0.790, 7.789	0.024
PT	-0.411	-0.0300, 0.049	0.129
aPTT	-0.078	-0.069, 0.052	0.731
TPA	-0.441	-0.041, 0.008	0.148
PAI-1	-0.657	-0.003, 0.000	0.069
TAFI	0.251	0.000, 0.000	0.355
D-dimer	0.314	-0.098, 0.292	0.270
IL-1β	0.341	-0.005, 0.020	0.195
IL-2	2.375	0.138, 0.227	0.011*
IL-6	0.107	-0.002, 0.004	0.445
IL-7	-0.344	-0.010, 0.002	0.149
IL-10	0.006	-0.034, 0.035	0.975
CCL2	-0.066	-0.001, 0.000	0.666
CCL3	0.366	-0.007, 0.020	0.297
CXCL10	-0.037	-0.001, 0.001	0.907
G-CSF	0.359	-0.001, 0.006	0.134
TNF-α	2.080	0.003, 0.022	0.016*

*p<0.05 indicates a significant difference

DISCUSSION

Comorbidities including cardiovascular diseases, diabetes, hypertension, and obesity have been associated with the worst outcomes from COVID-19. In addition, older adults with an aging immune system may not respond as effectively to infections and vaccines, leading to a higher risk of complications. Our findings demonstrated a higher percentage of COVID-19 patients with underlying conditions and the majority were hospitalized and categorized as stage four and five which indicated severe complications of the disease. Notably, obesity was associated with high severity of COVID-19 infection (7). This condition is related to high hospitalization or mortality in COVID-19 patients and is considered a risk factor for severe infections (9). The present study compared only hospitalized low-risk and high-risk patients with clinical stage three to five of COVID-19 to non-COVID-19 with and without comorbidities in order to evaluate the potential severity of the disease across these groups. Therefore, no lower COVID-19 clinical stage patient's samples were collected. Our findings demonstrate a significant increase in BMI among the high-risk COVID-19 cohort, supporting obesity as a potential risk factor for severe COVID-19 outcomes. Similar to the results here, the prevalence of severe COVID-19 infection has been found in various studies to be higher in males than females (10). In addition, the mortality rate due to infections is also higher in males (4.8%) than in females (2.8%), which indicates different immune responses depending on gender (11) suggesting the importance of considering gender as a prognostic marker in COVID-19.

Coagulopathy and inflammation are key features of severe COVID-19 and contribute significantly to its complications. The interaction between these pathways has been particularly evident in COVID-19 patients with obesity (12). One of the important consequences of COVID-19 infection is thrombosis, which plays a central role in the high mortality of patients due to damage to vital vessels and organs (12). The association between coagulation biomarker levels in COVID-19 patients and non-COVID high-risk and healthy individuals was investigated. In the non-COVID-19 group, high-risk individuals exhibited significantly higher levels of TPA, TAFI, fibrinogen, and D-dimer compared to their low-risk counterparts. Within the COVID-19 groups, only D-dimer was significantly elevated in the high-risk compared to the low-risk patients. Elevated levels of D-dimer in COVID-19 patients reflects ongoing clot formation and breakdown and have been reported as a biomarker of severe disease (12, 13). Increased in the levels of D-dimer is associated with prolonged PT (14), a finding that was observed in the COVID-19 group in this study. Fibrinolysis involves the activation of plasmin production by TPA (15). Previous studies have shown that high levels of TPA, fibrinogen, TAFI, and PAI-1 are present in ICU-admitted COVID-19 patients and are associated with severe COVID-19 infection (16). On the other hand, reduced clot lysis in COVID-19 patients has been correlated with elevated PAI-1, which supresses plasmin production and promotes a hypofibrinolytic state (16). Alterations in PAI-1, TPA, and TAFI levels among high-risk individuals may therefore indicate impairment of the fibrinolytic system. Consequently, elevated levels of these coagulation markers could contribute to the development of fibrinolytic system disorder, predisposing patients with severe COVID-19 to thrombosis.

Severe inflammation observed in critically ill COVID-19 patients is associated with increased levels of certain inflammatory cytokines. For instance, high IL-7 levels have been associated with lymphopenia in COVID-19 patients (17). In the current study, overproduction of inflammatory cytokines and chemokines including IL-1β, IL-2, IL-6, IL-7, IL-10, CXCL10, CCL-3, and GCSF was observed in COVID-19 patients, suggesting a state of hyperinflammation. In addition, elevation of IL-2, IL-6, IL-7, and CXCL10 have been linked to adverse outcomes and poor prognosis in COVID-19 patients (18). Notably, increased IL-10, an anti-inflammatory cytokine, was also detected in COVID-19 patients, which may represent a compensatory mechanism aimed at suppressing excessive pro-inflammatory cytokine production (19).

One of the important findings of this study was the significant overexpression of cytokines and chemokine in high-risk COVID-19 patients compared to low-risk COVID-19 which indicated severe inflammatory responses in COVID-19 patients with comorbidities. This can be attributed to the pre-existing inflammatory condition in these patients, aggravating COVID-19 infection (20). For example, hyperglycaemia is a risk factor for modulating inflammatory responses and this has a central role in the high severity of disease or COVID-19-induced mortality (21). Moreover, both insulin resistance and reduction in insulin secretion by the pancreas can contribute to the high severity of infection (22). Inflammatory reactions following the COVID-19 infection have also led to a change in insulin sensitivity (23), associated with the intensification of inflammatory responses in these patients. High levels of IL-6 in high-risk COVID-19 patients compared to low-risk counterparts can indicate hyperinflammation in various body tissues, and it is found that this cytokine also plays a role in insulin resistance (24).

The pre-existing low-grade inflammation in patients with comorbidities may provide a favourable background for the occurrence of severe inflammatory responses when affected by COVID-19. The overexpression of pro-inflammatory cytokines such as TNF-α, IL-1β, and IL-8 are the predictors for survival of COVID-19 patients (25). In the current study, high levels of IL-1β, IL-6, TNF-α, and CCL2, CCL3, and CCL10 chemokines, as well as G-CSF

were monitored in the high-risk subgroup of COVID-19 patients, indicating the activation of inflammatory pathways and inflammatory cells such as macrophages, which provides the basis for the development of cytokine storm. In addition, high levels of these inflammatory factors are correlated with the ARDS in COVID-19 patients (26). Therefore, the high levels of cytokines and chemokines studied in the present study in high-risk COVID-19 patients indicated that comorbidities can be a risk factor for severe outcomes of this infection, as confirmed by the regression analysis.

Evaluating TNF-α, IL-1β, and MCP-1 levels is relevant for predicting COVID-19 mortality (27). Previous reports showed that these cytokine levels were significantly higher in non-survivors of COVID-19 than in survivors (27). In addition, high levels of IL-6 were related to the progression of the disease of COVID-19 (28). Del Valle et al. (2020) found that high serum levels of IL-8, IL-6, and TNF-α with a range of other inflammatory markers and comorbidities as significant predictors of disease severity and death (25). IL-2 and IL-1β immune responses are key factors influencing the outcomes of COVID-19 infection (29). The increase of IL-2 during COVID-19 leads to a severe inflammatory response and contributes to poor prognosis in immunocompromised patients. Upregulation of IL-2 in patients without comorbidities correlates with the clinical outcome of the disease and intensifies as disease severity increases (30). Our present study showed that IL-2, TNF-α, and IL-1β can predict the severity of the COVID-19 disease. Furthermore, it turned out that IL-2 and TNF-α can also predict ICU admission. This data suggests the association of comorbidities and cytokines with adverse outcomes and high severity of COVID-19.

The present study did not account for genetic variability, including single nucleotide polymorphisms (SNPs) and other genetic polymorphisms, which may influence host susceptibility to COVID-19, the magnitude of the inflammatory response, and disease progression. Variations in genes regulating immune responses, cytokine production, endothelial function, and coagulation pathways could contribute to inter-individual differences in clinical outcomes, even among patients with comparable comorbidities. Future studies integrating genomic profiling with biochemical and clinical parameters are warranted to elucidate the role of host genetic factors in modulating COVID-19 severity.

Conclusion

COVID-19 patients with comorbidities have altered levels of coagulation and inflammatory parameters, which make them more susceptible to the adverse consequences of this infection, such as coagulopathy and cytokine storm. Moreover, it turned out that IL-2, TNF-α, and IL-1β significantly associated with severe COVID-19 and could be potentially used for predicting the disease complications.

FUNDING

This research is funded by the Ministry of Higher Education Malaysia under the Fundamental Research Grant Scheme (FRGS/1/2021/SKK0/UPM/02/14).

REFERENCES

1. Poggiali E, Bastoni D, Ioannilli E, Vercelli A, Magnacavallo A. Deep Vein Thrombosis and Pulmonary Embolism: Two Complications of COVID-19 Pneumonia? Eur J Case Rep Intern Med [Internet]. 2020 Apr 8;7(5):1. Available from: https://www.ejcrim.com/index.php/EJCRIM/article/view/1646
2. Iba T, Levy JH, Levi M, Thachil J. Coagulopathy in COVID-19. Journal of Thrombosis and Haemostasis. 2020 Sep;18(9):2103–9.
3. Lin S hui, Zhao Y si, Zhou D xing, Zhou F chun, Xu F. Coronavirus disease 2019 (COVID-19): cytokine storms, hyper-inflammatory phenotypes, and acute respiratory distress syndrome. Genes Dis. 2020 Dec;7(4):520–7.
4. Nagy É, Cseh V, Barcs I, Ludwig E. The Impact of Comorbidities and Obesity on the Severity and Outcome of COVID-19 in Hospitalized Patients—A Retrospective Study in a Hungarian Hospital. Int J Environ Res Public Health. 2023 Jan 12;20(2):1372.
5. Pereira SS, Alvarez-Leite JI. Low-Grade Inflammation, Obesity, and Diabetes. Curr Obes Rep. 2014 Dec 23;3(4):422–31.
6. Ministry of Health Malaysia. COVID-19 Management Guidelines in Malaysia No.5/2020. [Internet]. Vol. (Online), COVID-19 Management Guidelines in Malaysia No.5/2020. 2020 [cited 2024 Nov 25]. Available from: Available from: https://covid-19.moh.gov.my/garis-panduan/garis-panduan-kkm
7. Kristensen NM, Gribsholt SB, Andersen AL, Richelsen B, Bruun JM. Obesity augments the disease burden in COVID-19: Updated data from an umbrella review. Clin Obes. 2022 Jun 8;12(3).
8. Charan J, Biswas T. How to Calculate Sample Size for Different Study Designs in Medical Research? Indian J Psychol Med. 2013 Apr 15;35(2):121–6.
9. Kondili LA, Quaranta MG, Viganò M, Tata X, D'Angelo F, Lo Noce C, et al. Obesity and Dysmetabolic Factors among Deceased COVID-19 Adults under 65 Years of Age in Italy: A Retrospective Case-Control Study. Viruses. 2022 Sep 7;14(9):1981.
10. Ciarambino T, Para O, Giordano M. Immune system and COVID-19 by sex differences and age. Women's Health. 2021 Jan 6;17.
11. Zaher K, Basingab F, Alrahimi J, Basahel K, Aldahlawi A. Gender Differences in Response to COVID-19 Infection and Vaccination. Biomedicines. 2023 Jun

9;11(6):1677.
12. Talebabadi N, Mohd Tohit ER, Abdullah M, Zahari Sham SY, Zailan NFZ, Amin Nordin S, Ismail IZ, Gazali AM, Hassan M. Crosstalk Between Coagulopathy and Inflammation in Obesity-Related Severe COVID-19 Infection. Hemato. 2025; 6(1):4.
13. Conway EM, Mackman N, Warren RQ, Wolberg AS, Mosnier LO, Campbell RA, et al. Understanding COVID-19-associated coagulopathy. Nat Rev Immunol. 2022 Oct 5;22(10):639–49.
14. Tang N, Li D, Wang X, Sun Z. Abnormal coagulation parameters are associated with poor prognosis in patients with novel coronavirus pneumonia. Journal of Thrombosis and Haemostasis. 2020 Apr;18(4):844–7.
15. Chen N, Zhou M, Dong X, Qu J, Gong F, Han Y, et al. Epidemiological and clinical characteristics of 99 cases of 2019 novel coronavirus pneumonia in Wuhan, China: a descriptive study. The Lancet. 2020 Feb;395(10223):507–13.
16. Jönsson Rylander A -C., Lindgren A, Deinum J, Bergstrɯm GML, Bɯttcher G, Kalies I, et al. Fibrinolysis inhibitors in plaque stability: a morphological association of PAI-1 and TAFI in advanced carotid plaque. Journal of Thrombosis and Haemostasis. 2017 Apr;15(4):758–69.
17. Whyte CS, Simpson M, Morrow GB, Wallace CA, Mentzer AJ, Knight JC, et al. The suboptimal fibrinolytic response in COVID-19 is dictated by high PAI-1. Journal of Thrombosis and Haemostasis. 2022 Oct;20(10):2394–406.
18. Adamo S, Chevrier S, Cervia C, Zurbuchen Y, Raeber ME, Yang L, et al. Profound dysregulation of T cell homeostasis and function in patients with severe COVID-19. Allergy. 2021 Sep 30;76(9):2866–81.
19. Lorè NI, De Lorenzo R, Rancoita PM V., Cugnata F, Agresti A, Benedetti F, et al. CXCL10 levels at hospital admission predict COVID-19 outcome: hierarchical assessment of 53 putative inflammatory biomarkers in an observational study. Molecular Medicine. 2021 Dec 18;27(1):129.
20. Diao B, Wang C, Tan Y, Chen X, Liu Y, Ning L, et al. Reduction and Functional Exhaustion of T Cells in Patients with Coronavirus Disease 2019 (COVID-19). 2020.
21. Callender LA, Curran M, Bates SM, Mairesse M, Weigandt J, Betts CJ. The Impact of Pre-existing Comorbidities and Therapeutic Interventions on COVID-19. Front Immunol. 2020 Aug 11;11.
22. Yang Y, Cai Z, Zhang J. Hyperglycemia at admission is a strong predictor of mortality and severe/critical complications in COVID-19 patients: a meta-analysis. Biosci Rep. 2021 Feb 26;41(2).
23. Gangadharan C, Ahluwalia R, Sigamani A. Diabetes and COVID-19: Role of insulin resistance as a risk factor for COVID-19 severity. World J Diabetes. 2021 Sep 15;12(9):1550–62.
24. Ilias I, Diamantopoulos A, Pratikaki M, Botoula E, Jahaj E, Athanasiou N, et al. Glycemia, Beta-Cell Function and Sensitivity to Insulin in Mildly to Critically Ill Covid-19 Patients. Medicina (B Aires). 2021 Jan 14;57(1):68.
25. El-Kadre LJ, Tinoco ACA. Interleukin-6 and obesity: the crosstalk between intestine, pancreas and liver. Curr Opin Clin Nutr Metab Care. 2013;16(5):564–8.
26. Del Valle DM, Kim-Schulze S, Huang HH, Beckmann ND, Nirenberg S, Wang B, et al. An inflammatory cytokine signature predicts COVID-19 severity and survival. Nat Med. 2020 Oct 24;26(10):1636–43.
27. Ronit A, Berg RMG, Bay JT, Haugaard AK, Ahlstrɯm MG, Burgdorf KS, et al. Compartmental immunophenotyping in COVID-19 ARDS: A case series. Journal of Allergy and Clinical Immunology. 2021 Jan;147(1):81–91.
28. Kumboyono K, Chomsy IN, Iskandar A, Aryati A, Parwati I, Wihastuti TA. The Potential Predictive Role of Tumour Necrosis Factor-α, Interleukin-1β, and Monocyte Chemoattractant Protein-1 for COVID-19 Patients Survival. Infect Drug Resist. 2022 Mar;15:821–9.
29. Faraj SS, Jalal PJ. IL1β, IL-6, and TNF-α cytokines cooperate to modulate a complicated medical condition among COVID-19 patients: case-control study. Annals of Medicine & Surgery. 2023 Jun;85(6):2291–7.
30. Fawzy S, Ahmed MM, Alsayed BA, Mir R, Amle D. IL-2 and IL-1β Patient Immune Responses Are Critical Factors in SARS-CoV-2 Infection Outcomes. J Pers Med. 2022 Oct 17;12(10):1729.
31. Ghanbari Naeini L, Abbasi L, Karimi F, Kokabian P, Abdi Abyaneh F, Naderi D. The Important Role of Interleukin-2 in COVID-19. J Immunol Res. 2023 Aug 22;2023:1–13.