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Identification of oxylipins and lipid mediators in pulmonary embolism

Fei Chen[1†], Daibao Peng[2†], Yanyan Xia[3], Haixuan Sun[4], Han Shen[3*] and Mao Xia[5*]

Abstract

Background This study aimed to investigate the role of oxylipins and lipid mediators in Pulmonary Embolism (PE), a serious cardiovascular condition associated with high morbidity and mortality rates.

Methods A total of 6,365 hospitalized patients with thrombosis and 200 healthy individuals were recruited as the control group from 2015 to 2023. Thrombus type, coagulation, and lipid-related parameters were statistically analysed. Additionally, lipidomic characteristics of serum samples from the PE and control groups were examined via LC-MS/MS for the first time.

Results Among the 6,365 hospitalized patients with thrombosis, 72.1% (4,587/6,365) had venous thromboembolism (VTE). Within the VTE group, the incidence of PE was 12.1% (555/4,587). In comparison to the healthy control (HC) group, the PE group exhibited significant elevations in coagulation-related parameters, such as factor VIII (F VIII) and von Willebrand factor (vWF) activities, while antithrombin III (AT III) and factor XII (F XII) activities were notably reduced. Lipid-related parameters, including serum cholesterol (TC), high-density lipoprotein cholesterol (HDL-C), low-density lipoprotein cholesterol (LDL-C), apolipoprotein A (apoA), were significant reductions in PE patients ($P < 0.0001$), with areas under the curve (AUCs) exceeding 0.9. LC-MS/MS analysis of serum samples revealed 118 oxidized lipid metabolites. Compared to the HC group, the PE group exhibited 10 upregulated oxidized lipid metabolites, with the most significant difference observed in 20-hydroxyPGF2α derived from arachidonic acid (ARA). The study identified upregulated oxidized lipid metabolites primarily linked to the ARA metabolism signalling pathway.

Conclusion This research indicates a notable correlation between lipid metabolism and the occurrence and development of PE. Specifically, upregulation of the arachidonic acid metabolism signalling pathway may be an important pathogenic factor for PE, and 20-hydroxyPGF2α derived from ARA has potential as a biomarker for PE disease.

Keywords Thrombosis, Pulmonary embolism, Lipids, Oxylipins, Arachidonic acid

†Fei Chen and Daibao Peng contributed equally to this work.

*Correspondence:
Han Shen
shenhan@njglyy.com
Mao Xia
xiamao0126@163.com
[1]Department of Clinical Laboratory, Nanjing Drum Tower Hospital Clinical College of Nanjing Medical University, Nanjing, China
[2]Department of Clinical Laboratory Medicine, Affiliated Hospital of Medical School, Taikang Xianlin Drum Tower Hospital, Nanjing University, Nanjing 210008, China
[3]Department of Clinical Laboratory Medicine, Affiliated Hospital of Medical School, Nanjing Drum Tower Hospital, Nanjing University, Nanjing 210008, China
[4]Suzhou Institute of Biomedical Engineering and Technology, Chinese Academy of Sciences, Suzhou 215163, China
[5]Department of Clinical Laboratory Medicine, The Affiliated Hospital of Nantong University, Nantong 226001, Jiangsu Province, China

Background

Pulmonary embolism (PE) is a prevalent cardiovascular disease in China and ranks among the top three fatal cardiovascular diseases in Western countries, following heart disease and stroke [1]. Pulmonary embolism occurs when pulmonary arteries are blocked by emboli, such as thrombi, tumors, or fat, leading to disruption of normal pulmonary circulation. DVT is the primary cause of thrombi that result in PE. The "triad" of thrombosis formation, first suggested by German scientist Rudolph Virchow in 1856, includes venous stasis, injury to endothelial cells, and a hypercoagulable state, can account for most pulmonary embolism cases [2]. The symptoms of PE can vary significantly, with some patients being asymptomatic and others presenting severe symptoms such as hypotension, shock, or sudden death. It is estimated that approximately half of all PEs are asymptomatic [3, 4]. A study of 1,880 individuals diagnosed with PE across 22 medical facilities in the United States found an overall 30-day mortality rate of 5.4% [5]. The cornerstone of treating venous thromboembolism is anticoagulation therapy. However, approximately 4% of survivors of pulmonary embolism may develop post-PE syndrome [6]. PE represents a significant clinical concern, with issues such as misdiagnosis, underdiagnosis, delayed diagnosis, and inadequate treatment, especially concerning thrombolysis and anticoagulant therapy, contributing to high mortality rates.

Lipids are a class of natural compounds with numerous biological functions, including forming cell membranes or acting as signaling molecules and sources of energy. They exist in various types, such as free fatty acids, sphingolipids, and sterol lipids [7]. Exploring the potential link between lipids and the development of venous blood clotting, along with its pathophysiology, is a significant clinical concern. In a recent investigation, Yuan S and colleagues utilized a 2-step network Mendelian randomization method to analyze genetic data from a large cohort of 81,190 individuals with VTE. This study revealed a correlation between HDL receptor-related protein 4 and VTE, suggesting possible cardiovascular implications [8]. Alexander Brill and his team demonstrated that the lack of the HDL receptor (SR-BI), exacerbates venous thrombus formation in an animal model of deep vein thrombosis [9]. Additionally, evidence indicates that medications that lower lipids, particularly statins like rosuvastatin, are linked to a reduced risk of developing blood clots in veins [10, 11]. Oxylipins are compounds derived from polyunsaturated fatty acids through enzymatic and nonenzymatic routes, which require the participation of different enzymes such as lipoxygenases (LOXs), cyclooxygenases (COXs), and cytochrome P450s (CYPs), along with other subsequent enzymes [12]. Oxylipins are acknowledged as potent and short-lived compounds that are synthesized on demand and tightly regulated, exerting their effects through paracrine or autocrine pathways [13]. Studies have linked oxylipins to various diseases, such as autoimmune diseases [14], cardiovascular diseases [15], and cancer [12]. Recent studies have also revealed that oxylipins play a role in either promoting or inhibiting thrombus formation. Therefore, the role of oxylipins in thrombosis, especially in the pathogenesis of PE, is still unclear. Jennifer Yeung and colleagues demonstrated that Omega-6 DPA and its oxidized lipids from 12-lipoxygenase can effectively hinder platelet activation and the formation of blood clots after vascular injury [16]. Livia Stanger and coworkers reported that the oxylipin analogue CS585 can prevent platelet activation and clot formation by stimulating prostacyclin receptors [17]. Additionally, Yeung J and colleagues observed that 12(S)-HETrE, an oxylipin produced by 12-lipoxygenase from dihomogamma-linolenic acid, can prevent thrombosis by activating the Galphas signaling pathway in platelets [18]. Nevertheless, the precise involvement of oxylipins in the pathogenesis of PE remains uncertain.

This study initially conducted a statistical analysis of the incidence, in-hospital mortality, and related comorbidities of PE from 2015 to 2023. The coagulation-related indicators of PE and the levels of six lipid parameters were also explored. Additionally, for the first time, this study examined the varying oxylipin profiles in two groups via LC-MS/MS. The findings suggest a significant link between lipid metabolism and the onset and progression of PE. Specifically, upregulation of the arachidonic acid metabolism signaling pathway may be an important pathogenic factor for PE, and 20-hydroxyPGF2α derived from ARA has potential as a biomarker for PE disease.

Methods

Study participants

The study involved 6,365 adult thrombosis patients (≥18 years old) hospitalized at Nanjing Drum Tower Hospital from January 2015 to December 2023. Thrombotic events were confirmed using imaging techniques like Doppler ultrasound, computed tomography, magnetic resonance imaging or angiography. Among these patients, 555 were hospitalized with PE diagnosed by CT pulmonary angiography (CTPA), comprising 260 males and 295 females. Additionally, 200 healthy individuals who underwent physical examinations were matched for age and gender during the same period were chosen as the HC group.

Serum collection and preparation

The first fasting peripheral blood samples were acquired from enrolled patients during their initial hospitalization prior to the commencement of any drug treatment. These samples were then centrifuged at 3,500 rpm for 10 min at 4 °C to obtain serum. The serum was promptly preserved

at -80 °C for future research. *To minimize treatment interference with laboratory parameters, this study collected the initial set of laboratory data from patients upon admission.* Demographic information, clinical symptoms, and laboratory results were obtained from the hospital's records system for medical and laboratory management. The laboratory results encompassed coagulation-related and lipid-related indicators. The lipid-related indicators consisted of TC, HDL-C, LDL-C, apoA, apoB, and triglycerides. Table 1 presents a synopsis of the demographic and clinical traits of all individuals involved.

Table 1 Clinical and demographic characteristics of patients with PE ($n=555$) and healthy controls ($n=200$)

Characteristics	HC	PE	*P*-
Category (*N*)	**200**	**555**	**value**
Age, median (IQR), years	65 (57–71)	66 (54–73)	ns
Male, n (%)	94 (47.0)	260 (46.8)	ns
BMI, median (IQR), Kg/m2	-	24.2 (22.0-26.3)	-
Comorbidities, n (%)			
DVT	-	280 (50.5)	-
Varicose veins	-	28 (5.0)	-
Cancer	-	83 (15.0)	-
Hypertension	-	234 (42.2)	-
Diabetes	-	91 (16.4)	-
Orthopedic diseases	-	115 (20.7)	-
Pneumonia	-	276 (49.7)	-
Pulmonary arterial hypertension	-	108 (19.5)	-
Respiratory failure	-	119 (21.4)	-
Atherosclerosis	-	45 (8.1)	-
Cerebral infarction	-	78 (14.1)	-
Myocardial infarction	-	15 (2.7)	-
Cardiac insufficiency	-	51 (9.2)	-
Renal insufficiency	-	20 (3.6)	-
Other conditions			
Dementia	-	10 (1.8)	-
Pregnancy	-	9 (1.6)	-
Anticardiophospholipid syndrome	-	7 (1.3)	-

Abbreviations: PE: pulmonary embolism; HC: healthy control; IQR: interquartile range; BMI: body mass index; DVT: deep venous thrombosis; ns: no significance

Sample pretreatment and extraction

Serum samples were thawed in an ice bath after being removed from a -80 °C freezer. All subsequent steps were conducted in the same cold environment. Once thawed, the liquid samples were evenly vortexed, and 100 µL was combined with 200 µL of internal standard extraction solution (composed of methanol (Merck, Germany) and acetonitrile (Merck, Germany) in a 1:1 ratio). *Following 5 min of vortexing, the mixture was placed in a freezer at -20 ℃ and left to stand for 30 min for the proteins to precipitate. Following centrifugation at 12,000 rpm/min for 10 min at 4 ℃, the mixture was subjected to separation, resulting in the collection of the supernatant.* An activated and equilibrated Poly-Sery MAX SPE column (60 mg/3 cc, ANPEL, Shanghai, PRC) was used for solid-phase extraction, with the sample being applied, rinsed, eluted, and the eluate collected. The eluent should be concentrated to dryness, followed by dissolution in 100 µL of methanol and water in a 1:1 ratio. *After vortexing the solution for 30 s, the resulting supernatant can be extracted for further analysis using UPLC (Ultra Performance Liquid Chromatography) combined with MS/MS (Tandem Mass Spectrometry).*

LC-MS/MS analysis

The samples taken were subjected to analysis through liquid chromatography-electrospray ionization-tandem mass spectrometry (LC-ESI-MS/MS) using a UPLC system (ExionLC AD, Sciex) connected to a mass spectrometer (QTRAP® 6500+System, Sciex). The testing conditions involved an HPLC phase utilizing a Waters ACQUITY UPLC HSS T3 C18 column (100 mm × 2.1 mm i.d., 1.8 µm particle size). The liquid blend comprised of water with 0.04% acetic acid (Merck, Germany) (Solvent A) and acetonitrile with 0.04% acetic acid (Solvent B). The temperature of the column was adjusted to 40 °C, the flow rate maintained at 0.4 mL/min, and an injection volume of 10 µL was implemented. Linear ion trap (LIT) and triple quadrupole (QQQ) scans were acquired on a triple quadrupole-linear ion trap mass spectrometer (QTRAP), QTRAP® 6500+LC-MS/MS System, equipped with an ESI Turbo Ion-Spray interface, operating in negative ion mode and controlled by Analyst 1.6.3 software (Sciex). The parameters for the ESI source operation included an ion source set at ESI- with a temperature of 550 ℃, an ion spray voltage (IS) of -4500 V, and a curtain gas (CUR) set at 35 psi. Quantification was performed using the multiple reaction monitoring mode (MRM) of triple quadrupole mass spectrometry. Following the acquisition of mass spectrometry data from various samples, chromatographic peaks were integrated and quantitatively analyzed using a standard curve. Standard solutions (Merck, Germany) with concentrations ranging from 0.001 nmol/L to 400 nmol/L were prepared to correlate each concentration with the corresponding chromatographic peak intensity. Utilizing the concentration ratio of the external standard to the internal standard as the x-axis and the peak area ratio of the external standard to the internal standard as the y-axis, standard curves can be plotted for different substances. The integrated peak area ratio of all detected samples was substituted into the linear equation of the standard curve to calculate the substance content in the actual sample.

Statistical analysis

Medians with interquartile ranges (IQRs) were used to represent continuous variables, while percentages were employed for categorical variables. Statistical analyses were carried out utilizing the SPSS 23.0 software package (SPSS Inc., USA) and GraphPad Prism 9 (GraphPad Software Inc., USA). The chi-square test was used to compare qualitative data, while the Mann–Whitney U test was employed for analyzing variable comparisons between two groups. Statistical significance was considered at a level of $P<0.05$. In this study, statistical methods like Spearman rank correlation and receiver operating characteristic (ROC) analysis were applied to evaluate specific indicators' relationships and diagnostic efficiency. Furthermore, binary logistic regression in SPSS was utilized to predict combined diagnostic probability based on these indicators. The data obtained from mass spectrometry underwent processing with Analyst 1.6.3 software and MultiQuant 3.0.3. To assess the existence of statistically significant differences among groups, Orthogonal partial least squares-discriminant analysis (OPLS-DA) was employed. The Metabolites were initially assessed utilizing the Variable Importance in Projection (VIP). Subsequent analysis of metabolites with different abundance levels considered fold change values. The identification of differentially abundant metabolites was determined by meeting the criteria of a VIP>1 and fold change≥2, or a VIP>1 and fold change≤0.5 concurrently.

Pathway analysis

An analysis using the Kyoto Encyclopedia of Genes and Genomes (KEGG) database was carried out to better understand the functions of different metabolites. These metabolites were annotated and matched to pathways within the database. By utilizing Metabolite Set Enrichment Analysis (MSEA) and determining significance through hypergeometric test *P*values, pathways with significantly changed metabolites were further examined.

Results

Population demographics and clinical characteristics of hospitalized pulmonary embolism patients

A total of 6,365 hospitalized patients with thrombosis were enrolled in the study. Based on Fig. 1A-B, the proportion of venous thromboembolism (VTE) was 72.1% (4,587/6,365), and the proportion of arterial thromboembolism (ATE) was 27.9% (1,778/6,365). Among patients with VTE, 70.7% (3,242/4,587) had lower extremity deep venous thrombosis (LEDVT), with PE ranking second at 12.1% (555/4,587). The in-hospital mortality rate among the 555 patients with PE was 7.6% (42/555), as illustrated in Fig. 1C. The analysis from Fig. 1D and Table S1 indicated that the in-hospital mortality rate of PE patients rose with age. Specifically, the mortality rate was 3.8% (3/80) in the 18–44 age group, 6.1% (10/165) in the 45–64 age group, 7.6% (21/275) in the 65–84 age group, and 22.9% (8/35) in the age group of 85 and above. The mortality rate of male PE patients was greater than that of female patients at each stage. Table 1 shows that the most prevalent comorbidity among the patients was DVT, with a frequency of 50.5% (280/555), followed by pneumonia at 49.7% (276/555), hypertension at 42.2% (234/555), and respiratory failure at 21.4% (119/555). In Fig. 1E and Table S1, it is indicated that the highest in-hospital mortality rate across all comorbidities was 19.3% for cancer (16/83), followed by 17.6% for respiratory failure (21/119), and 14.3% for diabetes (13/91).

Coagulation parameters associated with pulmonary embolism

To investigate the characteristics of coagulation parameters associated with PE, a statistical analysis of relevant coagulation parameters was conducted in 555 enrolled patients with PE, including prothrombin time (PT), activated partial thromboplastin time (APTT), thrombin time (TT), fibrinogen (FIB), D-dimer, antithrombin III (AT III), protein C (PC), protein S (PS), von Willebrand factor (vWF), factor VIII (F VIII) and factor XII (F XII). According to Table 2, among these parameters, D-dimer showed the greatest increase compared to the normal reference range, with 78.0% (433/555) of patients exhibiting elevated D-dimer levels. This was followed by increased levels of F VIII activity and vWF activity, which were observed in 76.0% (422/555) and 58.9% (327/555) of patients, respectively. In contrast, AT III and F XII activities showed more pronounced reductions in 84.0% (466/555) and 54.1% (300/555) of patients, respectively.

Association of lipid levels with pulmonary embolism

The levels of six serum lipids were carried out to investigate the differences in two groups. Figure 2A-F show that individuals with PE had notably reduced serum concentrations of apoA, HDL-C, LDL-C, and TC in comparison to the HC group ($P<0.0001$). Additionally, there was a substantial increase in TG levels ($P<0.0001$) among the PE group, while apoB levels did not show a noteworthy distinction. The specific lipid levels and *P*values are listed in Table S2. According to Fig. 2G-H and Table S3, the six serum lipid levels were correlated with coagulation-related laboratory parameters. For instance, PT showed a negative correlation with serum TC, HDL-C, LDL-C, and apoA levels, with absolute correlation coefficients>0.3 ($P<0.0001$). The study identified a positive correlation between PC activity and serum levels of TC, LDL-C, apoA, apoB, and TG, with correlation coefficients exceeding 0.3 and statistical significance at $P<0.0001$. Additionally, the diagnostic performance of differential

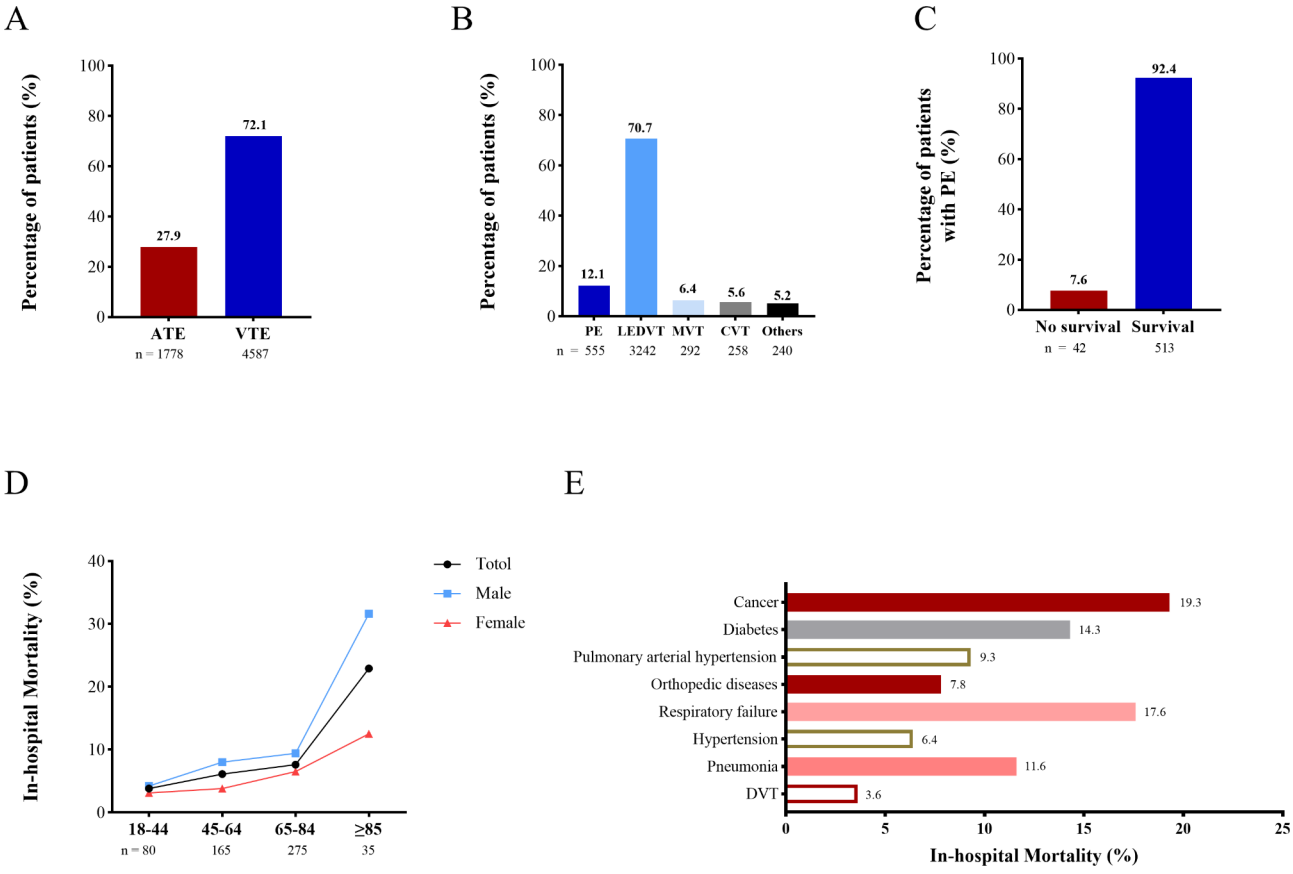

Fig. 1 Bar charts of pulmonary embolism incidence. (**A**) Bar chart showing the incidence ratio of ATE to VTE. (**B**) Bar chart showing the incidence rates of different types of VTE. (**C**) Bar chart comparing the incidence rates between the PE nonsurviving group and survival group. (**D**) Line graph showing the in-hospital mortality rates of PE patients across different age groups and genders. (**E**) Bar chart showing the in-hospital mortality rates of the top 8 comorbidities

Abbreviations: VTE: venous thromboembolism; ATE: arterial thromboembolism; LEDVT: lower extremity deep venous thrombosis; PE: pulmonary embolism; MVT: mesenteric venous thrombosis; CVT: cerebral venous thrombosis

Table 2 Main blood coagulation indexes of patients with PE ($n = 555$)

Index (unit)	Median (IQR)	Increase, *n* (%)	Decrease, *n* (%)	Reference range
PT (s)	12.2 (11.4–13.6)	111 (20.0)	6 (1.1)	9.8–12.1
APTT (s)	29.8 (26.8–33.3)	216 (38.9)	56 (10.1)	25.0-31.3
TT (s)	18.1 (17.1–19.4)	100 (18.0)	0 (0.0)	13–21
FIB (g/L)	3.4 (2.7–4.4)	161 (29.0)	39 (7.0)	2.0–4.0
D-Dimer (mg/L)	4.34 (1.93–8.17)	433 (78.0)	0 (0.0)	< 0.5
AT III (%)	88.8 (77.4–97.6)	33 (6.0)	466 (84.0)	103.2-113.8
PC (%)	97.7 (79.6-112.1)	61 (11.0)	39 (7.0)	60–130
PS (%)	89.6 (68.1-118.6)	72 (13.0)	155 (27.9)	63.5–149.0
vWF (%)	196 (167.9-260.3)	327 (58.9)	0 (0.0)	49.5–187.0
F VIII (%)	175.8 (130.9-225.3)	422 (76.0)	11 (2.0)	77.3-128.7
F XII (%)	47.6 (35.6–66.9)	11 (2.0)	300 (54.1)	50–120

Abbreviations: PE: pulmonary embolism; IQR: interquartile range; n: number; PT: prothrombin time; APTT: activated partial thromboplastin time; TT: thrombin time; FIB: fibrinogen; AT III: antithrombin III; PC: protein C; PS: protein S; vWF: von Willebrand factor; F VIII: factor VIII; F XII: factor XII

lipids for PE was evaluated through ROC analysis. Based on Fig. 3A-E, the AUC values for TC, apoA, HDL-C, LDL-C, and TG were 0.763, 0.893, 0.886, 0.721, and 0.622, respectively. Additionally, according to Fig. 3G, the combined AUC values for TC, apoA, HDL-C and LDL-C were 0.903. Furthermore, the AUC for D-dimer was 0.945 according to Fig. 3F. When combined with the aforementioned 4 serum lipids (TC, apoA, HDL-C, and LDL-C), the AUC increased to 0.979 as indicated in Fig. 3H. To investigate the correlation among lipid levels and PE

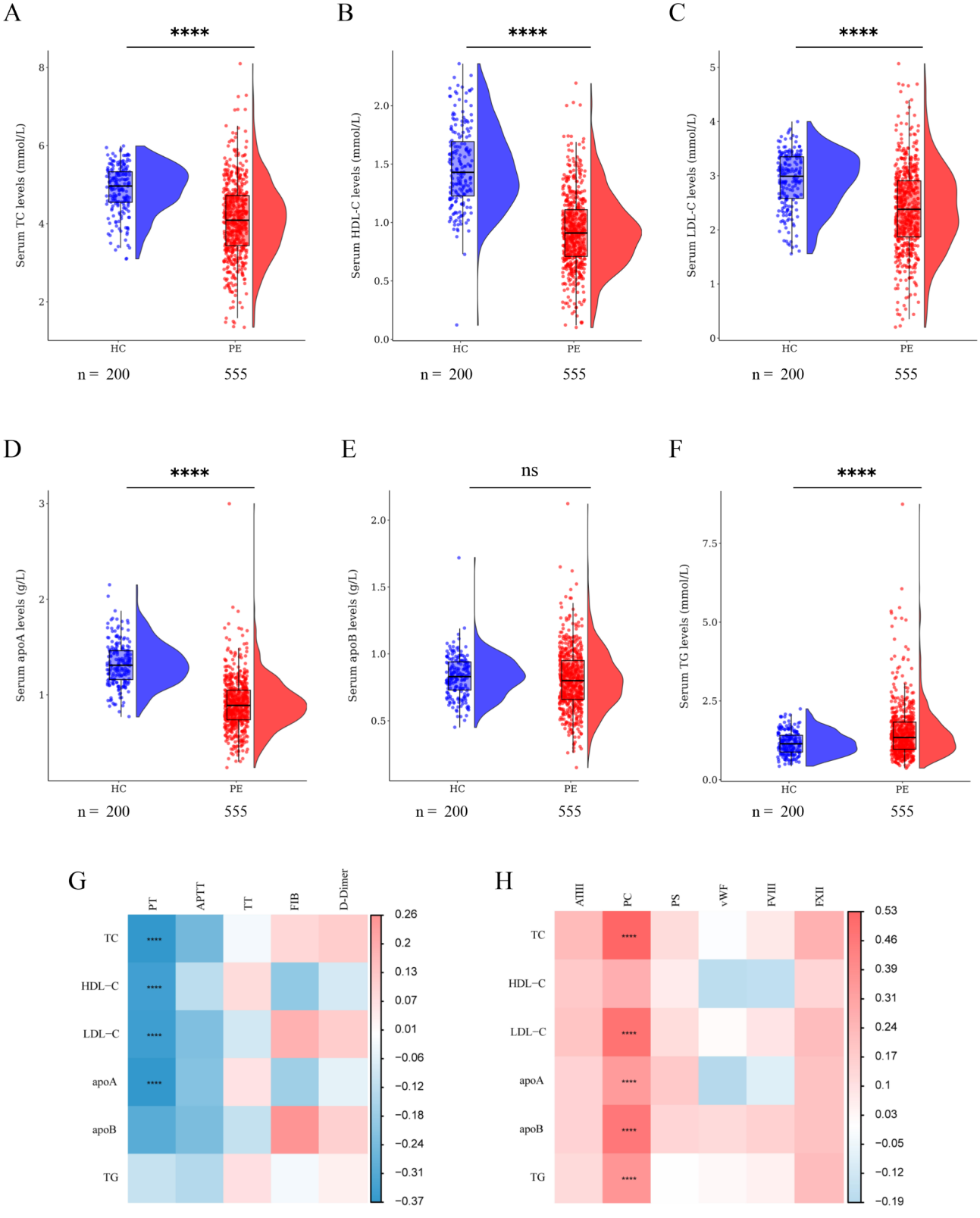

Fig. 2 (See legend on next page.)

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Fig. 2 Cloud and rain plots illustrating the differences in serum lipid levels between the PE group ($n=555$) and the HC group ($n=200$), along with a heatmap showing the correlation analysis between serum lipid levels and coagulation-related laboratory indicators within the PE group. (**A**) Cloud and rain plot showing the difference in serum TC levels between the PE group and HC group. (**B**) Cloud and rain plot showing the difference in serum HDL-C levels between the PE group and HC group. (**C**) Cloud and rain plot showing the difference in serum LDL-C levels between the PE group and HC group. (**D**) Cloud and rain plot showing the difference in serum apoA levels between the PE group and HC group. (**E**) Cloud and rain plot showing the difference in serum apoB levels between the PE group and HC group. (**F**) Cloud and rain plot showing the difference in serum triglyceride (TG) levels between the PE group and HC group (Mann-Whitney U test for quantitative data comparison between two groups, **** represents $P<0.0001$, ns represents no significance). (**G**) Heatmap showing the correlation between serum lipid levels (6 items) and coagulation-related indicators (5 items). The legend represents the correlation coefficient, with red and blue indicating positive and negative correlations, respectively, and the depth of color indicating the degree of correlation. The presence of "*" indicates an absolute correlation coefficient ($|r|>0.3$, ****$P<0.0001$, Spearman rank correlation analysis). (**H**) Heatmap showing the correlation between serum lipid levels (6 items) and coagulation factor levels. The legend represents the correlation coefficient, with red and blue indicating positive and negative correlations, respectively, and the depth of color indicating the degree of correlation. The presence of "*" indicates an absolute correlation coefficient ($|r|>0.3$, ****$P<0.0001$, Spearman rank correlation analysis)

Abbreviations: HC: healthy control; PE: pulmonary embolism; TC: total cholesterol; HDL-C: high-density lipoprotein cholesterol; LDL-C: low-density lipoprotein cholesterol; apoA: apolipoprotein A1; apoB: apolipoprotein B; TG: triglyceride; PT: prothrombin time; APTT: activated partial thromboplastin time; TT: thrombin time; FIB: fibrinogen; AT III: antithrombin III; PC: protein C; PS: protein S; vWF: von Willebrand factor; F VIII: factor VIII; F XII: factor XII

severity, the 555 hospitalized patients with PE were categorized into survival and nonsurviving groups. As shown in Fig. 4, the nonsurviving group displayed significantly lower levels of TC, HDL-C, LDL-C, and apoA compared to the survival group ($P<0.05$), with HDL-C and apoA exhibiting the most pronounced differences ($P<0.0001$). Additionally, the AUC values for serum HDL-C and apoA were 0.704 and 0.711, respectively, with a combined AUC of 0.712 as depicted in Fig. 3I-K. Detailed lipid levels and corresponding *P* values can be found in Table S4.

Serum oxylipin profiling analysis between pulmonary embolism and healthy control groups and upregulated differentially oxidized lipid metabolites widely concentrated in the ARA metabolism signaling pathway

120 samples each from the PE group and HC group were randomly selected to compare their serum oxylipin levels. Oxylipin profiling analysis on these serum samples were performed using LC-MS/MS. In total, 118 oxylipin metabolites were detected, and detailed information on the linear equation of the standard curve, retention time (RT), m/z values, and fragmentation pattern for each oxidized lipid was provided in Table S5. Furthermore, Figures S1 and S2 illustrated the individual extracted ion chromatogram (EIC) from a healthy individual and a patient with PE, respectively. Additionally, the characteristics and main clinical laboratory profiles for these 120 patients with PE and 120 healthy controls were detailed in Tables S7 and S8. These tables indicated that there were no significant differences in the clinical characteristics and primary laboratory indicators between the 120 PE patients and the 555 PE patients, nor between the 120 healthy controls and the 200 healthy controls. Using the OPLS-DA method, VIP scores were obtained, and differences were considered significant when the VIP was >1 and the fold change was ≥2 or ≤0.5. As shown in Fig. 5A-B, differences in the oxylipin profiles among the two groups were identified. Specifically, 10 upregulated (PE vs. HC) and 6 downregulated (PE vs. HC) oxylipin metabolites were identified, while 102 metabolites showed nonsignificant differences. According to Fig. 5C, the categories of differentially abundant metabolites included arachidonic acid (ARA), dihomogamma-linolenic acid (DGLA), docosahexaenoic acid (DHA), eicosapentaenoic acid (EPA), and linoleic acid (LA). Figure 5D illustrated the original intensities of the 16 metabolites with varying abundance, and more information about these 16 oxylipin compounds can be found in Tables S5 and S6. Based on the Fig. 5E and Table S6, this study found that the oxylipin with the most significant increase in abundance in the PE group was 20-hydroxyPGF2α, while the most notable decrease was observed in LXA4. Using the metabolite abundance analysis, an enrichment analysis of KEGG pathways was then performed. The results shown in Fig. 5F revealed that these 16 different metabolites were mainly enriched in the ARA pathway. Additionally, the transformation of Polyunsaturated fatty acids (PUFAs) into oxylipins via primary enzymatic pathways was depicted in Figure S3.

Discussion

PE represents a significant clinical concern, with issues such as misdiagnosis, underdiagnosis, delayed diagnosis, and inadequate treatment, especially concerning thrombolysis and anticoagulant therapy, contributing to high mortality rates [4]. According to a study conducted by Lee and colleagues, the incidence of venous thromboembolism in Asian populations was approximately 15–20% of that reported in Western countries between 1995 and 2016 [19]. However, there have been few reports on recent data specifically focusing on pulmonary embolism in China. In this study, the incidence, mortality, and associated comorbidities of PE were first analyzed in our hospital from 2015 to 2023 and found that over the past nine years, the incidence of PE accounted for 12.1% of venous thromboembolism cases. Among the 555 patients diagnosed with PE, the mortality rate during hospitalization was 7.6%. Furthermore, the rate of mortality during

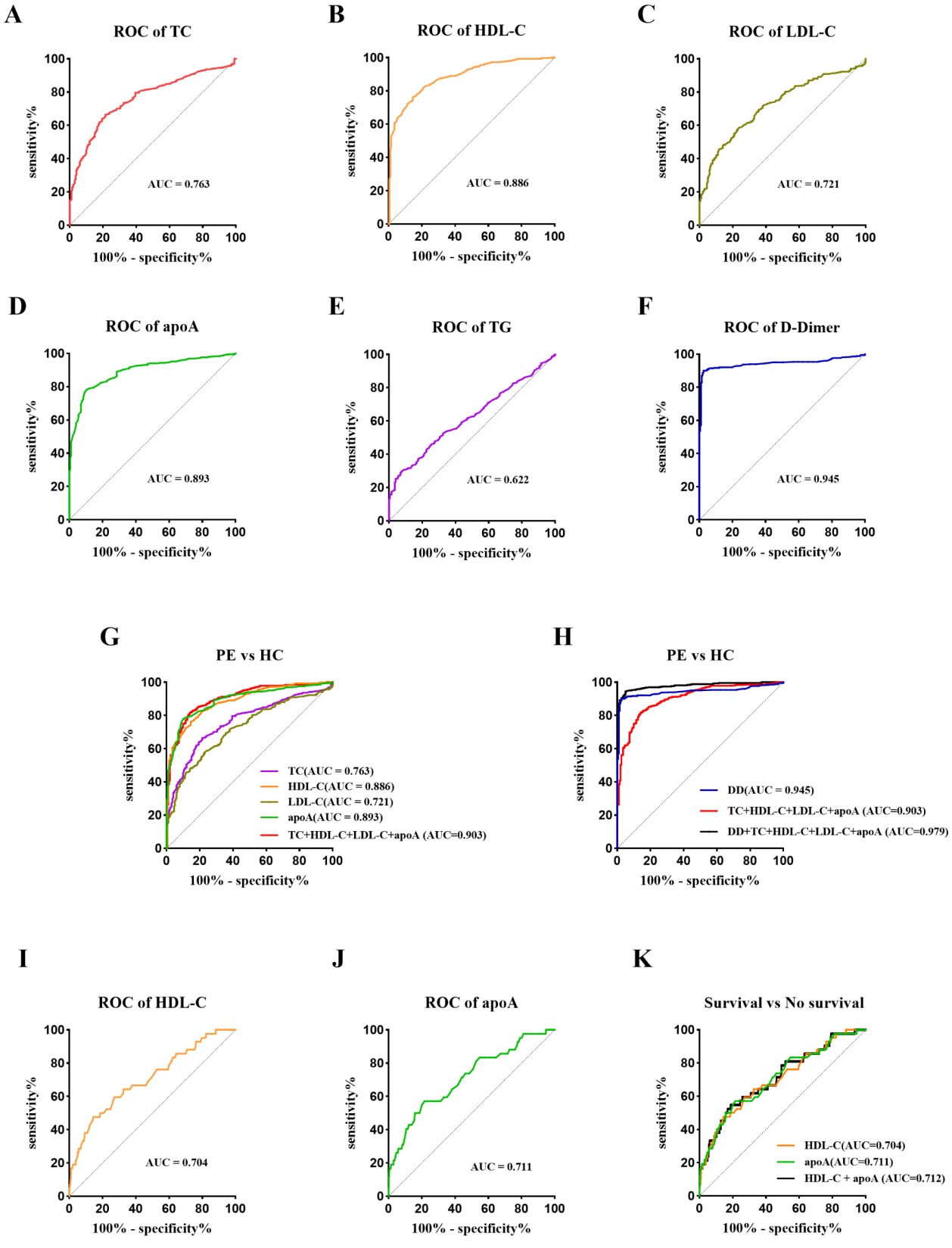

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Fig. 3 Receiver operating characteristic (ROC) curves of patients with PE ($n=555$), comprising the survival group ($n=513$) and the non-survival group ($n=42$), alongside the HC group ($n=200$). (**A**) ROC curve of serum TC for distinguishing patients with PE from HCs. (**B**) ROC curve of serum HDL-C for distinguishing patients with PE from HCs. (**C**) ROC curve of serum LDL-C for distinguishing patients with PE from HCs. (**D**) ROC curve of serum apoA for distinguishing patients with PE from HCs. (**E**) ROC curve of serum TG for distinguishing patients with PE from HCs. (**F**) ROC curve of serum D-dimer for distinguishing patients with PE from HCs. (**G**) ROC joint diagnostic analysis of serum TC, HDL-C, LDL-C, and apoA for distinguishing patients with PE from HCs. (**H**) ROC joint diagnostic analysis of serum TC, HDL-C, LDL-C, apoA, and serum D-dimer for distinguishing patients with PE from HCs. (**I**) ROC curve of serum HDL-C for distinguishing between the PE nonsurviving group and the survival group. (**J**) ROC curve of serum apoA for distinguishing between the PE nonsurviving group and the survival group. (**K**) ROC joint diagnostic analysis of serum HDL-C and apoA for distinguishing between the PE nonsurviving group and the survival group

Abbreviations: HC: healthy control; PE: pulmonary embolism; TC: total cholesterol; HDL-C: high-density lipoprotein cholesterol; LDL-C: low-density lipoprotein cholesterol; apoA: apolipoprotein A1; apoB: apolipoprotein B; TG: triglyceride

hospitalization due to PE increased with age, peaking at 22.9% in the 85 years and older age group. Age is the greatest risk factor for thrombus formation, possibly due to factors such as hypercoagulability, endothelial cell aging, venous stasis, and increased chronic inflammation [20]. The study revealed that male patients suffered from a greater mortality rate in comparison to females across all age groups. Some studies have also noted these gender differences [21, 22]. Compared to the HC group, the PE group showed significantly elevated coagulation-related parameters such as F VIII and vWF activities, while AT III and F XII activities were notably reduced. Lipid-related parameters including TC, HDL-C, LDL-C, and apoA exhibited a significant decrease in patients with PE, with AUCs exceeding 0.9. By analyzing 118 oxidized lipid metabolites in serum samples, PE patient serum revealed 10 upregulated oxidized lipid metabolites, with the most significant difference observed in 20-hydroxyPGF2α derived from ARA, and upregulated differential oxidized lipid metabolites were predominantly concentrated in the ARA metabolism signaling pathway.

Previous studies have shown that coagulation factors associated with a predisposition to PE thrombosis include F V Leiden mutation; elevated levels of F VIII and F IX; as well as insufficiencies in AT III, protein C, and protein S [23, 24]. In this study, the coagulation-related indicators of all hospitalized PE patients in our hospital were analyzed. It was found that compared to those in the normal reference range, the indicators with greater elevations in PE patients were D-dimer, F VIII activity, and vWF activity, while AT III and F XII activity were notably decreased. *Elevated blood D-dimer levels occur during thrombosis formation* [25]. D-Dimer testing exhibits a negative predictive value ranging from 97 to 100% in the diagnosis of PE and is typically part of the diagnostic strategy for ruling out PE [26, 27]. The F VIII circulates binds with vWF, enhancing the stability of F VIII, and binds with the subendothelial matrix, facilitating platelet adhesion at the injury site [28]. F VIII is recognized as a possible risk factor for both initial and recurrent VTE [29]. Over the last ten years, various extensive studies on genome-wide associations have shown a link between genetic predisposition to PE, and vWF levels, identified through single nucleotide polymorphisms [30, 31]. AT III deficiency can lead to venous thrombosis [32]. The activation of F XII plays a role in stabilizing thrombi, whereas a deficiency in F XII renders thrombi more prone to embolism [33, 34]. These findings are consistent with this finding that the indicators of elevated levels in PE patients are D-dimer, F VIII activity, and vWF activity, while AT III and F XII activity are significantly reduced.

Increasing evidence suggests a mutual association between coagulation and lipid metabolism. Hiroshi Deguchi et al. reported that blood lipids can regulate coagulation factor activity and may influence the risk of thrombosis formation [35]. Numerous investigations have performed proteomic studies on isolated lipoproteins, unveiling proteins that control lipid metabolism. This includes a variety of proteins that participate in the process of blood clotting [36, 37]. According to this research, the TC, HDL-C, LDL-C, and apoA levels upon initial admission were notably reduced among 555 hospitalized patients with PE compared to the HC group ($P<0.0001$). Conversely, TG levels were observed to be elevated in the PE group ($P<0.0001$). This trend aligns with a previous study that included 246 DVT patients and 254 non-DVT patients [38]. Furthermore, for the first time, it was observed that the combined diagnostic AUC of TC, HDL-C, LDL-C, and apoA exceeded 0.9. D-dimer levels increase during thrombus formation; it was found that the AUC for D-Dimer alone was 0.945. Interestingly, the combined diagnostic AUC for serum HDL-C, TC, LDL-C, and apoA levels reached 0.979. Additionally, for the first time the correlation between the six lipid indicators and coagulation-related indicators in the PE group were analyzed and found that PT values showed a negative correlation with TC (r = -0.3705, $P<0.0001$), HDL-C (r = -0.3436, $P<0.0001$), LDL-C (r = -0.3513, $P<0.0001$), and apoA (r = -0.3670, $P<0.0001$) levels, while PC activity exhibited a positive correlation with TC ($r=0.5304$, $P<0.0001$), LDL-C ($r=0.4780$, $P<0.0001$), apoA ($r=0.3396$, $P<0.0001$), apoB ($r=0.4633$, $P<0.0001$), and TG ($r=0.3654$, $P<0.0001$) levels. Protein C (PC) is an important physiologic anticoagulant that depends on vitamin K and is capable of inactivating F Va and F VIIIa. Deficiency in protein C increases the risk of

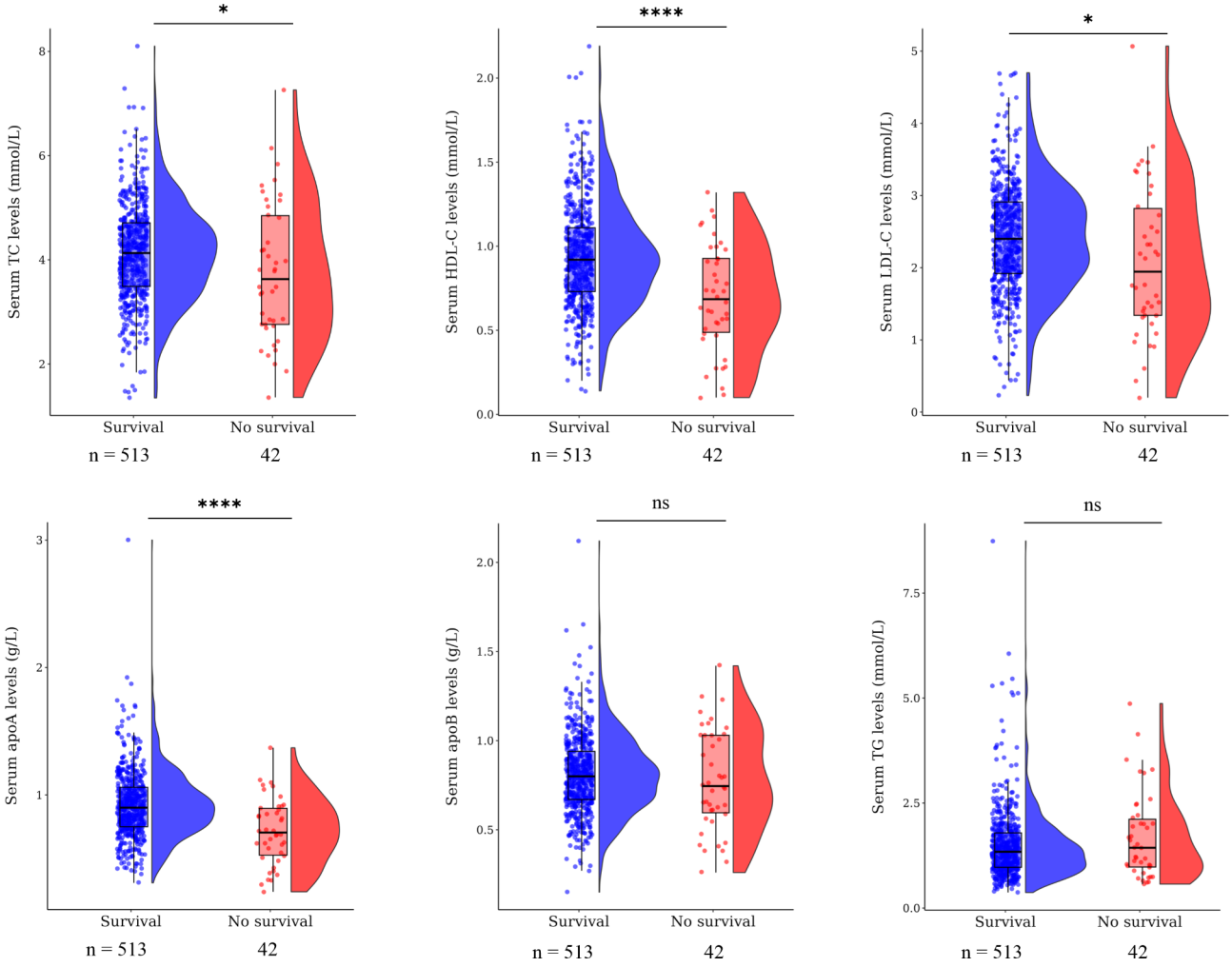

Fig. 4 Cloud and rain plots showing differences in the levels of six blood lipids between the survival group ($n=513$) and non-survival group ($n=42$) within the PE group. (Quantitative data differences between the two groups were compared using the Mann–Whitney U test, where **** $P<0.0001$, * $P<0.05$, and ns means not significant.)
Abbreviations: TC: total cholesterol; HDL-C: high-density lipoprotein cholesterol; LDL-C: low-density lipoprotein cholesterol; apoA: apolipoprotein A1; apoB: apolipoprotein B; TG: triglyceride

thrombosis [23]. The strong correlation between lipids and PCs reflects a certain association between lipids and coagulation. Subsequently, the PE group was divided into survival and nonsurviving groups. Our analysis revealed that the nonsurviving group had notably lower serum levels of TC, HDL-C, LDL-C, and apoA in comparison to the survival group ($P<0.05$). Among them, the most notable differences were observed for HDL-C and apoA ($P<0.0001$), with the combined diagnostic AUC of serum HDL-C and apoA surpassing 0.7. This finding aligns with previous research conducted by Karataş MB et al., indicating that the levels of HDL-C and apoA during admission could potentially serve as prognostic indicators for short-term mortality in patients with PE [39].

Unbound polyunsaturated fatty acids (PUFAs) undergo oxidation primarily through three enzymes: cyclooxygenase (COX), lipoxygenase (LOX), and cytochrome P450 (CYP), leading to the formation of various oxylipins with either pro-thrombotic or anti-thrombotic effects [40]. *The COX pathway produces prostanoids such as prostaglandins (PG) and thromboxanes (Tx). The LOX pathway generates hydroperoxy-PUFAs, which can be rearranged into monohydroxy-PUFAs or further converted through LOX-catalyzed reactions into leukotrienes (LT) and various dihydroxy- and trihydroxy-PUFAs, including specialized pro-resolving mediators such as lipoxins, resolvins, and protectins. Additionally, the CYP pathway primarily produces ω-/ω-1 hydroxy- and epoxy-PUFAs, with the epoxides being further transformed into vicinal (i.e., adjacent or 1,2-) dihydroxy-PUFAs* [41]. The study identified a total of 118 metabolites of oxylipin, with 10 upregulated (PE vs. HC) and 6 downregulated metabolites (PE vs. HC). The categories of upregulated differentially abundant metabolites included LXB4, 12-HHT, TXB2, 6-keto-PGF1α,

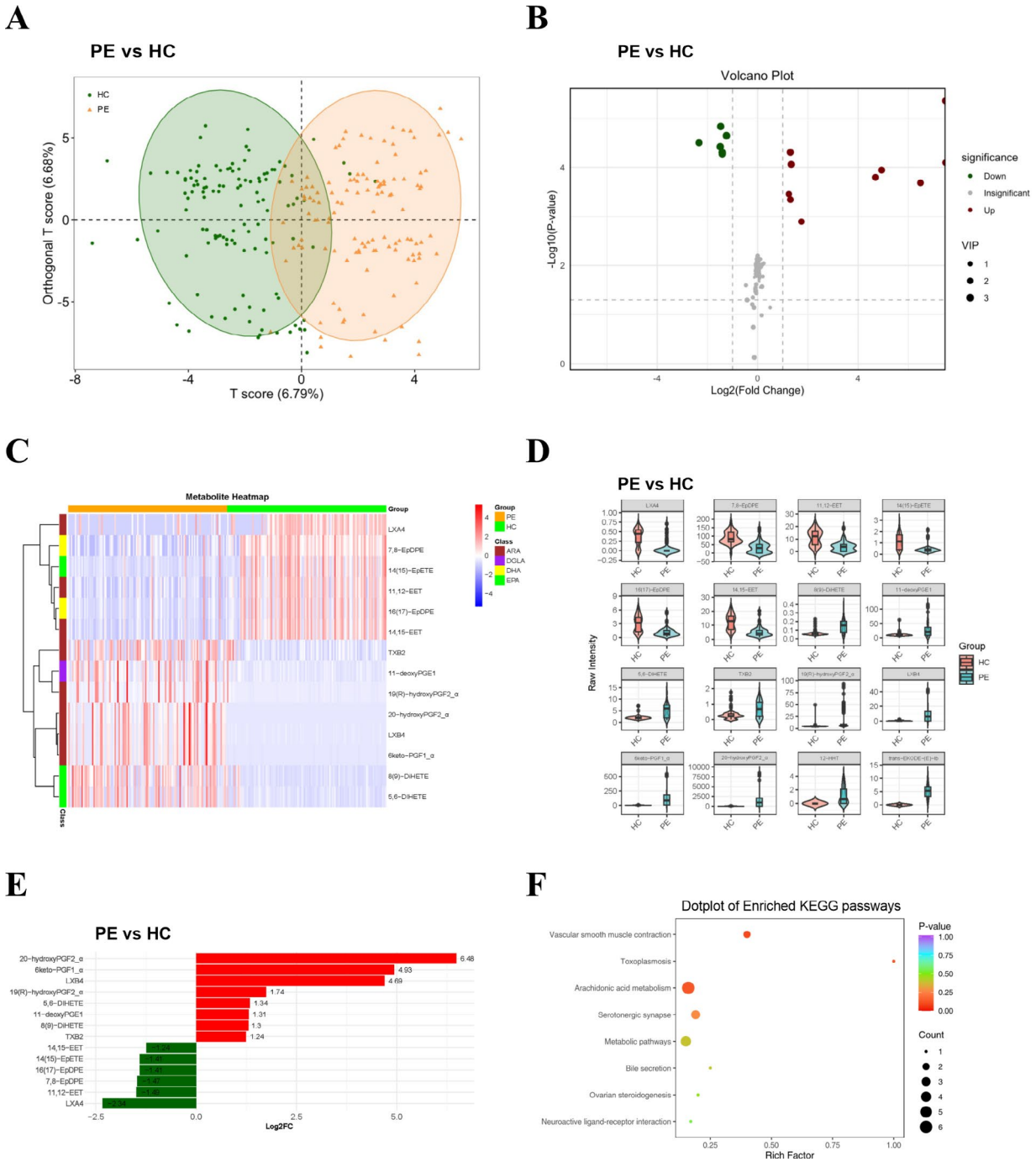

Fig. 5 Serum oxylipin spectrum analysis of patients with PE ($n = 120$) compared to healthy controls ($n = 120$). (**A**) The score plot of OPLS-DA comparing PE patients with HCs. (**B**) Volcano plot of differentially expressed oxylipins in patients with PE vs. HCs. Each point in the volcano plot represents a metabolite, where red points indicate upregulated differentially expressed metabolites (VIP > 1, fold change ≥ 2), green points indicate downregulated differentially expressed metabolites (VIP > 1, fold change ≤ 0.5), and gray points indicate detected but not significantly different metabolites. The size of the circle represents the VIP value. (**C**) Heatmap showing the expression levels of 16 differentially expressed oxylipins in patients with PE vs. HCs. The scale represents normalized expression levels, with red indicating higher expression and green indicating lower expression. (**D**) The raw intensity of 16 differentially expressed oxylipins in patients with PE vs. HCs. (**E**) Bar chart showing the fold changes in the levels of differentially expressed oxylipins. Red bars represent upregulated differentially expressed metabolites (VIP > 1, fold change ≥ 2), while green bars represent downregulated differentially expressed metabolites (VIP > 1, fold change ≤ 0.5). (**F**) KEGG pathway enrichment analysis of the 16 differentially expressed oxylipins in patients with PE vs. HCs. The x-axis represents the Rich factor corresponding to each pathway, the y-axis represents the pathway name, the color of the points represents the *P* value, with redder colors indicating more significant enrichment, and the size of the points represents the number of differentially expressed metabolites enriched in the pathway

20-hydroxyPGF2α, and 19(R)-hydroxyPGF2α derived from ARA. 5,6-DiHETE and 8(9)-DiHETE are derived from eicosapentaenoic acid (EPA), 11-deoxyPGE1 is derived from dihomoγ-linolenic acid (DGLA), and trans-EKODE-(E)-Ib is derived from linoleic acid (LA). Among these differential oxylipins, 3 are part of the PG family. PGs are metabolites of arachidonic acid that are oxygenated and produced sequentially by COX and their corresponding synthases [42], and PGF2a is one of the most stable PGs and that acts as a key signaling and biologically active molecule in regulating complex physiological processes. Hitoshi Kashiwagi and colleagues discovered in mouse models that PGF2 enhances platelet activation via the EP3 and TP receptors for PGE2 and thromboxane A2, respectively [43]. *Apolipoprotein A1 (APO A-1) is the primary apolipoprotein found in the serum of HDLs.* Recent research has demonstrated its positive impact on both atherosclerosis and thrombosis [44]. *Jones WL et al. investigated the*in vivo*impact of apoA-1 on carotid artery thrombosis and pulmonary embolism in mouse models.* Their findings showed that exogenous human apoA-I inhibited platelet aggregation triggered by arachidonic acid and collagen, Additionally, it reduced the levels of P-selectin and inhibited Akt activation on the platelet surface, resulting in decreased thrombus strength [45]. This is consistent with this finding that serum apoA levels are decreased and ARA-derived oxylipins are significantly increased in the PE group. When comparing our results to those of previous studies, it should be noted that upregulation of the arachidonic acid metabolism signaling pathway may be an important pathogenic factor for PE. This finding suggests the potential utility of ARA-derived metabolites in the treatment or as biomarkers of PE. *This study utilized lipidomic techniques to analyze the initial serum samples collected from enrolled patients during their first hospitalization, prior to the commencement of any drug treatment. It offers new insights into the mechanisms underlying blood clot formation, highlighting the significance of lipids in the initiation and progression of pulmonary embolism.*

Study strengths and limitations

This study possessed numerous unique advantages. First, the data used in this study were obtained from different populations of thrombotic patients, including VTE, ATE and PE patients and so on, leading to representative results. Second, the research focused on exploring the relationship between lipid mediators and PE, with a specific emphasis on the different types of oxidized lipids discovered. Finally, this study offers valuable insights into the pathogenesis of PE, offering potential avenues for predicting and diagnosing PE and reducing its high mortality rate. However, this study was constrained by its sample size and observational methodology, suggesting that any connection between oxidized lipids and PE can be inferred only through correlation analysis. Moreover, additional investigation is essential to elucidate the specific mechanisms of various oxidized lipids in PE.

Conclusions

Overall, this study revealed a significant correlation between lipid mediators, particularly oxylipins, and PE. *Importantly, the upregulation of the arachidonic acid metabolism signaling pathway may be a significant pathogenic factor for PE. Among the 16 differentially oxidized lipid metabolites identified, the oxylipin with the most significant increase in abundance in the PE group was 20-hydroxyPGF2α. Notably, 20-hydroxyPGF2α, derived from arachidonic acid (ARA), shows promise as a potential biomarker for PE. These findings provide new insights into the pathogenesis of PE and present compelling evidence for potential preventive measures to reduce mortality rates.* Healthcare providers should regularly monitor the lipid status of individuals at high risk of clinical PE. Nevertheless, additional mechanistic studies are needed to improve the comprehension of the complex connection between PE and oxidized lipids.

Supplementary Information

The online version contains supplementary material available at https://doi.org/10.1186/s12944-024-02315-6.

Supplementary Material 1

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Author contributions

M.X. and H.S. designed the study. D.B.P. and F.C. conducted the experiments. H.S., M.X. and F.C. were responsible for data collection and writing the paper. D.B.P., Y.Y.X., and S.H.X. assisted with the experiments and statistical analysis. The final manuscript was reviewed and approved by the authors.

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Data availability

No datasets were generated or analysed during the current study.

Declarations

Ethics approval and consent to participate

This study was conducted in accordance with the ethical principles described in the Declaration of Helsinki and was approved by the Ethics Committee of the Nanjing Drum Tower Hospital affiliated with Nanjing University Medical School (Project Number: 2022-360-03). Informed written consent was obtained from all participants prior to their enrollment in this study.

Consent for publication

Not applicable.

Competing interests
The authors declare no competing interests.

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References

1. Tsao CW, Aday AW, Almarzooq ZI, et al. Heart disease and stroke statistics-2022 update: a report from the American Heart Association. Circulation. 2022;145:e153–639.
2. Wolberg AS, Rosendaal FR, Weitz JI, et al. Venous thrombosis. Nat Rev Dis Primers. 2015;1:15006.
3. Walter K, What. Is pulmonary embolism? JAMA. 2023;329:104.
4. Goldhaber SZ, Visani L, De Rosa M. Acute pulmonary embolism: clinical outcomes in the International Cooperative Pulmonary Embolism Registry (ICOPER). Lancet. 1999;353:1386–9.
5. Pollack CV, Schreiber D, Goldhaber SZ, et al. Clinical characteristics, management, and outcomes of patients diagnosed with acute pulmonary embolism in the emergency department: initial report of EMPEROR (Multicenter emergency medicine pulmonary embolism in the real world registry). J Am Coll Cardiol. 2011;57:700–6.
6. Luijten D, de Jong CMM, Ninaber MK, et al. Post-pulmonary embolism syndrome and functional outcomes after acute pulmonary embolism. Semin Thromb Hemost. 2023;49:848–60.
7. Ding WY, Protty MB, Davies IG, et al. Relationship between lipoproteins, thrombosis, and atrial fibrillation. Cardiovasc Res. 2022;118:716–31.
8. Yuan S, Xu F, Zhang H, et al. Proteomic insights into modifiable risk of venous thromboembolism and cardiovascular comorbidities. J Thromb Haemost. 2024;22:738–48.
9. Brill A, Yesilaltay A, De Meyer SF, et al. Extrahepatic high-density lipoprotein receptor SR-BI and apoA-I protect against deep vein thrombosis in mice. Arterioscler Thromb Vasc Biol. 2012;32:1841–7.
10. Kunutsor SK, Seidu S, Khunti K. Statins and primary prevention of venous thromboembolism: a systematic review and meta-analysis. Lancet Haematol. 2017;4:e83–93.
11. Glynn RJ, Danielson E, Fonseca FA, et al. A randomized trial of rosuvastatin in the prevention of venous thromboembolism. N Engl J Med. 2009;360:1851–61.
12. Meng YW, Liu JY. Pathological and pharmacological functions of the metabolites of polyunsaturated fatty acids mediated by cyclooxygenases, lipoxygenases, and cytochrome P450s in cancers. Pharmacol Ther. 2024;256:108612.
13. Tourdot BE, Ahmed I, Holinstat M. The emerging role of oxylipins in thrombosis and diabetes. Front Pharmacol. 2014;4:176.
14. Rodriguez-Carrio J, Coras R, Alperi-Lopez M, et al. Profiling of serum oxylipins during the earliest stages of rheumatoid arthritis. Arthritis Rheumatol. 2021;73:401–13.
15. Back M. Icosapent ethyl in cardiovascular prevention: resolution of inflammation through the eicosapentaenoic acid - resolvin E1 - ChemR23 axis. Pharmacol Ther. 2023;247:108439.
16. Yeung J, Adili R, Yamaguchi A, et al. Omega-6 DPA and its 12-lipoxygenase-oxidized lipids regulate platelet reactivity in a nongenomic PPARalpha-dependent manner. Blood Adv. 2020;4:4522–37.
17. Stanger L, Yamaguchi A, Yalavarthi P, et al. The oxylipin analog CS585 prevents platelet activation and thrombosis through activation of the prostacyclin receptor. Blood. 2023;142:1556–69.
18. Yeung J, Tourdot BE, Adili R, et al. 12(S)-HETrE, a 12-lipoxygenase oxylipin of dihomo-gamma-linolenic acid, inhibits thrombosis via galphas signaling in platelets. Arterioscler Thromb Vasc Biol. 2016;36:2068–77.
19. Lee LH, Gallus A, Jindal R, et al. Incidence of venous thromboembolism in Asian populations: a systematic review. Thromb Haemost. 2017;117:2243–60.
20. Esmon CT. Basic mechanisms and pathogenesis of venous thrombosis. Blood Rev. 2009;23:225–9.
21. Lee CH, Cheng CL, Lin LJ, et al. Epidemiology and predictors of short-term mortality in symptomatic venous thromboembolism. Circ J. 2011;75:1998–2004.
22. Gillum RF. Pulmonary embolism and thrombophlebitis in the United States, 1970–1985. Am Heart J. 1987;114:1262–4.
23. Seligsohn U, Lubetsky A. Genetic susceptibility to venous thrombosis. N Engl J Med. 2001;344:1222–31.
24. Goldhaber SZ. Pulmonary embolism. Lancet. 2004;363:1295–305.
25. Freund Y, Cohen-Aubart F, Bloom B. Acute pulmonary embolism: a review. JAMA. 2022;328:1336–45.
26. van Es N, van der Hulle T, van Es J, et al. Wells rule and d-Dimer testing to rule out pulmonary embolism: a systematic review and individual-patient data meta-analysis. Ann Intern Med. 2016;165:253–61.
27. Carrier M, Righini M, Djurabi RK, et al. VIDAS D-dimer in combination with clinical pre-test probability to rule out pulmonary embolism. A systematic review of management outcome studies. Thromb Haemost. 2009;101:886–92.
28. Samuelson Bannow B, Recht M, Negrier C, et al. Factor VIII: long-established role in haemophilia A and emerging evidence beyond haemostasis. Blood Rev. 2019;35:43–50.
29. Vormittag R, Simanek R, Ay C, et al. High factor VIII levels independently predict venous thromboembolism in cancer patients: the cancer and thrombosis study. Arterioscler Thromb Vasc Biol. 2009;29:2176–81.
30. Sabater-Lleal M, Huffman JE, de Vries PS, et al. Genome-wide association transethnic meta-analyses identifies novel associations regulating coagulation factor VIII and von willebrand factor plasma levels. Circulation. 2019;139:620–35.
31. Smith NL, Rice KM, Bovill EG, et al. Genetic variation associated with plasma von Willebrand factor levels and the risk of incident venous thrombosis. Blood. 2011;117:6007–11.
32. Hirsh J. Blood tests for the diagnosis of venous and arterial thrombosis. Blood. 1981;57:1–8.
33. Konings J, Govers-Riemslag JW, Philippou H, et al. Factor XIIa regulates the structure of the fibrin clot independently of thrombin generation through direct interaction with fibrin. Blood. 2011;118:3942–51.
34. Kuijpers MJ, van der Meijden PE, Feijge MA, et al. Factor XII regulates the pathological process of thrombus formation on ruptured plaques. Arterioscler Thromb Vasc Biol. 2014;34:1674–80.
35. Deguchi H, Elias DJ, Griffin JH. Minor plasma lipids modulate clotting factor activities and may affect thrombosis risk. Res Pract Thromb Haemost. 2017;1:93–102.
36. Dashty M, Motazacker MM, Levels J, et al. Proteome of human plasma very low-density lipoprotein and low-density lipoprotein exhibits a link with coagulation and lipid metabolism. Thromb Haemost. 2014;111:518–30.
37. Lin M, Li M, Zheng H, et al. Lipoprotein proteome profile: novel insight into hyperlipidemia. Clin Transl Med. 2021;11:e361.
38. Huang Y, Ge H, Wang X, et al. Association between blood lipid levels and lower extremity deep venous thrombosis: a population-based cohort study. Clin Appl Thromb Hemost. 2022;28:10760296221121282.
39. Karatas MB, Gungor B, Ipek G, et al. Association of serum cholesterol levels with short-term mortality in patients with acute pulmonary embolism. Heart Lung Circ. 2016;25:365–70.
40. Stanger L, Holinstat M. Bioactive lipid regulation of platelet function, hemostasis, and thrombosis. Pharmacol Ther. 2023;246:108420.
41. Massey KA, Nicolaou A. Lipidomics of oxidized polyunsaturated fatty acids. Free Radic Biol Med. 2013;59:45–55.
42. Hirata T, Narumiya S. Prostanoid receptors. Chem Rev. 2011;111:6209–30.
43. Kashiwagi H, Yuhki KI, Imamichi Y, et al. Prostaglandin F2alpha facilitates platelet activation by acting on prostaglandin E2 receptor subtype EP3 and thromboxane A2 receptor TP in mice. Thromb Haemost. 2019;119:1311–20.
44. Cochran BJ, Ong KL, Manandhar B, et al. APOA1: a protein with multiple therapeutic functions. Curr Atheroscler Rep. 2021;23:11.
45. Jones WL, Ramos CR, Banerjee A, et al. Apolipoprotein A-I, elevated in trauma patients, inhibits platelet activation and decreases clot strength. Platelets. 2022;33:1119–31.

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