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ORIGINAL RESEARCH

Biomarkers of Prothrombotic State and Risk Assessment of Exacerbations in Patients with Chronic Obstructive Pulmonary Disease

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Background: Epidemiologic studies have shown that patients with acute exacerbation of COPD (AECOPD) suffer from morbidity and mortality from venous thromboembolism (VTE) and poor diagnosis. Von Willebrand factor (vWF) and plasminogen activator inhibitor type-1 (PAI-1) are frequently investigated in COPD as crucial parameters for coagulation and fibrinolysis. Nevertheless, the role of vWF and PAI-1 in AECOPD needs further exploration.

Objective: We sought to evaluate the hypercoagulability in AECOPD and investigate the association of plasma vWF and PAI-1 with occurrence and exacerbation risk of AECOPD patients.

Methods: Fifty-seven AECOPD patients and 34 control subjects were enrolled in our study. The concentrations of plasma vWF and PAI-1 antigens were measured by ELISA kit. Independent samples *t*-test or Wilcoxon rank sum test was applied for group comparison. Spearman correlation analysis, subject work curve (ROC) analysis, and Logistic regression were used to evaluate the role of the plasma vWF and PAI-1 in AECOPD.

Results: We observed increased vWF (770.15 ± 325.52 vs 327.62 ± 210.97 ng/mL, $P < 0.001$) and PAI-1 (0.47 vs 0.17 ng/mL, $P < 0.001$) levels in AECOPD patients compared with control subjects. Both vWF and PAI-1 are closely related to COPD (vWF: AUC = 0.8741, $P < 0.001$; PAI-1: AUC = 0.8222, $P < 0.001$). Moreover, elevated vWF could be an independent risk factor for COPD (OR = 1.01, 95% CI: 1.00–1.01, $P = 0.01$). We also discovered higher plasma levels of vWF and PAI-1 in the COPD "E" group in contract to "AB" group (vWF: 966.29 ± 251.18 vs 552.21 ± 253.28, $P < 0.0001$; PAI-1: 1.02 vs 0.38, $P = 0.003$). And vWF levels increased with increasing COPD exacerbation risk, moreover, plasma vWF positively related with patients' CAT scores and SGRQ scores. In addition, plasma vWF and PAI-1 correlated with each other in total participants and AECOPD subgroup analysis.

Conclusion: This study demonstrated that AECOPD patients have a prothrombotic state, as demonstrated by vWF and PAI-1 levels in plasma compared with those in control subjects, and the prothrombotic state increases with increasing COPD exacerbation risk.

Keywords: chronic obstructive pulmonary disease, exacerbation, vWF, PAI-1, coagulation, fibrinolysis

Introduction

Chronic obstructive pulmonary disease (COPD), one of the chronic respiratory diseases (CRDs), is a heterogeneous pulmonary condition characterized by chronic respiratory symptoms (such as dyspnea, cough, and sputum production) associated with airway abnormalities (bronchitis, bronchiolitis) and/or alveoli (emphysema) that contribute to persistent, progressive and aggravated airflow obstruction.[1] Until now, COPD remains an increasingly important cause of morbidity, disability, and mortality worldwide, associated with a substantial burden and cost.[2–4] Once the diagnosis of COPD has been established by spirometry, the GOLD ABE assessment tool is applied to the initial assessment of COPD to guide therapy. COPD patients will be divided into ABE subgroups according to the levels of symptoms and history of

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exacerbations in the previous year. Moreover, exacerbation is regarded as the independent risk factor for the prognosis of COPD patients, which is associated with more significant morbidity and mortality.[5,6]

Inpatients with AECOPD are at increased risk for vascular events,[7–9] including venous thromboembolism (VTE), an umbrella term of deep venous thrombosis (DVT) and pulmonary embolism (PE).[10,11] Furthermore, COPD patients are at 2–3 times greater risk for cardiovascular mortality risk factor for the prognosis of COPD patients, which risk factor for the prognosis of COPD patients, which.[12] Clinicians should therefore be confident in approaching the patient with suspected VTE. Although VTE scoring and routine detection of hemostatic parameters (APTT, PT, TT, FIB) are widely used in clinical practice, the incidence of vascular events in COPD patients is still underestimated, which affects patients' prognosis and increases mortality. Consequently, more clinical tests need to be carried out to help predict the VTE risk in COPD patients, and more research should be conducted on the relationship between the biomarkers of prothrombotic state and exacerbations in COPD patients as a premise.

Our study aimed to compare coagulation and fibrinolysis parameters between controls and AECOPD subjects, GOLD "AB" group and "E" group in respective. During the homeostasis of coagulation and fibrinolysis, von Willebrand factor (vWF) and plasminogen activator inhibitor type-1 (PAI-1) were well understood and were found to have a pivotal part to play. vWF, a large multimeric glycoprotein, plays a crucial role in both primary and secondary hemostasis by platelet activation and was deemed to be a central component of coagulation initiation.[13,14] PAI-1 belonged to the serine protease inhibitor (serpin) family and was revealed to regulate fibrinolysis by inhibiting tissue plasminogen activator and urokinase-type plasminogen activator[15] Furthermore, these two parameters are also affected by many factors, such as obesity, smoking, liver and kidney diseases, and blood system diseases. Indeed, despite vWF and PAI-1 having been previously evaluated in COPD patients, whether these biological parameters could be reliable biomarkers in independently evaluating the VTE risk of AECOPD remains partially understood. Thus, we detected the markers of hemostasis (vWF and PAI-1) in their peripheral blood. Our study hypothesized that AECOPD patients have a prothrombotic state, which increased with the risk of exacerbations and is related to the COPD Assessment Test (CAT) scores and St.George's Respiratory Questionnaire (SGRQ) scores.

Materials and Methods

Study Design

This case–control observational study was performed at the First Affiliated Hospital of Ningbo University. Patients admitted to our hospital's Respiratory and Critical Care Medicine Department between Sep 2019 and May 2022 were selected as the study population, and healthy medical examiners were collected as the control group during the same period. All study subjects signed an informed consent form before the study. The First Affiliated Hospital Ethics Committee of Ningbo University approved this study. The ethical approval number is 2017-R023.

Participants

All study subjects were required to meet the following inclusion criteria: 1. Aged 45–75 years; 2. Met the 2019 Global Initiative for Chronic Obstructive Lung Disease (GOLD) diagnostic definition: dyspnea, shortness of breath, chronic cough, or a history of exposure to risk factors for the disease, persistent airflow limitation, and pulmonary function tests suggesting Forced expiratory volume in the first second /forced vital capacity (FEV1/FVC) <0.7 after inhaled bronchodilators; 3. Pulmonary function FEV1 as a percentage of predicted (%pred) <80%; 4. Complete clinical information, examination, and test results were available; 5. Good patient compliance and agreement to sign an informed consent form.

The exclusion criteria were: 1. Requiring tracheal intubation mechanical or long-term immobilization, currently using antagonists and antiplatelet therapy; 2. Combined with other lung diseases: pneumonia, asthma, lung malignancy, interstitial lung disease, bronchiectasis, active tuberculosis, etc.; 3. Combined with severe liver and kidney failure, other systemic tumors, chronic neuromuscular disease, and other conditions that may cause respiratory symptoms; 4. Combined with immune-related inflammatory diseases (such as SLE, RA, ulcerative colitis), congenital coagulopathy, a history of acute embolism in the last three months (such as myocardial infarction, cerebral stroke, etc)., and a history of surgery or fracture in the last 1 to 3 months.

Clinical Data Collection

The clinical profile of the study population contained demographic information, underlying illnesses, comorbidities, coagulation test indicators, and lung function indicators. Among these, the lung function indicators included FEV1/FVC and FEV1%pred. The patient's symptoms were assessed using the medical research council (mMRC) dyspnea scale, CAT, and SGRQ. And Exacerbation history refers to exacerbations suffered the previous year collected. Then ABE assessment tool was applied according to the latest version of the Global Strategy for the Diagnosis, Treatment, and Prevention of Chronic Obstructive Lung Disease 2023 (GOLD 2023). AECOPD patients enrolled in our study were divided into two subgroups (GOLD AB group and E group). The Padua score was calculated to evaluate the VTErisk.

Plasma Sample Collection and Laboratory Examination

The enrolled AECOPD patients had received informed consent on admission, and these patients had not received any medication prior to blood drawing. All blood samples were collected using anticoagulant tubes, left to stand for 1 hour at room temperature, then centrifuged at 4°C for 10 minutes at 3000 rpm/min, and the supernatant was aspirated and dispensed. Plasma was kept at −80 °C until analysis. Plasma levels of vWF antigen (Elabscience, E-EL-H2168c) and PAI-1 antigen (Elabscience, E-EL-H2104c) were measured by enzyme immunoassay kit following the manufacturer's specification.

Statistical Analysis

All data were statistically analyzed and graphically plotted using GraphPad Prism (v 6.1 GraphPad Software, Inc., San Diego, CA, USA). Continuous variables were expressed as mean and standard deviation, using the independent samples *t*-test if they satisfied a normal distribution, or expressed as medians with interquartile, and the Wilcoxon rank sum test was applied if they did not satisfy a normal distribution; Count data were expressed as frequencies (%) and compared using the $\chi 2$ test. Correlations between variables were analyzed using the Spearman correlation test. Logistic regression was used for risk factor analysis. Receiver operating characteristic curve (ROC) analyses were performed to obtain the area under the curves (AUC).

$P < 0.05$ was considered statistically significant.

Results

Analysis for AECOPD Patients and Healthy Controls

Baseline Characteristics of Study Population

In this study, we extracted 160 patients with COPD as their primary diagnosis from September 2019 to May 2022. A total of 57 patients with COPD who met the inclusion criteria were further screened, and 34 age- and sex-matched healthy physical examiners with no history of COPD or other diseases during the same period were selected as the controls. Clinical data for the control group and AECOPD patients are shown in Table 1, where there were no statistically significant between-group differences in general information, except for the number of non-smokers ($P < 0.001$) and BMI ($P = 0.005$), which differed between the two groups.

Coagulation and Fibrinolysis Parameters

For hemostasis parameters, platelet count (PLT) and activated partial thromboplastin time (APTT) were not statistically different between the control and AECOPD groups. Prothrombin time (PT) and fibrinogen concentration (FIB) were elevated in the AECOPD group, while prothrombin time (TT) was lower in the COPD group than in the control group (PT, $P = 0.036$; FIB, $P = 0.001$; TT, $P < 0.001$, Table 1). Figure 1 gives comparative results of plasma vWF and PAI-1 concentration in control and AECOPD patients. Compared with the control group, increased plasma levels of vWF and PAI-1 are observed in AECOPD patients (vWF, $P < 0.001$, Figure 1A; PAI-1, $P < 0.001$, Figure 1B). ROC curve analysis (Figure 1C and D) shows that the area under the curve (AUC) of vWF is 0.8741, $P < 0.001$, the AUC of PAI-1 is 0.8222, $P < 0.001$, further demonstrating that both vWF and PAI-1 have a close relationship with COPD. Moreover, the elevated value of combined vWF and PAI-1 (Figure 1E) was presented (AUC = 0.9226, $P < 0.001$). We conducted a multivariate logistic regression analysis to furthermore evaluate the value of vWF and PAI-1 in COPD presented in Table 2, adjusting

Table 1 Baseline Characteristics of the Study Population

Parameters	Control (n=34)	COPD (n=57)	*P*-value
Age (y)	63.12±6.95	65.47±4.90	0.089
Sex n(%)			0.074
Male	23.00(67.60%)	48.00(84.20%)	
Female	11.00(32.40%)	9.00(15.80%)	
Never smokers n(%)	27.00(79.40%)	10.00(17.50%)	P<0.001
BMI(kg/m^2)	22.38(20.44–24.17)	20.76(17.99–22.43)	0.005
Disease history n(%)			
Hypertension	9.00(26.50%)	17.00(29.80%)	0.813
Diabetes mellitus	4.00(11.80%)	6.00(10.50%)	1.000
Coronary heart disease	0.00(0.00%)	1.00(1.80%)	1.000
PLT (×10^9/l)	230.00(190.00–282.00)	225.00(174.00–300.00)	0.857
PT (s)	11.10(10.60–12.10)	11.80(11.10–12.60)	0.036
FIB (g/l)	2.95(2.60–3.60)	4.10(3.10–5.40)	0.001
APTT (s)	31.15(28.70–33.90)	30.90(29.60–34.30)	0.661
TT (s)	15.35(14.20–16.50)	14.00(13.40–14.70)	P<0.001
VWF (ng/mL)	327.62±210.97	770.15±325.52	P<0.001
PAI-1 (ng/mL)	0.17(0.12–0.19)	0.47(0.28–1.11)	P<0.001

Notes: Continuous variables with a normal distribution are shown as mean ±standard deviation, or expressed as median (25th percentile–75th percentile). Categorical variables are shown as percentages with numbers in brackets.

Abbreviations: COPD, chronic obstructive pulmonary disease; BMI, body mass index; PLT, platelet; PT, prothrombin time; FIB, fibrinogen; APTT, activated partial thromboplastin time; TT, thrombin time, vWF, von Willebrand factor; PAI-1, plasminogen activator inhibitor type-1.

for smoking history, BMI, PLT, PT, APTT, FIB, and TT, the factors that were shown to be differed between the AECOPD patients and controls described above. And these variables were verified to be related to the vWF and PAI concentration. The analysis shows that increased plasma level of vWF is associated with COPD and is an independent risk factor for COPD. Although PAI-1 was not statistically significant between the two groups after multivariate analysis, the level of PAI-1 was positively associated with the concentration of vWF ($r = 0.518$, $P < 0.001$, Figure 1F).

Analysis for AECOPD Subgroups ("AB" Group and "E Group")2.1 Population Characteristics

According to GOLD 2023, we divided AECOPD patients into "AB" and "E" groups based on their mMRC score, CAT score, and acute exacerbation history collected on admission, and made a variation analysis on the clinical characteristics between "AB" group and "E" group displayed in Table 3. Except for CAT score, other variations such as age, sex, smoking history, BMI, mMRC score, spirometry parameters, and disease history have no significant difference. The results showed that the CAT score in the "E" group is higher than those in the "AB" group ($P = 0.046$).

Hemostasis Parameters

For all the parameters relevant to hypercoagulabilities, such as PLT, APTT, PT, TT, fibrinogen, and VTE scores, while they had no significance in the two subgroups. As shown in Figure 2A and B, the plasma concentration of vWF ($P < 0.001$) and PAI-1 ($P = 0.003$) was significantly increased in the "E" group. We plotted ROC curves for vWF and PAI-1

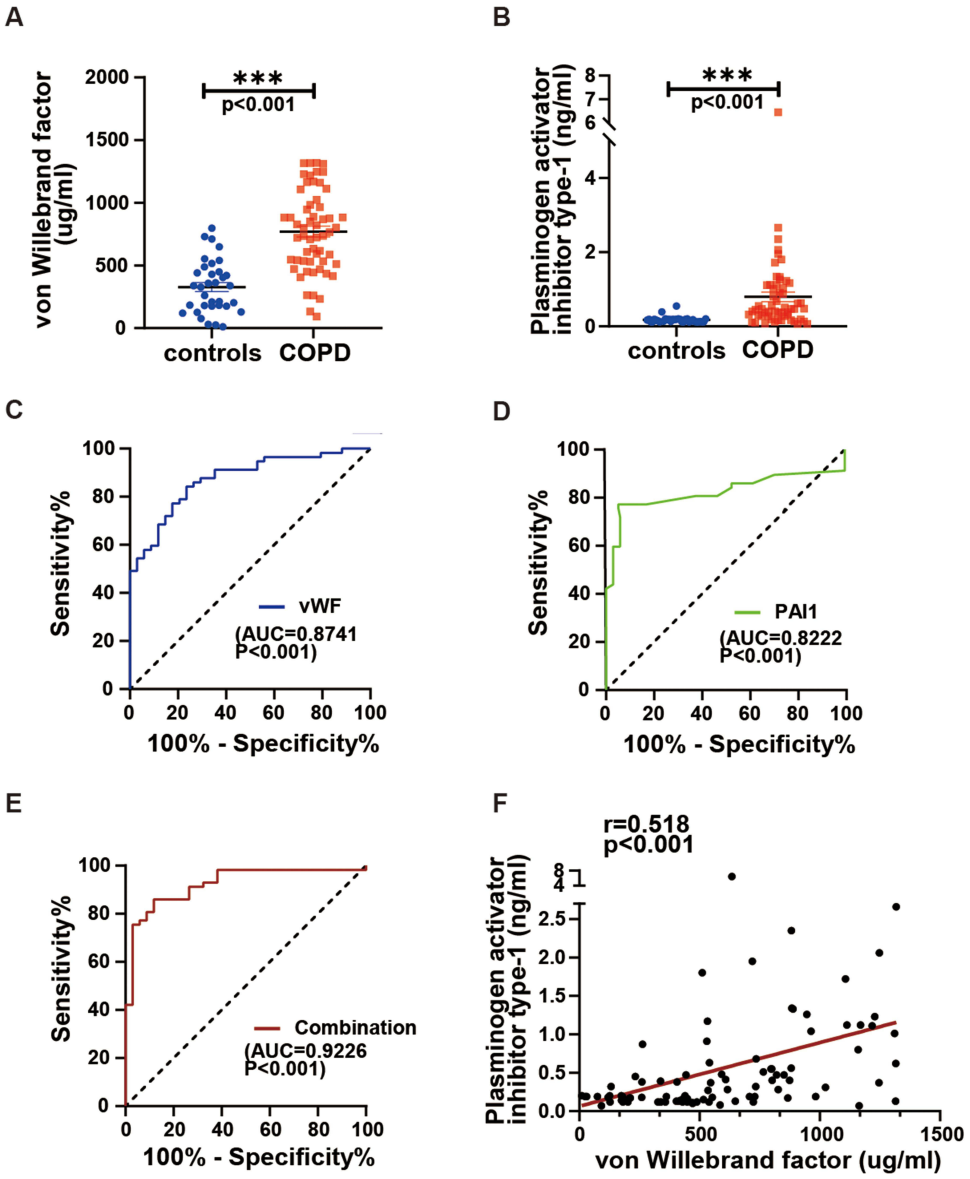

Figure 1 All the analysis concluded for COPD patients and control subjects in this study. The levels of vWF (**A**) and PAI-1 (**B**) in plasma were evaluated by ELISA kit. ROC curve analysis demonstrated the value of vWF (**C**), PAI-1 (**D**) and vWF+PAI-1 (**E**) in the exacerbation risk of COPD. The correlation between vWF and PAI-1 levels in plasma were also analyzed by Spearman correlation analysis (**F**). ***P <0.001.

Abbreviations: vWF, von Willebrand factor; PAI-1, plasminogen activator inhibitor type-1; AUC, area under the curve; ROC, receiver operating characteristic.

(vWF: AUC = 0.8802, P < 0.001; PAI-1: AUC = 0.7914, P < 0.001, Figure 2C). Then, the relevance of combined vWF and PAI-1 and exacerbation risk of COPD were also demonstrated simultaneously (AUC = 0.8593, P < 0.001, Figure 2E).

We also analyzed the correlation of vWF and PAI-1 with COPD-related clinical data such as CAT score, SGRQ score, mMRC, and spirometry variables. The results showed that vWF positively correlated with patients' CAT and SGRQ scores, with r of 0.37 and 0.34, a P value of 0.011 and 0.007 respectively (Table 4 and Figure 2D and E). We also evaluated the correlation between vWF and PAI-1 and found that the level of PAI-1 was positively associated with the concentration of vWF (r = 0.374, P = 0.004, Figure 2F).

Table 5 presents a multivariate analysis of vWF and PAI-1 concentration and COPD exacerbation risk, corrected for PLT, PT, FIB, APTT, TT, CAT score, SGRQ score, mMRC score, and VTE scores. The results show that vWF could be an independent risk factor for the exacerbation of COPD.

Table 2 Adjusted Multivariate Analysis for the Risk Factors in COPD Patients

Variables	B	*P*	OR	95% CI for OR	
				Min	Max
vWF (ng/mL)	0.01	0.01	1.01	1.00	1.01
PAI-1 (ng/mL)	5.79	0.10	326.57	0.35	307,643.19
BMI(kg/m2)	0.16	0.30	1.18	0.87	1.60
Smoking history	−4.24	0.00	0.01	0.00	0.24
PLT ($\times 10^9$/l)	0.00	0.96	1.00	0.98	1.02
PT (s)	−0.21	0.71	0.81	0.27	2.46
FIB (g/l)	0.71	0.31	2.03	0.52	7.98
APTT (s)	−0.01	0.94	0.99	0.77	1.28
TT (s)	−0.81	0.15	0.44	0.15	1.33
Constant	6.11	0.59	450.23		

Note: Binary logistic regression was used.

Abbreviations: Binary logistic regression was used. OR, odds ratio; CI, confidence interval; Max, maximum; Min, minimum; vWF, von Willebrand factor; PAI-1, plasminogen activator inhibitor type-1; BMI, body mass index; PLT, platelet; PT, prothrombin time; FIB, fibrinogen; APTT, activated partial thromboplastin time; TT, thrombin time.

Table 3 Characteristics of the COPD "AB" and "E" Groups

Parameters	"AB" group (n=27)	"E" group `(n=30)	*P*-value
Age (y)	64.67±4.44	66.20±5.25	0.242
Sex n(%)			0.149
Male	25.00(92.60%)	2.00(7.40%)	
Female	23.00(76.70%)	7.00(23.30%)	
Smoking n(%)			0.304
Smokers	24.00(88.90%)	23.00(76.70%)	
Nonsmokers	3.00(11.10%)	7.00(23.30%)	
BMI(kg/m^2)	21.04±3.63	20.35±3.99	0.501
CAT score	19.50±6.77	23.40±7.14	0.046
SGRQ score	38.84±24.33	50.09±21.49	0.086
mMRC score	2.00(1.00–3.00)	2.00(1.00–3.00)	0.189
FEV1(L)	0.90(0.62–1.42)	1.04(0.75–1.26)	0.523
FEV1/FVC(%)	48.35(37.90–60.10)	49.42(40.61–55.52)	0.936
FEV1/Pred(%)	34.20(21.80–53.30)	39.80(29.10–46.50)	0.462
Disease history n(%)			
Hypertension	7.00(25.90%)	10.00(33.30%)	0.576
Diabetes mellitus	3.00(11.10%)	3.00(10.00%)	1.000
Coronary heart disease	0.00(0.00%)	1.00(3.30%)	1.000
PLT ($\times 10^9$/l)	241.30±101.30	238.87±86.09	0.922

(*Continued*)

Table 3 (Continued).

Parameters	"AB" group (n=27)	"E" group `(n=30)	*P*-value
PT (s)	11.30(11.10–12.35)	12.20(11.15–12.95)	0.263
FIB (g/l)	3.80(3.00–4.55)	4.40(3.25–5.50)	0.263
APTT (s)	31.30(29.95–33.90)	30.20(29.55–33.75)	0.507
TT (s)	14.21±1.24	14.07±1.11	0.635
vWF (ng/mL)	552.21±253.28	966.29±251.18	0.000
PAI-1 (ng/mL)	0.38(0.18–0.52)	1.02(0.43–1.29)	0.003
VTE	2.00(2.00–3.00)	2.50(2.00–3.00)	0.245

Notes: Continuous variables with a normal distribution are shown as mean ±standard deviation, or expressed as median (25th percentile–75th percentile). Categorical variables are shown as percentages with numbers in brackets.

Abbreviations: COPD, chronic obstructive pulmonary disease; E, exacerbation; BMI, body mass index; CAT, COPD Assessment Test; SGRQ, St.George's Respiratory Questionnaire; mMRC, Modified British Medical Research Council; FEV1, forced expiratory volume in one second; FVC, forced vital capacity; Pred, predicted, PLT, platelet; PT, prothrombin time; FIB, fibrinogen; APTT, activated partial thromboplastin time; TT, thrombin time, vWF, von Willebrand factor, PAI-1, plasminogen activator inhibitor type-1; VTE, venous thromboembolism.

Discussion

COPD is an increasing contributor to chronic morbidity, disability, and mortality worldwide, associated with significant burden.[3,4,16] The morbidity and mortality from VTE in patients with COPD are not surprising for the presence of

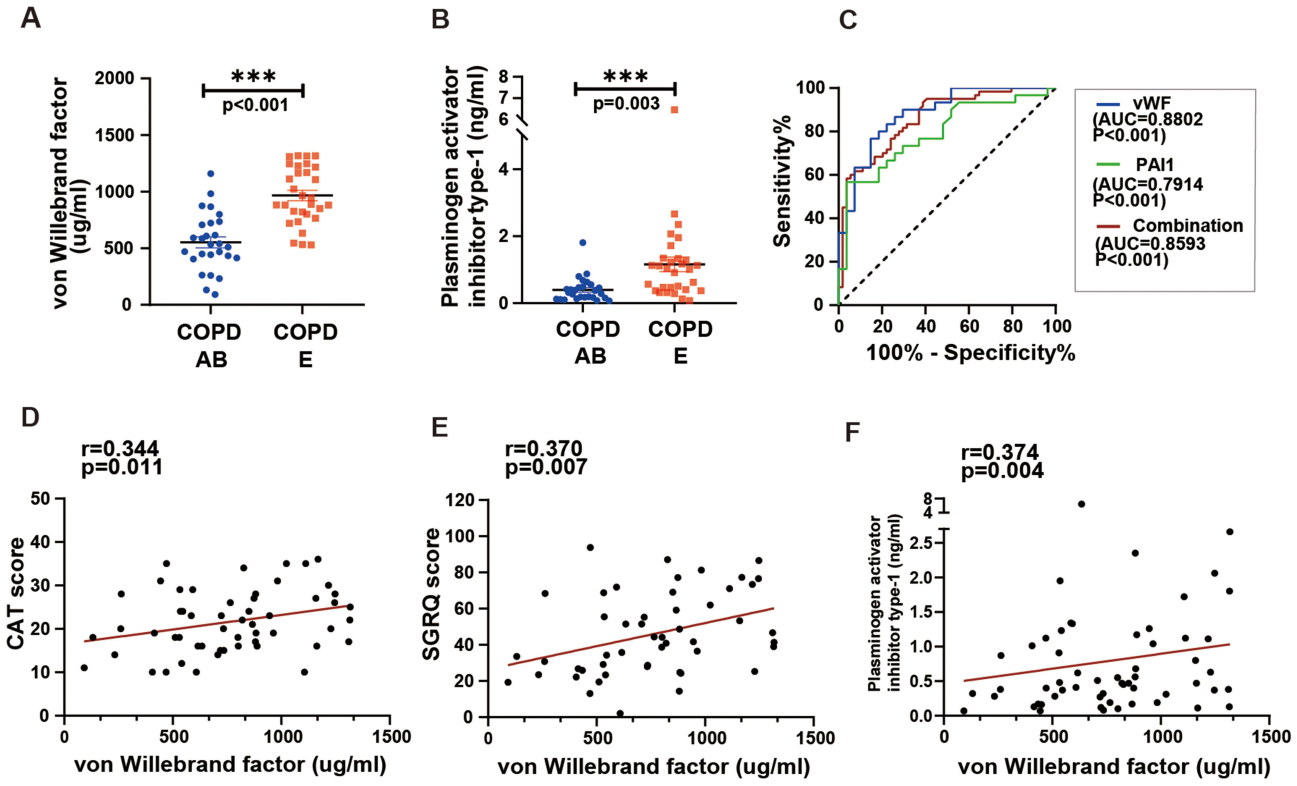

Figure 2 All the analysis conducted for COPD "AB" group and "E" group in this survey. The levels of vWF (**A**) and PAI-1 (**B**) in plasma were evaluated by corresponding ELISA assay. ROC curve analysis of vWF, PAI-1 and vWF+PAI-1 for evaluate the exacerbation risk of COPD was shown (**C**). The correlation of vWF levels in plasma with CAT score (**D**), SGRQ (**E**) and PAI-1(**F**) were evaluated by Spearman correlation analysis. ***P <0.001.

Abbreviations: E, exacerbation; vWF, von Willebrand factor; PAI-1, plasminogen activator inhibitor type-1; AUC, area under the curve; ROC, receiver operating characteristic.

Table 4 Correlations of vWF and PAI-1 with Symptom and Lung Function of COPD Patients

Variables		CAT score	SGRQ score	mMRC score	FEV1	FEV1/pred	FEV1/FVC
vWF (ng/mL)	*r*	0.37	0.34	0.24	−0.05	0.02	−0.04
	P	0.01	0.01	0.07	0.69	0.87	0.78
PAI-1 (ng/mL)	*R*	0.23	0.09	0.02	−0.05	0.05	−0.10
	p	0.11	0.53	0.90	0.71	0.74	0.44

Note: Spearman correlation analysis was used.
Abbreviations: vWF, von Willebrand factor, PAI-1, plasminogen activator inhibitor type-1; COPD, chronic obstructive pulmonary disease; CAT, COPD Assessment Test; SGRQ, St.George's Respiratory Questionnaire; mMRC, Modified British Medical Research Council; FEV1, forced expiratory volume in one second; FVC, forced vital capacity; Pred, predicted.

Table 5 Multivariate Analysis for Risk of Exacerbations in COPD Patients

Variables	B	*P-value*	OR	95% CI for OR	
				Min	Max
vWF (ng/mL)	0.01	0.01	1.01	1.00	1.01
PAI-1 (ng/mL)	1.86	0.07	6.41	0.89	46.34
PLT ($\times 10^9$/l)	0.00	0.94	1.00	0.99	1.01
PT (s)	0.95	0.20	2.58	0.61	10.91
FIB (g/l)	−0.18	0.74	0.84	0.29	2.41
APTT (s)	−0.24	0.12	0.79	0.58	1.07
TT (s)	−0.26	0.65	0.77	0.26	2.33
CAT score	0.25	0.15	1.29	0.91	1.82
SGRQ score	−0.06	0.20	0.94	0.86	1.03
mMRC score	−0.05	0.94	0.95	0.22	4.07
VTE score	−0.11	0.88	0.90	0.23	3.50
Constant	−6.56	0.57	0.00		

Note: Binary logistic regression was used.
Abbreviations: E, exacerbation; OR, odds ratio; CI, confidence interval; Max, maximum; Min, minimum; vWF, von Willebrand factor; PAI-1, plasminogen activator inhibitor type-1; BMI, body mass index; PLT, platelet; PT, prothrombin time; FIB, fibrinogen; APTT, activated partial thromboplastin time; TT, thrombin time; CAT, COPD Assessment Test; SGRQ, St. George's Respiratory Questionnaire; mMRC, Modified British Medical Research Council; VTE, venous thromboembolism.

coagulation abnormalities,[12,17,18] especially in the exacerbation stage, which then brings difficulties for treatment.[12,17,18] Therefore, vascular events should be looked for routinely and treated proactively in AECOPD patients. Among these, seeking potential biomarkers for assessing hypercoagulability and COPD exacerbation is significant.

At present, there are several researches linking vWF to COPD. Van der Vorm et al[19] investigated the level of vWF in the peripheral blood of COPD patients during the exacerbation phase and convalescence phase, respectively, and found that vWF in the peripheral blood was significantly higher during the exacerbation phase than during the convalescence phase. Furthermore, a similar study was conducted by Polosa, who designed a matched pair study of COPD patients during exacerbation and clinical stability and revealed elevation of the level of vWF during COPD exacerbations closely related to acute inflammation[20] While these studies lack the comparison with controls. Langholm et al[21] demonstrated a significantly increased level of vWF in symptomatic COPD subjects compared to asymptomatic/mild symptomatic COPD patients and found to be independently associated with increased mortality risk of COPD. And Bártholo et al[22] found that absolute vWF levels were significantly lower in the control group than in the smoker and SCOPD (Stable COPD) group. Some of the studies focus far more on the relationship between vWF and inflammation or smoking in COPD. And several researches demonstrated that the level of vWF was significantly increased during COPD

exacerbation, which is in line with our result, while they did not further explore the relationship between vWF and exacerbation risk of COPD.

For PAI-1 exploration, during the past decades, numerous clinical and laboratory studies aimed to evaluate the various functions of PAI-1, especially the vital traditional function of regulating plasma fibrinolysis in the process of thrombosis.[23,24] And PAI-1 is regarded as a risk factor for VTE in kinds of diseases such as obesity, diabetes, cancer, and inflammatory diseases.[25–28] As a chronic inflammatory disease, elevated serum levels of PAI-1 were found in COPD patients, which were associated with lung function decline and systemic inflammation.[29,30] Researchers supposed that PAI-1 might be a potential biomarker candidate for COPD diagnosis because moderate-severe airflow limitation and systemic inflammation could be independent predictors of an increased PAI-1 level[30] Besides blood-borne PAI-1, sputum PAI-1 level was also investigated in COPD for its potential influence.[29,31] However, the role of PAI-1 remains poorly understood. In this study, two types of grouping were adopted that were total AECOPD patients versus healthy controls, COPD "AB" group versus "E" group. On the one hand, we aimed to analyse the difference in hypercoagulable state between AECOPD patients and controls. On the other hand, we further analyzed the influence of the degree of acute exacerbation in the past on hypercoagulable state.

First, in our study, we demonstrated that plasma level of vWF and PAI-1 increased in AECOPD patients compared to controls, which means a prothrombotic state exists in AECOPD patients. These results further support other studies investigating previously and provide evidence for the potential biomarker of vWF.[19–22] While we preferred to focus more on the exacerbation risk of COPD and eliminate the interference factor by logistic regression analysis, which was not conducted in previous research. Actually the coagulation and fibrinolytic systems are extremely complex, many parameters cooperate with each other. Predictably other important hemostasis parameters such as PT, FIB, and TT, which are routinely tested in clinical work, were also increased. While after adjusting for these confounders, only vWF but not PAI-1 was found to be an independently risk factor associated with AECOPD. At the same time, we also analyzed the correlation of these hemostasis factors, among which PAI-1 is clearly correlated with vWF, that is to say, vWF plasma is relatively reliable for evaluating the coagulation status of AECOPD patients, and if the plasma level of PAI is analyzed together, the diagnostic value will be improved. Secondly, we found the plasma level of vWF and PAI-1 obviously increased in COPD "E" group comparing to "AB" group, while other hemostasis parameters such as PT, FIB, APTT, TT and even VTE score have no difference between these two groups. At the same time, we also performed logistic regression to exclude confounding factors as much as possible, and found that only vWF increased more significantly in COPD "E" group. Combined with the above information, we suggest that patients in COPD "E" group may be more prone to potential VTE, which is not captured by conventional coagulation relevant measures. In addition, when we did correlation studies, we found that the level of vWF was significantly correlated with the level of PAI, CAT score and SGRQ score in COPD patients. To our knowledge, these results did not appear in previous studies. In addition, on the one hand, we believe that although PAI-1 is also found to be elevated, this increase is more complementary to the increase of VWF, and the plasma level for PAI-1 alone is not enough to evaluate. On the other hand, the level of vWF is not only significantly correlated with the level of previous acute exacerbations in COPD patients but also significantly correlated with symptoms, that is, patients with multiple symptoms and high risk of acute exacerbations are more prone to VTE events. In patients with COPD, acute exacerbation in the past has been deemed to be an independent risk factor for assessing disease prognosis, so it can also speculate indirectly whether there is a correlation between the occurrence of hypercoagulability and future acute exacerbation. However, due to the lack of follow-up study, it is not possible to make a conclusion from this, but it also provides certain clues for relevant studies.

In conclusion, this study demonstrated that AECOPD patients have a prothrombotic state, as demonstrated by vWF and PAI-1 levels in plasma compared with those in control subjects, and the prothrombotic state increases with increasing COPD exacerbation risk. This might explain why patients with AECOPD, especially the patients with multiple symptoms and high risk of exacerbation, have an increased risk of venous thromboembolism. vWF could be a potential parameter for evaluating the potential VTE risk of AECOPD patients.

As a retrospective clinical study, we tried our best to minimize bias. During the enrollment of participants, we strictly excluded any factors that could potentially be homeostasis, including hematological diseases, tumors, co-existing respiratory diseases, existing VTE complications, recent history of surgery or trauma, etc. However, the existence of

a not-large-enough sample size must be confirmed. Additionally, compared to prospective studies, the limitations of this observational study leading to lower predictive values of these parameters in further exacerbation risk exploration also exists. More effort should be made to verify this conclusion in the future.

Data Sharing Statement

The datasets used and/or analysed during the current study are available from the corresponding author on reasonable request.

Ethics Approval and Consent to Participate

The study was approved by the Ethics Committee of Medicine, the First Affiliated Hospital of Ningbo University. All participants signed informed consent in accordance with the Declaration of Helsinki.

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Author Contributions

All authors made a significant contribution to the work reported, whether that is in the conception, study design, execution, acquisition of data, analysis and interpretation, or in all these areas; took part in drafting, revising or critically reviewing the article; gave final approval of the version to be published; have agreed on the journal to which the article has been submitted; and agree to be accountable for all aspects of the work.

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