Diagnostic value of platelet-derived microparticles in pulmonary thromboembolism: A population-based study

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Abstract. An early and accurate diagnosis of pulmonary thromboembolism (PTE) remains challenging. The present study aimed to evaluate the diagnostic value of platelet-derived microparticles in PTE based on a population study. A total of 102 patients with PTE, 102 healthy controls and 40 patients suspected with PTE were enrolled in this study. The platelet count, mean platelet volume and platelet distribution width were assessed using an automated hematology analyzer, P-selectin was assessed using an ELISA kit and PMPs were explored using flow cytometry using Megamix beads. Receiver operating characteristic curves were established to evaluate the diagnostic values of PMPs, D-dimer, PMPs combined with D-dimer, and multiple parameters (including PMPs, platelet distribution width, P-selectin and D-dimer in PTE). The PMP levels were significantly higher in the patients with PTE (609.10/μl) compared with those in the healthy controls (230.60/μl) and patients with suspicious PTE (166.70/μl; P<0.01). The accuracy (72.06%) of PMPs in the diagnosis of PTE was similar to those of D-dimer (P>0.05). The combination of D-dimer and PMPs significantly increased the sensitivity (86.27%) of D-dimer and the specificity of PMP for the diagnosis of PTE (P<0.01). The combination of PMPs, platelet distribution width, P-selectin and D-dimer exhibited high sensitivity (88.24%), specificity (91.18%) and accuracy (89.71%) in the diagnosis of PTE. These findings suggest that elevated PMP levels are an effective predictor of PTE. The combination of PMPs, platelet distribution width, P-selectin and D-dimer may be used in the diagnosis of PTE with high sensitivity and specificity.

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Introduction

Pulmonary thromboembolism (PTE), a blockage of the main pulmonary artery or one of its branches, is a potentially fatal disorder with a high mortality (1). Since the signs and symptoms of PTE are diverse, nonspecific, and sometimes silent, PTE is difficult to be diagnosed in a timely manner (2). Currently, computed tomography pulmonary angiography (CTPA) is the gold standard for the diagnosis of PTE (3). However, CTPA is associated with an increased risk of radiation exposure and is especially contraindicated in patients with renal insufficiency and in pregnant women (2). It has been reported that the prevalence of PTE in patients suspected of having this disorder and undergoing CTPA is only 5 to 10% in the United States and 20 to 30% in Europe (4,5). In addition, the high cost greatly limits the application of CTPA (6). Therefore, exploration of simple and feasible tests, which are less invasive, well-priced, and highly efficient in the diagnosis of PTE, has become a serious consideration in clinical practice.

According to the European Society of Cardiology (ESC) in 2014, assessing D-dimer is recommended as the first step in excluding PTE among patients who have a low or moderate likelihood of PTE. If the D-dimer result is positive, CTPA is then performed to confirm the diagnosis of PTE (7). The level of D-dimer, a soluble degradation product derived from cross-linked fibrin in the fibrinolytic system, is increased in acute thromboembolic events (8). It is known that a D-dimer level lower than 500 ng/ml in the peripheral blood can exclude the diagnosis of PTE (9). Although D-dimer has a high sensitivity in the diagnosis of PTE, its specificity (30 to 40%) is poor because it can be influenced by various factors, such as increasing age, cardiovascular disease, surgery, tumor, infection, and tissue necrosis (10,11). Therefore, novel non-invasive biomarkers with high sensitivity and specificity are urgently needed.

Microparticles are cellular vesicles of a heterogeneous size ranging from 0.1 to 1 μm and located in multiple cells, such as platelets, endothelial cells, and red cells (12,13). Phosphatidylserine (PS) and tissue factors on the surface of microparticles can promote the expression of Xase and

prothrombinase, thereby activating blood coagulation and inducing thrombogenesis (14). Elevated microparticles levels are associated with various diseases, such as chronic rhinosinusitis (15), autoimmune diseases (16), acute myocardial infarction (17), endothelial injury (18), and chronic obstructive pulmonary disease (COPD) (19). Platelet-derived microparticles (PMPs) are a large population of microparticles (70-90%) generated from the plasma membrane during platelet activation (20). Recent studies showed that PMPs are involved in the thrombin generation via PS exposure and activation of both the intrinsic and extrinsic pathway of coagulation, thus promoting blood coagulation (21-23). In addition, accumulating evidence demonstrated that phosphatidylserine (PS) positive PMPs can promote procoagulant activity (24,25). It has been reported that PMP levels are significantly elevated in patients with acute PTE compared with those in healthy controls who have no history of venous thromboembolism and/or cardiovascular risk factors (26). These PMPs are involved in the occurrence and development of PTE and may serve as a biomarker of PTE, as a new target for anti-platelet drugs, and as a new indicator for antithrombotic activity (27). However, the diagnostic value of PMPs in PTE still needs to be studied.

In the present study, the PMP levels of patients with PTE were assessed. The diagnostic values of PMPs, D-dimer, PMPs combined with D-dimer, and a combination of PMPs, platelet distribution width, platelet count, P-selectin and D-dimer in PTE were evaluated using a receiver operating characteristic (ROC) analysis. Our findings may reveal a novel non-invasive biomarker in the diagnosis of PTE with high sensitivity and specificity.

Materials and methods

Participants. A total of 102 patients with PTE were screened at Shenzhen People's Hospital between August 2015 and August 2017. The diagnosis of PTE was in accordance with the guidelines for the diagnosis and management of acute pulmonary embolism (7). A positive CTPA result was reported for these patients before admission or within 24 h after admission. Forty patients with suspicious PTE but negative results of CTPA were also included, they had similar symptom with PTE, such as increased D-dimer level, dyspnea, chest pain, hemoptysis. Patients who had histories of anticoagulant treatment, severe infectious disease, malignant tumor, hepatic function deficiency, transplantation, severe malnutrition, and hematological disorders were excluded from this study (these factors can affect the microparticles levels). A total of 102 healthy individuals (without a history of venous thromboembolism or vascular risk) recruited from the Physical Examination Department of the same hospital were enrolled as the control group. The clinical characteristics of the enrolled subjects were recorded, including sex, age, deep vein thrombosis (DVT), platelet counts, mean platelet volume, platelet distribution width, thrombophilia (protein C, protein S, antithrombin III, fibrinogen degradation product, and lupus-like anticoagulant) and baseline diseases. This study was approved by the Institutional Review Board of Shenzhen People's Hospital, and informed consents were obtained from all participants.

Assessment of the platelet count, mean platelet volume, and platelet distribution width and P-selectin. Ethylenediamine tetraacetic acid (EDTA) anticoagulated blood samples were collected from all the participants. Briefly, vacutainer was used to draw peripheral venous blood with a 0.7 x 25 TWLB venepuncture needle. The tourniquet was routinely used during the blood collection. The platelet count, mean platelet volume, and platelet distribution width were assessed using an automated hematology analyzer (XS-800i, Sysmex Corporation, Kobe, Japan). Platelet-free plasma supernatant was rapidly collected after the blood sampling (within 30 min) followed by 2,500 x g centrifugation for 15 min at room temperature; the plasma supernatant is then again rapidly centrifuged by 2,500 x g for 15 min at room temperature. Platelet-free plasma is obtained by collecting the supernatant, avoiding any contact with the platelet pellet. Plasma was stored frozen at -80˚C just before use (28). The level of P-selectin in the plasma was assessed using an enzyme linked immunosorbent assay kit (R&D Systems, Minneapolis, MN, USA) according to the manufacturer's instruction. Optical density was determined at 450 nm and corrected at 620 nm.

Flow cytometry analysis of PMPs. Phycoerythrin-conjugated antibodies to PMPs, CD41a, and Annexin V were used to label the PMPs. A total of 30 μl platelet-free plasma was incubated using 10 μl Mouse anti-Human CD41a (1:2, BD Pharmingen; BD Biosciences, San Jose, CA, USA) and 10 μl FITC-Annexin V (1:2, BD Pharmingen; BD Biosciences) antibodies in Annexin V-binding buffer at 37˚C for 30 min. Thereafter, the samples were diluted in 290 μl Annexin V-binding buffer and transferred to BD Trucount tubes (BD Pharmingen; BD Biosciences) containing counting beads. For flow cytometry (FACS LSRII, BD Pharmingen; BD Biosciences) analysis, the Megamix plus SSC beads (BioCytex, Marseille, France) were backgated in a forward scatter-side scatter plot (Fig. 1A), and a gate was defined for identifying large MPs sized 0.5 μm (P10) and small MPs sized 0.24 μm (P13) (Fig. 1A). The PMPs with double positive Annexin V and CD41a staining were divided into large PMPs (P9) (Fig. 1B) and small PMPs (P14) (Fig. 1C). Counting beads were analyzed using a free fluorescence channel of PerCP to avoid falling off-scale in the MP-optimized settings (P11) (Fig. 1D). The PMP level was calculated as follows: [(P9 events + P14 events)/P11 events] x (total bead counts/test volume). Isotype controls (PE Mouse IgG1, κ Isotype control) were used to distinguish true positive events from noise and increase the specificity of MP detection. Sample values of a patient with PTE are shown in Fig. 1E (PMPs=594.41/μl).

Statistical analysis. Statistical analysis was performed using SPSS version 22.0 (SPSS Inc., Chicago, IL, USA). Quantitative data with a normal distribution were expressed as means ± standard deviations and compared using the Student's t-test. Quantitative data with a non-normal distribution were expressed as medians (inter-quartile ranges) and compared using the Mann-Whitney test. Qualitative data were expressed as numbers (percentages) and compared using the χ^2 test. ROC curves were established to evaluate the diagnostic values of PMPs, D-dimer, PMPs combined with D-dimer, and a combination of PMPs, platelet distribution width, platelet

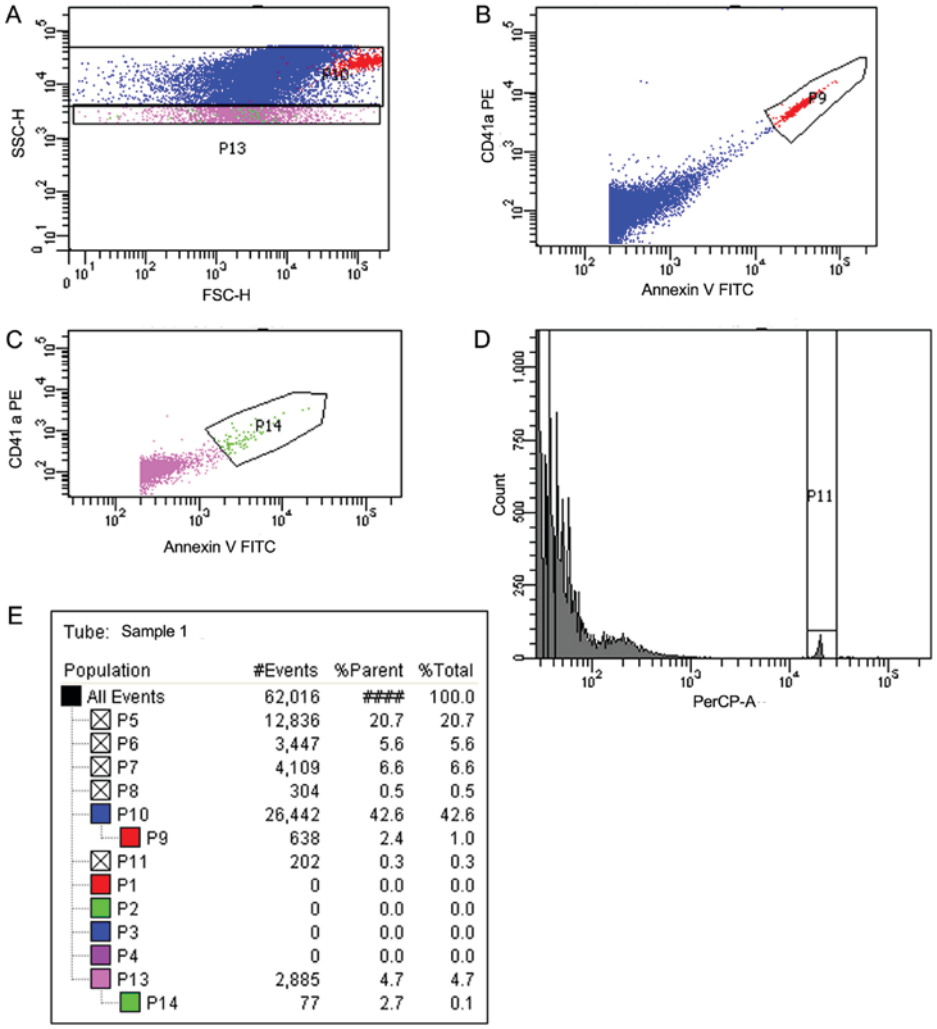

Figure 1. Flow cytometer analysis of the PMPs in the patients with PTE. (A) The Megamix beads were backgated in a forward scatter-side scatter plot, and a gate was defined for identifying large MPs sized 0.5 μm (P10) and small MPs sized 0.24 μm (P13). (B) Large PMPs (0.5 μm) with double positive Annexin V and CD41a staining. (C) Small PMPs (0.24 μm) with double positive Annexin V and CD41a staining. (D) Counting beads were analyzed using a free fluorescence channel of PerCP to avoid falling off-scale in the MP-optimized settings. (E) Sample values of a patient with PTE (PMP=594.41/μl). PMPs, platelet-derived microparticles; PTE, pulmonary thromboembolism.

count, P-selectin, and D-dimer in PTE. The diagnostic values were compared using MedCalc (version 11.4.2.0; MedCalc, Mariakerke, Belgium). A P-value <0.05 was considered to indicate a statistically significant difference.

Results

Clinical characteristics of the patients with PTE. The clinical characteristics of the patients with PTE are presented in Table I. No significant differences were revealed regarding sex and age among the patients with (without) PTE and healthy controls. The platelet count of the patients with (without) PTE were not significantly different from those of the healthy controls, while the mean platelet volume of patients with suspicious PTE were significantly higher than those of patients with PTE and healthy controls (P<0.01), the platelet distribution width of the patients with (without) PTE were significantly lower than that of the healthy controls (P<0.01). We observed DVT in 20 (19.61%) patients with PTE and 2 (5.00%) in patients with suspicious PTE. A total of 27 (26.47), 16 (15.69), and 17 (16.67%) patients with PTE exhibited decreased protein C (<65%), protein S (<63.5%), and antithrombin III (<83%) levels, respectively. A total of 48 (47.06%) patients with PTE exhibited increased fibrinogen degradation product (<80%) levels, and 27 (26.47%) patients had positive findings for lupus-like anticoagulant. Furthermore, 77 patients with PTE had at least one underlying disease, such as hypertension, diabetes, COPD, cerebral infarction, coronary heart disease, among others.

Diagnostic value of PMPs and D-dimer in PTE. Flow cytometry analysis showed that the PMP level was significantly higher in the patients with PTE (609.10/μl; 163.80-8501.00/μl) than in the healthy controls (230.60/μl; 20.91-790.00/μl) and patients with suspicious PTE (166.70/μl; 52.07-722.70/μl (P<0.01) (Fig. 2A). The ROC analysis, using patients with PTE as experimental group and healthy control as control group, showed that the sensitivity and specificity of the PMPs in the diagnosis of PTE were 93.14 and 51.96%, respectively [area under the curve (AUC), 0.822; cut-off point, 236.97/μl] (Fig. 2B). The PMPs had the following values in the diagnosis of PTE: Positive likelihood ratio (+LR) of 1.94, negative

Table I. Clinical characteristics of the participants enrolled in this study.

Variable	Patients (n=102)	Suspicious patients (n=40)	Healthy controls (n=102)	P-value
Male sex, no. (%)	54 (52.94)	17 (42.50)	43 (42.16)	0.256
Age, years	60.23±16.73	64.22±13.59	53.01±40.88	0.089
PLC ($x10^9$/l)	257.4±101.40	217.6±140.6	251.6±45.31	0.086
MPV (fl)	10.23±0.94	11.08±1.83	10.22±0.93	<0.01
PDW (fl)	11.36±2.06	11.37±1.77	13.52±2.09	<0.01
P-selectin	68.15±38.16	47.14±18.20	40.45±12.28	<0.01
Thrombophilia				
PC decrease, no. (%)	27 (26.47)	1 (2.50)	-	-
PS decrease, no. (%)	16 (15.69)	0 (0)	-	-
AT- III decrease, no. (%)	17 (16.67)	1 (2.50)		-
FDP increase, no. (%)	48 (47.06)	1 (2.50)	-	-
LA (+), no. (%)	27 (26.47)	2 (5.00)	-	-
Baseline diseases				
Hypertension, no. (%)	25 (24.51)	10 (25.00)	0 (0)	-
DVT, no. (%)	20 (19.61)	2 (5.00)	0 (0)	-
Diabetes, no. (%)	14 (13.73)	6 (15.00)	0 (0)	-
COPD, no. (%)	6 (5.88)	6 (15.00)	0 (0)	-
Cerebral infarction, no. (%)	7 (6.86)	3 (7.50)	0 (0)	-
Coronary heart disease, no. (%)	7 (6.86)	3 (7.50)	0 (0)	-
Pulmonary hypertension, no. (%)	9 (8.82)	5 (12.50)	0 (0)	-
Other diseases, no. (%)	13 (12.75)	4 (10.00)	4 (3.92)	0.066

DVT, deep vein thrombosis; PLC, platelet count; MPV, mean platelet volume; PDW, platelet distribution width; PC, protein C; PS, protein S; AT-III, antithrombin III; FDP, fibrinogen degradation product; LA, lupus-like anticoagulant; COPD, chronic obstructive pulmonary disease.

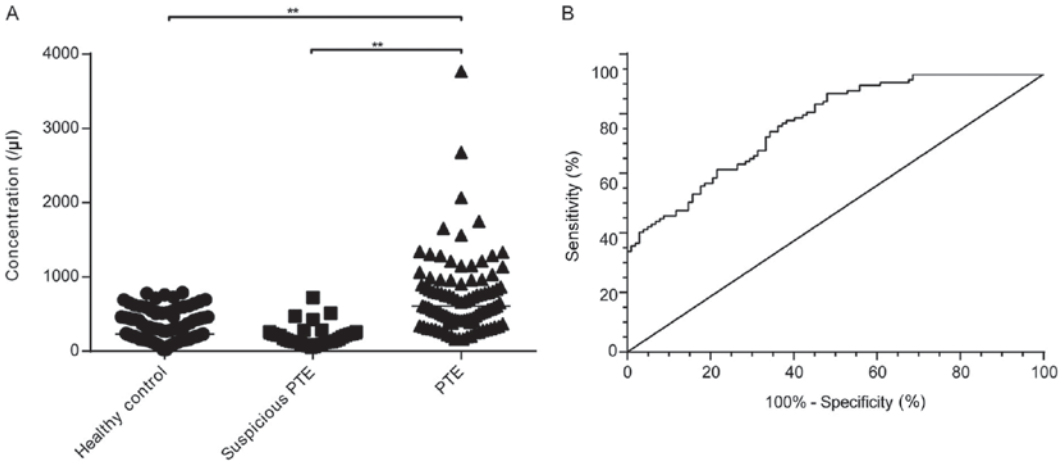

Figure 2. Diagnostic values of PMPs in PTE. **P<0.01. (A) PMP levels of the patients with PTE (n=102, median=607.10/μl; range: 163.80-3769.63/μl), healthy controls (n=102, median=230.60/μl; range: 20.91-790.00/μl) and suspicious PTE (n=40, median=166.70/μl; range: 52.07-722.70/μl. (B) Receiver operating characteristic curve of the PMPs. AUC=0.822; cut-off point, 236.97/μl, sensitivity=93.14%, specificity=51.96%. PMPs, platelet-derived microparticles; PTE, pulmonary thromboembolism; AUC, area under the curve.

likelihood ratio (-LR) of 0.13, positive predictive value (PPV) of 65.52%, negative predictive value (NPV) of 88.14%, and Youden's index of 45.10%. The accuracy rate of PMP values in the diagnosis of PTE was 72.06% (Table II).

The diagnostic value of D-dimer in PTE was also evaluated. The sensitivity and specificity of D-dimer in the diagnosis of PTE were 56.86%, 74.51%, respectively (AUC, 0.773; cut-off point, 500 ng/ml) when using patients with PTE as experimental group and healthy control as control group. The sensitivity was significantly lower than that of PMPs (P<0.01) (Fig. 3 and Table II). In addition, D-dimer showed +LR, -LR, PPV, NPV, and Youden's index of 2.23, 0.58, 69.05,

Table II. Diagnostic value of PMPs, D-dimer, PMPs combined with D-dimer, and combination of multiple parameters in PTE.

Groups	AUC	Sensitivity (%)	Specificity (%)	Accuracy (%)	PPV (%)	NPV (%)	Youden's index (%)	+LR	-LR
PMPs	0.822	93.14	51.96	72.06	65.52	88.14	45.10	1.94	0.13
D-dimer	0.773	56.86	74.51	65.69	69.05	63.33	31.37	2.23	0.58
PMPs & D-dimer	0.875	86.27	71.57	78.43	74.58	83.72	57.84	3.03	0.19
Multiple parameters	0.957	88.24	91.18	89.71	90.91	88.57	79.42	10.00	0.13
χ^2 value	-	53.96	41.21	35.69	21.33	27.85	-	-	-
P-value	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	-	-	-

The multiple combination included the combination of PMPs, platelet distribution width, P-selectin, and D-dimer. PMP, platelet-derived microparticles; PTE, pulmonary thromboembolism; AUC, area under the curve; PPV, positive predictive value; NPV, negative predictive value; +LR, positive likelihood rate; -LR, negative likelihood rate.

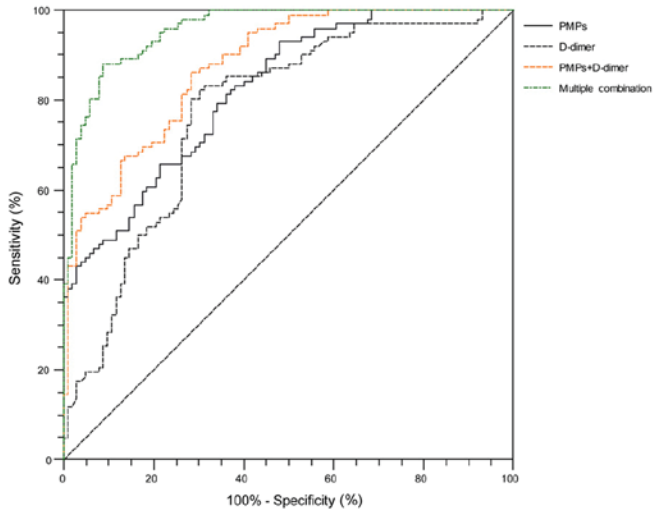

Figure 3. Receiver operating characteristic curve of platelet-derived microparticles (PMPs), D-dimer and multiple parameters (including PMPs, platelet distribution width, P-selectin, and D-dimer) in the diagnosis of PTE. The multiple combination showed the highest AUC, significantly higher than all the other three methods (P<0.01). AUC between PMPs and D-dimer had no significant difference (P>0.05). The AUC of PMPs combined with D-dimer was also higher than those of PMPs and D-dimer alone in diagnosis of PTE (P<0.01). PMPs, platelet-derived microparticles; PTE, pulmonary thromboembolism; AUC, area under the curve.

63.33 and 31.37%, respectively, with an accuracy rate of 65.69% (Table II).

Diagnostic value of the PMPs combined with D-dimer in PTE. The diagnostic value of the PMPs combined with D-dimer in PTE was further evaluated. A logit equation, logit(z)=-3.0452 + 0.001153 x D-dimer + 0.004932 x PMPs, was obtained, exhibiting a significant overall model fit (χ^2=99.349, P<0.01). The sensitivity and specificity of the PMPs combined with D-dimer in the diagnosis of PTE were 86.27 and 71.57%, respectively (AUC, 0.875). The PMPs combined with D-dimer showed the following values in the diagnosis of PTE: +LR of 3.03, -LR of 0.19, PPV of 74.58%, NPV of 83.72%, and Youden's index of 57.84%. The accuracy rate of the PMPs combined with D-dimer in the diagnosis of PTE was 78.43% (Fig. 3 and Table II). When compared with the D-dimder alone, the sensitivity of the PMPs combined with D-dimer for the diagnosis of PTE significantly increased (P<0.01), while the specificity significantly increased when compaired with PMP (P<0.01).

Diagnostic value of the combination of multiple parameters in PTE. Since the specificity of the PMPs combined with D-dimer in the diagnosis of PTE was relatively low, the combination of PMPs, platelet distribution width, P-selectin, and D-dimer was used to diagnose PTE. A logit equation, logit (z)=-3.4068 + 0.001317 x D-dimer + 0.006114 x PMPs-0.3751 x platelet distribution width + 0.09183 x P-selectin, was obtained, exhibiting a significant overall model fit (χ^2=169.47, P<0.01). The ROC analysis showed that the sensitivity and specificity of the combination of multiple parameters in the diagnosis of PTE were 88.24 and 91.18%, respectively (AUC: 0.957, cut-off point: P=0.4537), and such a combination showed the following values: +LR of 10.00, -LR of 0.13, PPV of 90.91%, NPV of 88.57%, and Youden's index of 79.42%. The accuracy rate of the combination of multiple parameters in the diagnosis of PTE was 89.71% (Fig. 3 and Table II). All these indices showed that this combination had a better diagnostic value in PTE than the PMPs, D-dimer, and PMPs combined with D-dimer (P<0.01).

Validation of the diagnostic value of multiple parameters in patients with suspicious PTE. In this study, a total of 40 patients with suspicious PTE were included. To investigated the potential application of the diagnostic value of the multiple parameters method, we applied the established logistic model in the patients with suspicious PTE and evaluated its performance in distinguishing clinical suspicious as PTE but negative patients with those positive patients. The possibility of being predicted as PTE was calculated following the equation: $P=e^z/(1+e^z)$, if P-value was higher than the cut off point (0.4537), the patient would be identified as PTE patients, otherwise, as non-PTE patients. Thirty-two (80%) of suspicious patients were correctly clarified as non-PTE patients.

Discussion

The PMPs are a large heterogeneous population of circulating microparticles released from the platelet as a result

of membrane phospholipid reconstruction or cytoskleton hydrolysis (20). Accumulating evidence has suggested that PMPs play an important role in thromboembolism through direct cell-to-cell contact interactions or release of active components (29). In this study, we found that the PMP level was significantly higher in the patients with PTE than in the healthy controls. Our finding is consistent with those of previous studies (26,29,30), and further illustrates the association between elevated PMP levels and PTE. When the blood vessels are injured, the release of PMPs can lead to the exposure of collagen and von Willebrand factor, thereby leading to the adhesion, aggregation, and activation of platelets (31). Thereafter, thromboxane A2 and endothelin produced by platelets can lead to contraction of the blood vessels, thus promoting thrombogenesis (32). Meanwhile, the membrane proteins of PMPs gathering on an anion phospholipid surface can enhance the assembly and catalytic activity of tissue factors, thereby exacerbating blood clotting responses (33). Since PMPs are associated with a strong procoagulant activity, elevated PMP levels may be considered as a potential indicator of PTE in clinical practice.

Microparticles are known as a biomarker of DVT (34,35). It has been reported that the sensitivity, specificity, and accuracy of total circulating microparticles in the diagnosis of DVT were 59, 62, and 61%, respectively (34). In addition, microparticles > P95 increased the venous thromboembolism risk from 1.63 (0.60-4.50) to 6.09 (1.03-36.1), and high levels of circulating microparticles may be a possible independent risk factor for venous thromboembolism (29). To date, related studies regarding PMPs are still limited, and the diagnostic role of PMPs in PTE has not been revealed. To reveal the diagnostic value of PMPs in PTE, a ROC analysis was performed in this study. The result showed that the sensitivity of PMPs in the diagnosis of PTE was 93.14%, higher than that of D-dimer in the diagnosis of PTE, suggesting a potential value of combination of these two makers in clinical practice.

Although the sensitivity of PMPs in the diagnosis of PTE was higher, the specificity and accuracy were still limited. Thus, we combined PMPs and D-dimer to diagnose PTE. The ROC analysis showed that the combination of PMPs and D-dimer significantly increased the specificity in the diagnosis of PTE. The findings indicate that using PMPs combined with D-dimer is useful for the diagnosis of PTE. However, the specificity of PMPs combined with D-dimer for the diagnosis of PTE was still low. This phenomenon may be attributed to the low specificity of PMPs and D-dimer. A previous study has shown that the guidelines recommending clinical probability and D-dimer assessment as the initial screening tests for venous thromboembolism diagnosis in low-risk patients are underused (36). Many risk factors of venous thromboembolism also increase the D-dimer level, including old age, cardiovascular disease, surgery, tumor, infections, and tissue necrosis (10,11). Therefore, we suspect that the combination of D-dimer and PMPs may not improve the diagnostic efficiency of PTE in clinical practice.

To eliminate the limitation of D-dimer, combinations of other biomarkers are used to diagnose thromboembolism. A sensitivity of 73%, specificity of 81%, and accuracy of 77% have been reported for the identification of DVT by combining D-dimer, soluble P-selectin, and total microparticles (34). The combination of D-dimer and MPV results in an increase in the AUC (0.799) in the diagnosis of PE (37). The combined measurement of D-dimer and FXIII helps to distinguish PE from serious diseases with similar symptoms (38). In this study, the combination of PMPs, D-dimer, platelet distribution width, and P-selectin was applied in the diagnosis of PTE. The ROC analysis showed high sensitivity (88.24%), specificity (91.18%), and accuracy (89.71%), indicating promising prospects in clinical practice.

The pro-coagulant properties of PMPs have been extensively studied (22-25). Zhao *et al* recently found that PMPs platelets and MPs from the colon cancer patients significantly enhanced intrinsic/extrinsic FXa and thrombin generation, greatly shortened coagulation time, and increased fibrin formation (24). Similarly, the study of Wang *et al* also suggested that PMPs formed in sepsis are a potent inducer of thrombin generation via PS exposure and activation of both the intrinsic and extrinsic pathway of coagulation (21). P-selectin, on the other hand, was another known marker of platelet procoagulant activity that exposed on the platelet membrane when the platelet was activated (39). Besides, P-selectin can mediate the adhesion of activated platelet with other cells, leading to the hypercoagulant state of the blood (40). More recently, Prakash *et al* found that P-selectin can even promote thrombus propagation independently of both von Willebrand factor and thrombospondin-1 in mice (41). A single assessment of P-Selectin, at baseline in prospective epidemiological studies is also suggested to be appropriate to investigate associations between platelet activation and risks of chronic diseases (42). In the current study, both PMPs and P-selectin were increased in PTE patients, indicating that they were involved in the procoagulant activity of platelets.

Currently, D-dimer is the major exclusion of PTE (9). Nevertheless, there are still a certain part of D-dimer positive patients found to be PTE negative after undergoing CTPA confirmation. Unnecessary CTPA will put the patients into radiation expose and may cause potential complication, and increase the expense of the patients. The findings in this study showed higher sensitivity of multiple parameters than D-dimer, and achieved 80% of accuracy in distinguishing patients suspected with PTE from positive PTE patients. In addition, some patients may be allergic to iodine or too severe to undergo CTPA, in which case, the method in this study might be an alternative option for the diagnosis of PTE in the clinical practice.

In conclusion, an elevated PMP level was an effective biomarker of PTE. The diagnostic value of PMPs was similar to that of D-dimer. The combination of D-dimer and PMPs significantly increased the sensitivity of D-dimer in the diagnosis of PTE. The combination of PMPs, D-dimer, platelet distribution width, and P-selectin presents a novel non-invasive strategy for the diagnosis of PTE with high sensitivity and specificity. However, this study was limited by its small population, further studies about the application of PMPs for the diagnosis of PTE based on larger populations are still needed.

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Availability of data and material

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

Author's contributions

YF conceived and designed the study. LXu, MW, LXi, QH, SLiu and YL recruited subjects and performed the experiments. MW and SLi analyzed the data. MW, YF and YY wrote the paper. YY and YL were assisted with flow cytometry, the adjustment of the scientific design of the study and the revision of the manuscript.

Ethics approval and consent to participate

All procedures performed in studies involving human participants were in accordance with the ethical standards of Institutional Review Board of Shenzhen People's Hospital. Informed consent were considered and obtained from all participants after approval by the research ethical review board.

Patient consent for publication

All the study participants provided informed consent for the publication of data.

Competeing interests

The authors declare that they have no competing interests.

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