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# Upregulation of Inflammatory Cytokines in Pulmonary Embolism Using Biochip-Array Profiling

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## Abstract

The complex pathophysiology of pulmonary embolism (PE) involves hemostatic activation, inflammatory processes, cellular dysfunction, and hemodynamic derangements. Due to the heterogeneity of this disease, risk stratification and diagnosis remains challenging. Biochip-array technology provides an integrated high throughput method for analyzing blood plasma samples for the simultaneous measurement of multiple biomarkers for potential risk stratification. Using biochip-array method, this study aimed to quantify the inflammatory biomarkers such as interleukin (IL)-1 $\alpha$ , IL-1 $\beta$ , IL-2, IL-4, IL-6, IL-8, IL-10, vascular endothelial growth factor (VEGF), interferon gamma (IFN- $\gamma$ ), tumor necrosis factor alpha (TNF- $\alpha$ ), monocyte chemoattractant protein-1 (MCP-1), and epidermal growth factor (EGF) in 109 clinically confirmed PE patients in comparison to the control group comprised of plasma samples collected from 48 healthy subjects. Cytokines IL-4, IL-6, IL-8, IL-10, IL-1 $\beta$ , and MCP-1 demonstrated varying level of significant increase ( $P < 0.05$ ) in massive-risk PE patients compared to submassive- and low-risk PE patients. The upregulation of inflammatory cytokines in PE patients observed in this study suggest that inflammation plays an important role in the overall pathophysiology of this disease. The application of biochip-array technology may provide a useful approach to evaluate these biomarkers to understand the pathogenesis and risk stratification of PE patients.

## Keywords

pulmonary embolism, biochip-array, biomarkers, inflammation

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## Introduction

Acute pulmonary embolism (PE) is a complex, multifactorial disease involving both systemic and localized processes. It is estimated nearly 600,000 Americans develop PE and deep vein thrombosis (DVT), leading to 100,000 deaths annually.<sup>1</sup> PE refers to a thrombus that detaches from a DVT and obstructs the pulmonary artery or peripheral arteries. This obstruction occludes blood flow and consequentially increases right ventricular (RV) pressure.<sup>2</sup> Failure of the pulmonary circuit leads to systemic hypoperfusion, hypoxia, ischemia, and eventually death.<sup>3,4</sup> Left untreated, PE results in a high likelihood of mortality, arrhythmia, and massive RV failure.<sup>4</sup> Even after effective treatment of acute PE, patients are at risk for developing persistent dyspnea, exercise limitations, and impaired quality of life.<sup>5</sup>

The diagnosis, risk-stratification, and treatment selection of PE patients remains difficult despite advancements in diagnostic approaches. As the severity of symptoms associated with PE can vary greatly and are largely non-specific, PE diagnostic criteria lack consensus and vary widely.<sup>6</sup> Clinical scoring criteria, such as the Geneva and Pulmonary Embolism Severity Index (PESI) scores are useful in the prediction of adverse outcomes of acute PE, yet do not utilize imaging nor biomarkers.<sup>7</sup> Recent studies have shown biomarkers to be useful for risk stratification, evaluation, and treatment strategies in many cardiopulmonary disorders, yet markers specific for PE have not been found.<sup>8</sup> Guidelines for the management of acute PE from the European Society of Cardiology (ESC) have found troponin levels in patients with PE to be useful in guiding anticoagulation therapy and classifying PE severity by subsequent right ventricular dysfunction and cardiac injury, yet the question arises whether additional biomarkers would be more useful, equivalent, or complementary to cardiac troponin levels.<sup>9-12</sup> Therefore, further development of risk stratification criteria through the use of biomarkers may be helpful to identify PE patients at low and high risk of mortality.

The complex pathophysiology of PE involves thrombogenesis, inflammation, endothelial cell dysfunction, and hemodynamic aberrations. These processes contribute to the formation, progression and outcome of PE.<sup>4,13,14</sup> The formation of thrombosis is likely due to the activation of endothelial cells, platelets, and leukocytes, resulting in inflammatory cytokine release and triggering of the coagulation system.<sup>15-17</sup> Additionally, the presence of an established embolism has also been shown to be a source of inflammation.<sup>3</sup>

Inflammation and thrombosis are closely associated and can cross activate one another.<sup>18,19</sup> This phenomenon has been observed in several complex hemostatic disorders, such as sepsis associated coagulopathy and coronavirus disease 2019 (COVID-19) associated thromboembolic complications. Pro-inflammatory cytokines, such as IL-1 $\beta$ , IL-6, IL-8 and TNF $\alpha$ , promote a pro-coagulant state through the induction of tissue factor.<sup>18,19</sup> Anti-inflammatory cytokines, including IL-2, IL-4, and IL-10, act to reduce coagulation induced by other pro-inflammatory factors.<sup>20</sup> This regulation of anti-inflammatory

and pro-inflammatory cytokines is disrupted during trauma, infection, tumors, and surgery.<sup>19</sup> Therefore, due to the vascular stress and thrombosis associated with PE, analyzing biomarkers of inflammation may aid in understanding the complex pathophysiology of this condition.

Biochip technology provides simultaneous measurement of multiple analytes, creating a panel from a single patient sample. Efficient and cost-effective, this technology allows for a large-scale and rapid evaluation of data.<sup>20</sup> Biochips have been used to investigate other disorders such as angina, various cancers, neurodegenerative diseases, and pneumonia.<sup>21</sup> Commercially available biochip technology, such as RANDOX biochip array approach, allows for simultaneous measurement of inflammatory markers present as a result of numerous dysregulated cellular processes, which may be useful in the evaluation of PE patient plasma.<sup>20,21</sup>

This study sought to quantify the inflammatory biomarkers interleukin (IL)-1 $\alpha$ , IL-1 $\beta$ , IL-2, IL-4, IL-6, IL-8, IL-10, vascular endothelial growth factor (VEGF), interferon gamma (IFN- $\gamma$ ), tumor necrosis factor alpha (TNF- $\alpha$ ), monocyte chemoattractant protein-1 (MCP-1), and epidermal growth factor (EGF) in PE patient plasma compared to healthy controls. We hypothesized that higher levels of pro-inflammatory biomarkers would correlate with greater severity scores in PE patients. Identification of significantly elevated cytokines may provide insight to the complex pathophysiology of PE as well as potential therapeutic targets. Additionally, correlation of cytokine concentration to PE severity may be useful for stratification models.

## Materials and Methods

### *Patient Selection and Data Collection*

Patients 18 years or older were recruited to participate in this study through enrollment conducted in conjunction with an ongoing IRB approved project by the Pulmonary Embolism Response Team (PERT) registry. Diagnosis of acute PE was confirmed by Computed Tomographic (CT) angiography or ventilation/perfusion imaging. Patients were classified into subcategories of low risk, submassive, and massive PE according to the American College of Cardiology (ACC)/ American Heart Association (AHA) guidelines.<sup>22</sup> Table 1 depicts the representative distribution of co-morbidities in PE patients, including demographic information, collected through the review of patient electronic medical records (EMR).

### *Blood Samples*

Whole blood samples were drawn from patients within 24 hours of confirmed diagnosis of acute PE and collected under an Institutional Review Board approved protocol. Samples were collected in 3.8% (0.109 mol/L) sodium citrate and EDTA tubes at the time of PE diagnosis, processed for platelet-poor plasma within 2 hours, and stored at -70°C prior to analysis. Control plasma samples from 50 healthy, non-smoking, adults,

**Table 1.** Representative Distribution of Co-Morbidities in Pulmonary Embolism Patients.<sup>a</sup>

Age (mean $\pm$ standard deviation)	62.8 $\pm$ 14.8
Female, n (%)	66 (60.5%)
Race	
White, n (%)	55 (50.4%)
Black, n (%)	47 (42.1%)
Hispanic, n (%)	2 (1.8%)
Other, n (%)	5 (4.5%)
Body mass index (kg/m <sup>2</sup> , median $\pm$ IQR)	32.6 (26.5-37.9)
PESI score (median $\pm$ IQR)	117 (81-149)
Hypertension, n (%)	62 (56.8%)
Diabetes mellitus, n (%)	20 (18.3%)
Chronic kidney disease, n (%)	23 (21.1%)
Cancer, n (%)	28 (25.6%)
Coronary artery disease, n (%)	13 (11.9%)
COPD, n (%)	12 (11%)
Prior stroke, n (%)	7 (6.4%)
Prior pulmonary embolism, n (%)	13 (11.9%)
Acute DVT, n (%)	68 (62.3%)
Lactate (median $\pm$ IQR)	1.7 (1.1-2.2)

Abbreviations: PESI, pulmonary embolism severity index; COPD, chronic obstructive pulmonary disease; DVT, deep vein thrombosis; IQR, interquartile range.

<sup>a</sup>The above analysis of the co-morbidities and demographic is based on the available data on 60 patients representing the cohort analyzed.

aged 19 to 53, were purchased from a commercially available source (George King Biomedical, Overland Park, Kansas).

### Biochip Assay

A Randox Investigator Cytokine and Growth Factors High-Sensitivity Array was utilized to measure IL-2, IL-4, IL-6, IL-8, IL-10, VEGF, IFN- $\gamma$ , TNF- $\alpha$ , IL-1 $\alpha$ , IL-1 $\beta$ , MCP-1 and EGF (Randox Laboratories, London, United Kingdom) per manufacturer guidelines. Quantification of all factors were tested simultaneously by utilizing a sandwich chemiluminescent immunoassay using a single patient sample.

### Statistical Analysis

Circulating levels of each biomarker in PE patient plasma were compared to control plasma. Descriptive statistics were calculated utilizing GraphPad Prism version 8.3.0 (GraphPad Software, La Jolla, California) and Microsoft Excel version 16.0 software. Statistical difference between PE groups and normal controls were evaluated utilizing nonparametric Mann-Whitney *t* tests and Kruskal-Wallis ANOVA. Correlations utilized Spearman correlation coefficients. Fold increase from the normal mean was calculated for each individual patient and averaged.  $P < 0.05$  was considered statistically significant.

### Results

Samples from 109 pulmonary embolism patients were used in this study. PE patients were categorized based on guidelines from the ACC/AHA and classified as low risk ( $n = 23$ ; 21.1%),

submassive ( $n = 76$ ; 69.7%), and massive PE ( $n = 10$ ; 9.2%). As shown in Figure 1, the levels of IL-4, IL-6, IL-8, IL-10, VEGF, IFN- $\gamma$ , TNF- $\alpha$ , IL-1 $\alpha$ , IL-1 $\beta$ , MCP-1 and EGF were significantly elevated in PE patients compared to normal individuals ( $P < 0.05$ ). Levels of IL-2 were not found to be significantly different between PE patients and healthy controls. Composite data, including the average, median, and range for each cytokine are shown in Table 2.

The mean fold difference between each individual PE samples and the normal sample mean are shown in Figure 2. Wide variation in the average fold difference was demonstrated in the PE population. IL-6 exhibited the highest average fold difference from the normal mean at 53-fold, followed by EGF and IL-8 at 22 and 20-fold higher, respectively.

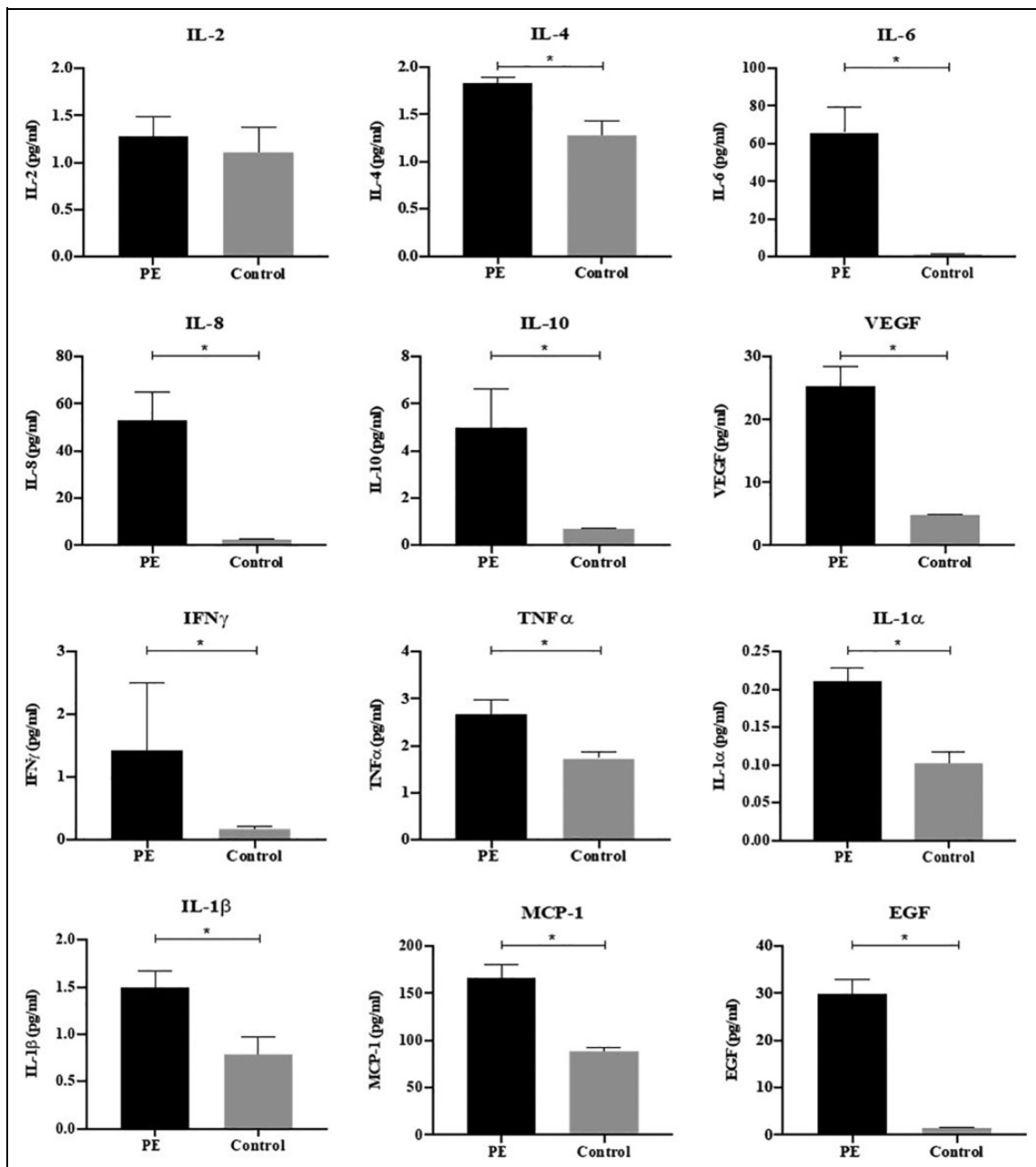
Table 3 provides a composite tabulation of various inflammatory biomarkers in accordance to low, submassive, and massive risk subgroups. The data is tabulated in terms of mean, median, and range for individual biomarkers in each subgroup. Moreover, individual differences in the range of the various biomarkers were evident. Figure 3 shows a composite of variations in several biomarkers for each subgroup as illustrated in graphic version. Such biomarkers including interleukin-4, IL-6, IL-8, IL-10, IL-1 $\beta$ , and MCP-1 were significantly elevated in the massive PE subgroups compared to submassive and low-risk PE ( $P < 0.05$ ). VEGF and EGF showed downwards trends in the massive group.

Correlations between cytokine concentrations for all biomarkers in PE patients are shown in Table 4, with bolded values indicating statistical correlation ( $P < 0.05$ ). At least one significant correlation was observed in all cytokines. Interleukin-8, TNF- $\alpha$ , and IL-1 $\beta$  correlated with four additional cytokines. The strongest correlation was observed between IL-6 and IL-8, with a Spearman correlation coefficient of 0.555 ( $P < 0.0001$ ).

### Discussion

Pulmonary embolism is a complex, multifactorial disease involving dysregulation in coagulation, fibrinolysis, and inflammatory processes, as well as hemodynamic insults and vascular cellular injury. Due to the difficulty in diagnosis and risk stratification, analyzing biomarkers involved in the pathophysiology of PE may allow for additional disease characterization. We have previously reported on the dysregulation of thrombo-inflammatory biomarkers in sepsis.<sup>22</sup> The markedly higher level of these biomarkers in the sepsis group are indicative of severe inflammatory response, as observed in the PE patients. Relative elevation of these markers may vary in other pathophysiologic states such as viral pneumonias, trauma, and unprovoked PE. Thus, utilizing biochip array technology allows for a comprehensive biochemical quantification to underscore the multifaceted processes involved in this disease.

Our study found significantly higher levels of both pro-inflammatory and anti-inflammatory biomarkers in PE patients compared to healthy controls, indicating the high amounts of cellular dysfunction and inflammation associated with PE. We speculated that these processes play complex roles within this



**Figure 1.** Comparison of cytokine levels between PE patients ( $n = 109$ ) and healthy individuals ( $n = 48$ ). Data is represented as mean  $\pm$  SEM. \* $P < 0.05$ . PE indicates pulmonary embolism; SEM, standard error of mean.

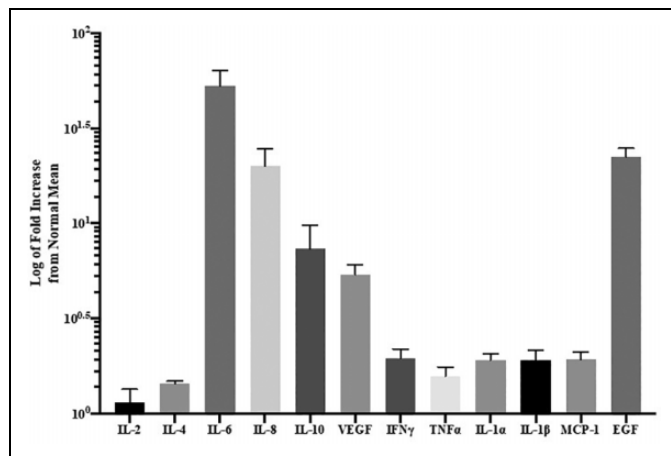
condition, thus increased concentrations of biomarkers associated with cellular dysfunction and inflammation as seen in this study would be expected to correlate with a higher PE severity and an overall systemic stress response. Increased intensity of inflammation associated with acute PE likely correlates with morbidity and mortality and may contribute to the poorer chronic health outcomes in these patients following PE resolution. Inflammation can also lead to activation of other systemic processes, such as coagulation and fibrinolysis, endothelial dysfunction, and hemodynamic changes, which also may contribute to the pathogenesis and propagation of this disease.

The cytokines demonstrating the largest average fold increase relative to healthy normals included IL-6, IL-8, and EGF. Interleukin-6 is a major pro-inflammatory cytokine linked to DVT progression.<sup>23</sup> The general mechanism of IL-6 is well understood and has been known to be closely related to tissue damage through inflammation, endothelial cell and platelet activation, and promoting coagulation without affecting fibrinolysis.<sup>7</sup> Elevated levels of IL-6 seen in PE patients increase the ability of platelets to respond to thrombin and thus promote the development of thrombosis.<sup>19,24</sup> This may explain the significantly increased levels of IL-6 observed in massive

**Table 2.** Inflammatory Biomarker Levels in PE Patients and Healthy Controls.

Marker	PE patients (n = 109)			Healthy controls (n = 48)		
	Mean $\pm$ SEM (pg/ml)	Median (pg/ml)	Range (pg/ml)	Mean $\pm$ SEM (pg/ml)	Median (pg/ml)	Range (pg/ml)
IL-2	1.27 $\pm$ 0.21	1.03	0-19.9	1.11 $\pm$ 0.26	0	0-6.25
IL-4	1.83 $\pm$ 0.05	1.74	0-4.62	1.28 $\pm$ 0.16	1.41	0-6.73
IL-6	65.8 $\pm$ 13.3	17.73	0.59-1003	1.25 $\pm$ 0.18	0.94	0.25-7.29
IL-8	52.8 $\pm$ 12.1	11.79	1.45-692	2.64 $\pm$ 0.13	2.585	0.985-7.4
IL-10	4.97 $\pm$ 1.64	0.9	0-131.24	0.68 $\pm$ 0.05	0.6	0-2.12
VEGF	25.7 $\pm$ 3.27	13.47	2.36-219.7	4.78 $\pm$ 0.19	4.585	2.14-7.77
IFN $\gamma$	0.33 $\pm$ 0.04	0.23	0-2.52	0.17 $\pm$ 0.04	0	0-1.76
TNF $\alpha$	2.72 $\pm$ 0.32	1.99	0-33.63	1.73 $\pm$ 0.15	1.77	0-6.55
IL-1 $\alpha$	0.19 $\pm$ 0.01	0.16	0-0.83	0.10 $\pm$ 0.02	0.1	0-0.53
IL-1 $\beta$	1.51 $\pm$ 0.19	1.21	0-17.48	0.79 $\pm$ 0.18	0	0-5.63
MCP-1	170 $\pm$ 15.2	129.87	7.79-746	88.60 $\pm$ 3.93	90.38	30.22-149.52
EGF	30.6 $\pm$ 3.24	19.95	0.51-216	1.36 $\pm$ 0.17	1.265	0-5.42

Abbreviations: EGF, epidermal growth factor; IFN, interferon; IL, interleukin; MCP, monocyte chemoattractant protein; TNF, tumor necrosis factor; VEGF, vascular endothelial growth factor.



**Figure 2.** Fold increase of inflammatory biomarkers in PE patients compared to the normal mean. Data is represented as mean  $\pm$  SEM. PE indicates pulmonary embolism; SEM, standard error of mean.

PE patients, compared to low-risk and submassive patients, since these patients have a more severe embolic event. Thus, our results suggest IL-6 may be useful in stratifying PE severity.

Interleukin-8 promotes the expression of integrins on the vascular endothelium and allowing for adhesion of neutrophils<sup>25</sup> and monocytes.<sup>26</sup> Integrin expression thus alters the homeostatic role of the endothelium to prevent clot formation. Previous studies have shown IL-8 to be involved in procoagulant activity<sup>27,28</sup> and elevated levels have been shown to correlate with first thrombotic events; elevated levels of this biomarker may be a risk factor for venous thrombosis.<sup>29,30</sup> Our study demonstrated significantly elevated levels of IL-8 in all PE patients compared to healthy individuals, and additionally, patients with massive PE to have significantly higher levels compared to low-risk and submassive patients. Therefore IL-8 elevation may be a potential indicator of thrombosis development. These results are supportive of previous research suggesting that IL-8 expression leads to thrombosis and therefore may be indicative of embolism severity.<sup>30</sup>

Additionally, this study found significantly elevated levels of EGF in PE patients compared to healthy controls. Studies have shown that particles of EGF, known as signal peptide-complement C1r/C1s, Uegf, and Bmp1-EGF domain-containing protein 1 (SCUBE1) to be higher in the PE population compared to non-PE and control groups.<sup>31,32</sup> Despite trends towards higher levels, significance was not reached among risk classifications. As epidermal growth factor is a prominent protein released from activated platelets during thrombus formation,<sup>33</sup> a possible explanation for the generalized higher levels of EGF seen in the PE population may be due to platelet activation involved in the formation of embolic lesions.

Additionally, the cytokines IL-4, IL-6, IL-8, IL-10, IL-1 $\beta$ , and MCP-1 were all significantly elevated in massive PE patients in comparison to those with low-risk and submassive disease, indicating the overwhelming inflammatory response with larger embolism. Amongst the cytokines analyzed, IL-2 was the only biomarker which was not significantly elevated in PE patient plasma compared to normal controls. IL-2 is known to be involved in suppressing the inflammatory response, and thus a non-significant elevation of this biomarker in our study may reflect dysregulation in the anti-inflammatory cascade in PE patients.

Most patients included in our study represent those recruited in PERT study with acute PE due to multiple factors including preexisting DVT, cancer, trauma, and surgical interventions. None of the patients had COVID-19. Because of the heterogeneous nature of our group, it is likely that some patients had preexisting increase in various biomarkers. The availability of such data will be helpful in further characterization of the pathophysiology of acute PE.

Correlations between these cytokines were demonstrated in this population, as shown in Table 4, and indicate a complex interconnection between multiple pathways within the inflammatory response of PE. The strongest correlation in this study was observed between IL-6 and IL-8, which further

**Table 3.** Inflammatory Biomarker Levels in PE Patients According to Low, Submassive and Massive Risk.

Marker	Low risk (n = 23)			Submassive (n = 76)			Massive (n = 10)		
	Mean $\pm$ SEM (pg/ml)	Median (pg/ml)	Range (pg/ml)	Mean $\pm$ SEM (pg/ml)	Median (pg/ml)	Range (pg/ml)	Mean $\pm$ SEM (pg/ml)	Median (pg/ml)	Range (pg/ml)
IL-2	0.73 $\pm$ 0.15	0.99	0-2.10	1.42 $\pm$ 0.30	1.03	0-19.9	1.37 $\pm$ 0.33	1.16	0-3.38
IL-4	1.66 $\pm$ 0.09	1.71	0-2.13	1.82 $\pm$ 0.07	1.71	0-4.62	2.30 $\pm$ 0.21	1.98	1.81-3.67
IL-6	45.02 $\pm$ 22.23	9.32	0.87-516.7	46.41 $\pm$ 9.99	17.73	0.59-594	265.12 $\pm$ 97.6	191.26	2.27-1003
IL-8	53.96 $\pm$ 27.94	9.62	2.49-626.1	44.57 $\pm$ 12.66	10.24	1.45-692	115.2 $\pm$ 65.44	49.66	10.36-692
IL-10	2.72 $\pm$ 1.42	0.61	0-32.9	1.56 $\pm$ 0.22	0.88	0-10.54	36.03 $\pm$ 14.83	15.03	0.4-131
VEGF	25.46 $\pm$ 6.62	15.83	2.77-150.7	25.55 $\pm$ 4.11	13.2	2.36-219	18.84 $\pm$ 5.25	15.81	3.69-55.56
IFN $\gamma$	0.33 $\pm$ 0.07	0.23	0-1.23	0.35 $\pm$ 0.05	0.24	0-2.52	0.27 $\pm$ 0.12	0.12	0-0.99
TNF $\alpha$	3.55 $\pm$ 1.42	1.79	0.9-33.63	2.36 $\pm$ 0.15	1.99	0-6.34	3.40 $\pm$ 0.68	2.55	1.71-8.66
IL-1 $\alpha$	0.19 $\pm$ 0.02	0.16	0-0.48	0.18 $\pm$ 0.01	0.15	0-0.61	0.35 $\pm$ 0.10	0.26	0-0.83
IL-1 $\beta$	1.74 $\pm$ 0.74	1.04	0-17.5	1.30 $\pm$ 0.13	1.18	0-7.03	2.61 $\pm$ 0.54	2.12	0.97-6.11
MCP-1	142.8 $\pm$ 16.3	125.75	28.4-334.8	149.53 $\pm$ 16.7	126.84	7.79-746	397.6 $\pm$ 72.9	421.41	57.18-746
EGF	34.09 $\pm$ 7.18	19.56	1.05-134.1	30.05 $\pm$ 4.05	19.95	0.51-216	21.23 $\pm$ 5.15	19.57	0.61-56.28

Abbreviations: EGF, epidermal growth factor; IFN, interferon; IL, interleukin; MCP, monocyte chemoattractant protein; TNF, tumor necrosis factor; VEGF, vascular endothelial growth factor.

underscores the importance of these cytokines in the progression of the severity of the observed pathogenesis of PE. Future studies investigating biomarker concentrations in relation to patient outcomes, such as mortality and long-term outcomes would be of interest. Additionally, assessing a combination of these biomarkers along with cardiac troponins may further facilitate the prediction of disease outcomes. Furthermore, evaluation of these biomarkers in patients following PE resolution may provide insight to the duration of inflammation and may be useful for guiding pharmacological and pharmacomechanical treatments and their effectiveness. The biochip array profile used in this study provides a high throughput technology with reproducibility and reliable sensitivity. The upper and lower limit of quantification have been established and the data generated in several studies was within these limits.

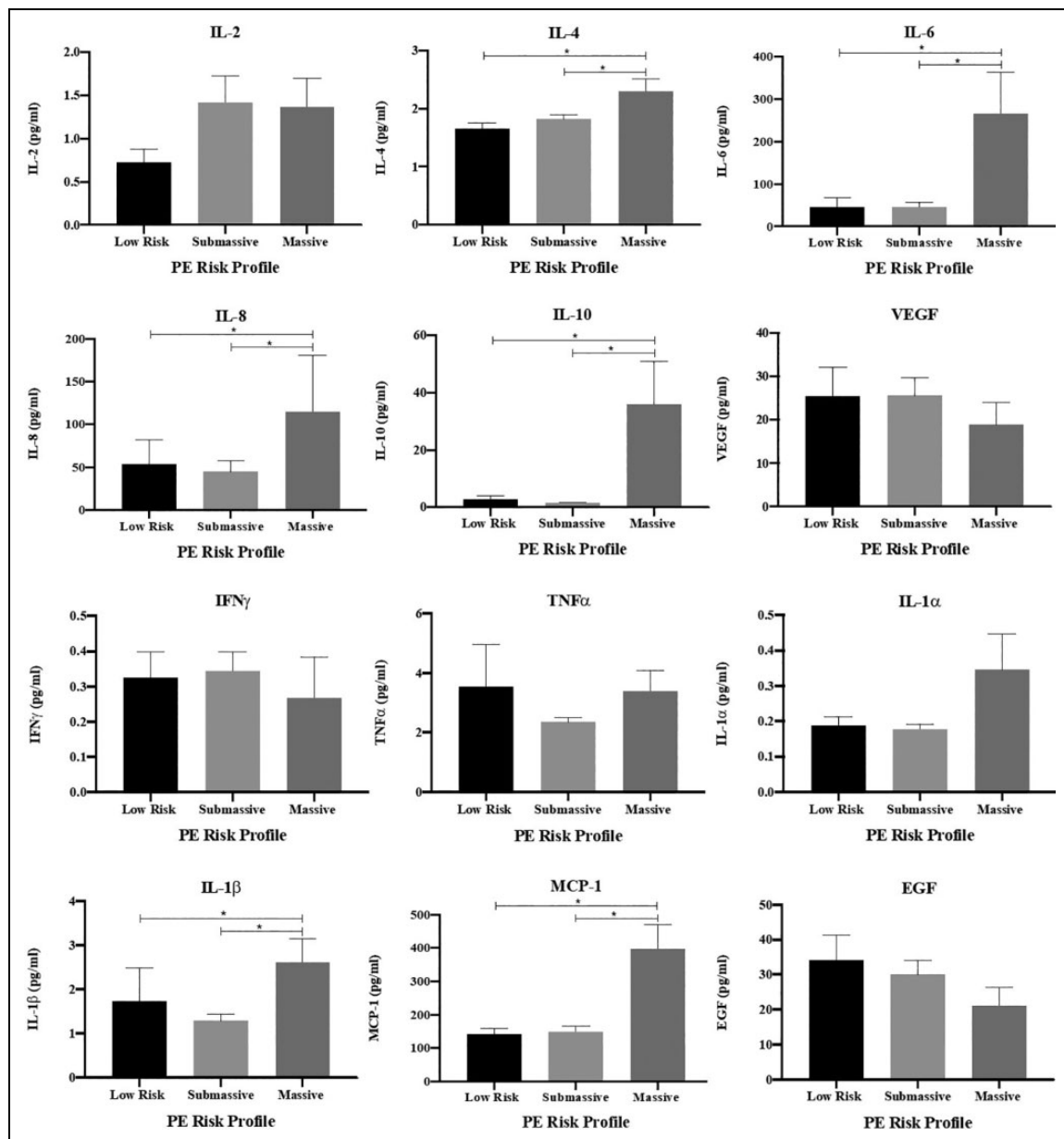
The biomarker profiling in COVID-19 patients with pulmonary manifestations has also validated the diagnostic and prognostic value of the biomarkers investigated in our study.<sup>34</sup> There is a growing awareness of the increased prevalence of PE in COVID-19 patients. Evaluation of biomarkers in COVID-19 patients can help in risk stratification and prediction of the clinical outcome in these patients.<sup>35</sup> The hemostatic abnormalities in COVID-19 result in complex thrombotic complications, including PE, and are associated with increased inflammatory and/or thrombotic cytokines. PE eventually contributes to the intravascular coagulopathy associated with marked increase in D-dimer, fibrin-degradation products, and endothelial dysfunction.<sup>36</sup> Profiling of biomarkers in COVID-19 patients is also helpful in the anticoagulant management of these patients, particularly those with PE and its complications. It is indeed true that COVID-19 associated PE also manifests other vascular co-morbidities. However, biomarker quantification provides comparable information on the dysregulation of hemostatic processes and the increased thrombo-inflammatory state observed in PE.<sup>37,38</sup> The biomarkers measured in our study were primarily

comprised of inflammatory cytokines. Similar multiparametric biochip analyses can also be developed for coagulation activation, fibrinolytic dysregulation, and platelet release products. Dedicated instruments with rapid turnaround and onsite monitoring will be developed to manage these critically ill patients with COVID-19 and other conditions resulting in PE.

### Study Limitations

As this study was observational, there are limitations to the applicability of these results in risk stratification. We did not follow these patients long-term and therefore are unable to correlate circulating levels of these biomarkers to clinical outcomes over periods of time. Additionally, as blood samples were collected at a single time, serial measurements of these biomarkers throughout resolution and treatment of PE may be useful to provide insight to the efficacy or dysregulation of inflammatory and hemostatic responses within this condition. In our study, we were not able to stratify the patient group in accordance to their smoking status in comparison to the control, which were non-smokers. Therefore, the role of smoking in modulating these biomarkers is not discussed. In future studies, such stratification will be helpful. Additionally, the data reported is based on only 109 representative cases which are further subcategorized into three groups. Therefore, a similar study with a larger cohort would balance subgroups and may provide further validation of the reported observations in our study. As noted previously, the difficulty of categorizing PE patients into massive, submassive, and low-risk may have had an impact in scoring the PE patients that participated in this study, yet utilizing the PE response team (PERT) and available guidelines, we hope to provide applicable definitions for the maximum utility of these results. This study represented a pilot investigation and the results warrant a need for additional trials in a larger cohort of patients.





**Figure 3.** Comparison of cytokine levels in PE patients with low, submassive, and massive PE risk. Data is represented as mean  $\pm$  SEM. \* $P < 0.05$ . PE indicates pulmonary embolism; SEM, standard error of mean.

## Conclusion

The primary purpose of this study was to quantify inflammatory cytokine levels in pulmonary embolism patient plasma according to patient risk categorization of low-, submassive-, and massive-risk based on the ACC/AHA guidelines.<sup>22</sup> Biochip array technology was used for the quantification of multiple inflammatory cytokines simultaneously. This study demonstrated an elevated systemic inflammatory response due to the elevation in anti-inflammatory and pro-inflammatory cytokines, including IL-4, IL-6, IL-8, IL-10, VEGF, IFN- $\gamma$ , TNF- $\alpha$ , IL-1 $\alpha$ , IL-1 $\beta$ , MCP-1 and EGF in PE patients compared to healthy individuals. IL-2 was the only biomarker found to not

reach significance in the PE population compared to controls. These results demonstrate that increased inflammation is associated with increased PE severity, allowing us to speculate that the pathogenesis of this condition largely involves multiple inflammatory processes. The use of biochip array technology may be a valuable and efficient means to evaluate multiple inflammatory markers and allow for rapid evaluation of these biomarkers for the use of guiding clinical decisions. The biochip array technology described in these studies can also be expanded to evaluate biomarkers of hemostatic dysregulation and immuno-activation. Future studies aimed in correlating these results to clinical outcomes may be useful for the evaluation of patients long term and provide additional insight to the pathogenesis of this condition.

**Table 4.** Spearman Correlation Coefficients for Inflammatory Biomarkers in PE Patients.<sup>a</sup>

	IL-2	IL-4	IL-6	IL-8	IL-10	VEGF	IFN $\gamma$	TNF $\alpha$	IL-1 $\alpha$	IL-1 $\beta$	MCP-1	EGF
IL-2		<b>0.270</b>	n.s.	<b>0.216</b>	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.
IL-4			n.s.	<b>0.322</b>	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.
IL-6				<b>0.555</b>	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.
IL-8					n.s.	n.s.	<b>0.201</b>	n.s.	n.s.	n.s.	n.s.	n.s.
IL-10						n.s.	n.s.	<b>0.404</b>	n.s.	<b>0.326</b>	<b>0.393</b>	n.s.
VEGF							n.s.	n.s.	n.s.	n.s.	n.s.	<b>0.267</b>
IFN $\gamma$								<b>0.267</b>	n.s.	n.s.	n.s.	n.s.
TNF $\alpha$									n.s.	<b>0.388</b>	<b>0.437</b>	n.s.
IL-1 $\alpha$										<b>0.498</b>	n.s.	n.s.
IL-1 $\beta$											<b>0.271</b>	n.s.
MCP-1												n.s.
EGF												n.s.

Abbreviations: EGF, epidermal growth factor; IFN, interferon; IL, interleukin; n.s., nonsignificant correlation; MCP, monocyte chemoattractant protein; TNF, tumor necrosis factor; VEGF, vascular endothelial growth factor.

<sup>a</sup>Compilation of the Spearman correlation coefficients for inflammatory cytokines in pulmonary embolism patients. Significant correlations ( $P < 0.05$ ) are bolded.

### Authors' Note

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