Role of mean platelet volume in patients with chronic obstructive pulmonary disease

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Background and objective Stable chronic obstructive pulmonary disease (COPD) is associated with low-grade systemic inflammation as demonstrated by an increase in blood leukocytes, acute-phase proteins such as C-reactive protein, and inflammatory cytokines. Mean platelet volume (MPV) is one of the platelet (PLT) function indexes. It reflects the PLT production rate and stimulation. The changes in MPV during an exacerbation of COPD versus stable COPD have not been clearly examined. The aim of the present study was to evaluate MPV in participants during their stable phase and during an exacerbation of COPD.

Patients and methods This study included a total of 80 COPD patients: group A included 40 patients in the stable state and group B included the remaining 40 who were admitted for exacerbation of COPD, who were followed-up for 6 weeks after recovery, and were matched with 40 healthy, nonsmoking controls. All patients underwent spirometry, laboratory investigations with respect to complete blood count, PLT count and MPV, and echocardiography.

Results MPV revealed a highly significant decrease within the exacerbated patient group than stable ones who had lower levels of MPV than the control volunteers. There was a highly significant increase in MPV during the stable follow-up visit than during exacerbation for the same included group of patients. There was a significant positive correlation between MPV and grade of severity of COPD; 60% of the stable COPD patients were found to have pulmonary arterial hypertension by echocardiography. There was a significantly higher MPV among patients with pulmonary hypertension (PH) than those without PH. Although PLT count increased with increased severity of PH, it did not reach significance, and there was a significant increase in MPV with increased severity of PH.

Conclusion MPV is a quick and reliable tool for the assessment of inflammatory response. PLT activation is an important prothrombotic manifestation of COPD, which may be a useful therapeutic target.

Egypt J Bronchol 2016 10:251–260

Egyptian Journal of Bronchology 2016 10:251–260

Keywords: chronic obstructive pulmonary disease, exacerbation, mean platelet volume, stable

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Received 30 April 2016 **Accepted** 26 May 2016

Introduction

Chronic obstructive pulmonary disease (COPD) is a major cause of morbidity and mortality around the world and it is primarily characterized by limited airflow resulting from inflammation and remodeling of the airways [1]. It has been established that stable COPD is associated with low-grade systemic inflammation as demonstrated by an increase in blood leukocytes, acute-phase proteins such as C-reactive protein (CRP), and inflammatory cytokines [2]. Cigarette smoking accelerates pathogenesis in different types of cancers such as lung, pancreas, breast, liver, and kidney cancers [3]. Similarly, it also enhances stomach pH causing peptic ulcers and gastric diseases [4]. Smoking has both acute and chronic effects on hematological parameters [5]. Epidemiological evidence suggests that impaired lung function is strongly correlated with increased cardiovascular disease-related deaths [6]. In addition, COPD and atherosclerosis often coexist and share common risk factors such as age and smoking. It is widely known that smokers have a higher risk for hypertension, inflammation, stroke, clotting disorders, and respiratory diseases as well [7]. Pulmonary thrombosis often appears to complicate the clinical course of patients with COPD (chronic obstructive airway disease) [8]. In an autopsy study, Alersandri and colleagues [8] found pulmonary thrombosis in ~25% of patients with chronic obstructive airway disease. Thrombosis could be the consequence of a prothrombotic condition occurring in these patients with vascular and alveolar lesions [9] or due to platelet (PLT) dysfunction leading to pulmonary hypertension (PH) and pulmonary thromboembolism. PLTs are involved in atherogenesis, inflammation, and atherothrombosis. A recent study found that an elevation in arginase activity in PLTs reflected alterations in nitric oxide metabolism and enhanced PLT activity in COPD patients [10]. Moreover, soluble P-selectin, a marker of PLT hyperactivity, was reported to be higher in COPD patients [11]. Thus, an increase in inflammatory markers such as blood leukocytes, CRP, erythrocyte sedimentation rate, and inflammatory cytokines is observed and is most likely associated with lung function decline [12]. Mean platelet

 DOI: 10.4103/1687-8426.193635

volume (MPV) is increased in patients at risk for atherothrombotic disease, and measurement of PLT volume has been suggested as an indicator of PLT activation [13]. MPV has been investigated as an indicator of inflammation in different diseases [14], and MPV has also been noted to be increased in cardiovascular disease, peripheral artery disease, and cerebrovascular disease [15]. Recent studies have found that an increased MPV was a predictor of unprovoked venous thromboembolism and arterial thrombosis [16]. There are other studies showing that chronic obstructive airway disease patients have altered PLT functions and clotting system activation. In particular, it has been shown that there is a shortened PLT half-life, increased PLT size, increased PLT aggregation, high levels of blood fibrinogen, and in-vitro and in-vivo PLT activation in these patients [17]. MPV is one of the PLT function indexes. It reflects the PLT production rate and stimulation [18]. MPV is a parameter generated by routine complete blood count (CBC) test that is usually overlooked by clinicians [19]. The changes in MPV during an exacerbation of COPD versus stable COPD have not been clearly examined. The aims of the present study were to evaluate MPV in participants during their stable phase and during an exacerbation of COPD and to compare the measurements with an appropriately matched control population.

Patients and methods

Participant selection

This study was approved by IRB/ethics committe. This study included a total of 80 COPD patients: group A included 40 patients in the stable state and group B included the remaining 40 who were recruited from among patients who sought medical advice in the outpatient clinic or were admitted to our outpatient and emergency clinics at the Pulmonary Diseases Department of Ain Shams Hospitals for exacerbation of COPD and were matched with 40 healthy, nonsmoking controls for age, sex, and BMI during the period between June 2011 and June 2013. The control group included healthy individuals with normal spirometry and no history of respiratory symptoms, and individuals with any major illness at present or in the past or having a ratio of forced expiratory volume in one second (FEV_1) to forced vital capacity (FVC) of less than 70% were excluded from the control group.

Inclusion criteria

Patients with a history of the disease, smoking history, clinical definition of chronic obstructive airway disease, and a ratio of postbronchodilator FEV_1 to FVC of less than 70% of the predicted value or FEV_1 percentage predicted less than 80% were included in the study. COPD was diagnosed and classified according to the Global Initiative for Chronic Obstructive Lung Disease criteria. Patients were evaluated on admission and in the stable state.

Individuals in the stable disease group (group A) (no exacerbation of COPD within the previous 6 weeks) were not prescribed regular oral steroid therapy or long-term oxygen therapy. Stable state was defined as the absence of significant changes in symptoms beyond the expected daily variation, along with no requirements for changes in treatment for 3 months [20]. Patients included in group A were not included in group B.

The acute exacerbation group (group B) was defined according to recommended international criteria [21]. The diagnosis of acute exacerbation of COPD was made by the admitting respiratory physician. An exacerbation of COPD was defined as sustained (48 h or more) worsening of dyspnea, cough, or sputum production leading to an increase in the use of maintenance medications and/or supplementation with additional medications [22]. They were studied within 24 h of admission and at least 2 weeks following discharge from the hospital when their condition was considered to be clinically stable. We recorded the follow-up data of the same patients in the stable period 6 weeks after an acute exacerbation. The participants examined during exacerbation (group B) were further re-evaluated and assessed when in the stable phase of COPD during their follow-up visit.

Exclusion criteria

Patients with suspected or proven alternative diagnosis for the acute deterioration in symptoms such as acute inflammatory diseases (pneumonia episodes of previous pulmonary embolism, heart failure), hematological disorders, autoimmune diseases or systemic inflammatory condition such as rheumatoid arthritis, asthma, bronchiectasis, pneumonia, pulmonary embolism, cystic fibrosis, active pulmonary tuberculosis, valvular diseases, coronary artery disease and peripheral arterial disease, hypertension, hypercholesterolemia, diabetes, acute respiratory failure, heart failure, hepatic and renal failure, cancer, patients with long-term oxygen therapy and medical treatment with anticoagulants, statins, angiotensin-converting enzyme inhibitors, acetylic salicylic acid, clopidogrel, theophylline, antibiotics and systemic glucocorticoids during the previous 8 weeks, systemic hypertension, diabetes

mellitus, cancer or having received anti-PLT or anticoagulant drugs in past 15 days were excluded from the study.

All participants provided written informed consent and were subjected to the following tests.

Clinical examination

All the participants underwent a complete clinical examination.

Spirometric function test

Spirometry was performed to confirm the COPD diagnosis when patients were in the stable phase (group A). Patients were instructed to perform forced expiration until three acceptable measurements were obtained according to the European Respiratory Society criteria [23]. Each recorded result was expressed as a percentage of the predicted value for that parameter. Predicted values were calculated according to the system developed by Quanjer *et al.* [24]. These results were used to define disease severity according to the Global Initiative for Chronic Obstructive Lung Disease classification – namely, stage I (mild), $FEV_1/FVC<70\%$ and $FEV_1\geq80\%$ predicted; stage II (moderate), $FEV_1/FVC<70\%$ and $50\%\leq FEV_1<80\%$ predicted; stage III (severe), $FEV_1/FVC<70\%$ and $30\%\leq FEV_1<50\%$ predicted; and stage IV (very severe), $FEV_1/FVC<70\%$ and $FEV_1<30\%$ predicted or $FEV_1<50\%$ predicted plus chronic respiratory failure. Baseline spirometric function parameters were obtained in group B during exacerbation and repeated during the follow-up visit after improvement 2 weeks later.

Biochemical measurements

Venous blood samples were collected from participants in the morning between 9.00 and 10.00 a.m. after an overnight fast of at least 10 h. Venous blood samples were collected into K3 EDTA (15%) Becton Dickinson Vacuum tubes (Shandong Weigao Group Medical Company, China) and mixed gently. CBC was measured within 1–2 h of blood sampling using Nihon Codon fully automatic hematological analyzer (Nihon Kohden, Tokyo, Japan). The hematological analyzer was calibrated using a standardized, commercially available calibrator kit. CBC [white blood cells (WBC), red blood cells (RBC), hemoglobin (Hb), mean corpuscular volume, mean corpuscular Hb, mean corpuscular Hb concentration, hematocrit (HCT), red cell distribution width, PLT, platelet crit, MPV, and platelet distribution width] was measured. CRP level was determined by the nephelometric method (Clauss, Dade Behring, Marburg, Germany). The expected MPV values in our laboratory ranged between 7.0 and 12.0 fl. CRP levels and CBC of patients with acute COPD exacerbation were recorded. CBC and CRP measurements were taken at first administration of medications for exacerbation and before initiation of any additional therapies and repeated 2 weeks later after improvement.

Arterial blood gases

Arterial blood gases were determined for the assessment and classification of disease severity.

Echocardiography

Transthoracic echocardiogram (TTE) (Philips IE33, instrument equipped with a 3–5 MHz transducer) was performed. TTE-derived mean pulmonary artery pressure (MPAP) was calculated as TTE-derived SPAP×0.61+2 mmHg, according to Chemla *et al.* [25] SPAP was estimated by TTE from the systolic right ventricular-to-right atrial pressure gradient using the modified Bernoulli equation, and the assessment of right atrial pressure was performed in accordance with previously described methods. Furthermore, isovolumic relaxation time and color M-mode Doppler flow propagation velocity (FPV) were measured. According to the following equation – 4.5(103/[2–VRT] +FPV)–9–pulmonary capillary wedge pressure (PCWP) was estimated noninvasively [26]. On the basis of TTE-derived MPAP and PCWP, the transpulmonary pressure gradient (MPAP–PCWP) was calculated noninvasively. According to the recommendations in the guidelines, the measurements of stroke volume and cardiac output (stroke volume×heart rate) were made at the level of the left ventricular outflow tract [27].

Statistical analysis

All data are presented as mean and SD or as medians (interquartile range) for continuous variables. The between-group differences were determined using Student's *t*-tests. The differences between the stable COPD group and the matched control group were determined using independent samples *t*-test. The differences between the exacerbated COPD group and the stable COPD group were determined using paired-samples *t*-test. The χ^2-test was performed for all categorical variables. Correlations between MPV and clinical parameters were tested by partial correlation with age, BMI, and smoking status as continuous variables and sex and presence of hypertension as dichotomous variables. CRP was logarithmically transformed before statistical analysis

to approximate normal distribution. A two-tailed *P* less than or equal to 0.05 indicated statistical significance. Statistical analyses were performed using SPSS, version 17.0 software (SPSS Inc., Chicago, Illinois, USA). All parameters were expressed as mean values ±SD. The Pearson's correlation analysis was used to investigate the relationship between the parameters. A *P* value less than 0.05 was considered to be statistically significant.

Results

Forty stable COPD patients (group A) and 40 patients with COPD exacerbation (group B) were included in this study and matched with 40 healthy, nonsmoking controls. There was no significant correlation regarding age, sex, or smoking habits among the three studied groups (P=0.564, 0.743, and 0.451, respectively) as shown in Table 1.

There was statistically highly significant correlation among the three studied groups (stable, group A; exacerbated COPD, group B; and control volunteers) regarding pulmonary functional abnormalities (P<0.001), in which FEV_1/FVC and FEV_1 were decreased in the exacerbated COPD patient group than in the stable and control groups. There was a significant correlation among the studied groups regarding RBC count and HCT. Although PLT count and Hb levels were increased among patients in group B compared with patients in group A, who showed higher ratios compared with controls, this was not significant (where P=0.346 and 0.451, respectively). CRP and WBC counts tended to increase significantly with disease progression, and thus they were higher in patients of group B compared with group A who had higher significant values compared with the controls as well (P<0.001 for each). Regarding MPV, a highly significant statistical correlation was observed among the studied groups, with a significant decrease in group B compared with group A patients who had lower levels than the control volunteers (P<0.00) as shown in Table 2.

Follow-up data of the patients in group B when they were clinically stable after an acute exacerbation 6 weeks from hospital discharge were recorded and further re-evaluated and assessed during their follow-up visit. There was a nonsignificant decrease in hematological parameters after group B became clinically stable, with respect to RBC, HCT, Hb, and PLT values (P=0.451, 0.342, 0.151, and 0.251, respectively). On the other hand, there was a statistically highly significant decrease in both CRP and WBCs during the stable period than during exacerbation for the same included group of patients (group B), when *P* values were less than 0.001 for both. MPV was significantly increased during the stable

Table 1 Demographic characteristics of COPD patients [stable (group A) and COPD exacerbation (group B)] and controls

Variables	Stable COPD (group A)	COPD exacerbation (group B)	Controls	*P* value
Number	40	40	40	
Age (mean±SD) (years)	56±5.3	58±6.5	54±4.2	0.564
Sex (male/female)	38/2	40/0	40/0	0.743
Smoking (mean±SD) (pack-years)	56.7±13.7	59.4±14.8	0	0.451

COPD, chronic obstructive pulmonary disease.

Table 2 Laboratory characteristics among stable, exacerbated COPD patients and controls

Variables	Mean±SD			*P* value
	Stable COPD (group A) (N=40)	COPD exacerbation (group B) (N=40)	Controls (N=40)	
FEV_1 (% predicted)	48.6±17.1	37.9±12.9	89.2±13.5	<0.001
FEV_1/FVC (%)	49.9±14.2	41.0±15.9	85.4±17.3	<0.001
PaO_2 (mmHg)	73.1±11.4	52.6±13.4	92.9±15.2	<0.001
$PaCO_2$ (mmHg)	39.8±11.6	46.5±15.7	34.6±11.6	<0.001
RBC (10^3/μl)	5.32±0.80	5.41±0.75	5.02±0.49	0.011
HCT	48.86±13.40	49.84±11.63	44.31±15.28	0.004
Hemoglobin (g/dl)	16.21±2.35	16.78±1.45	14.53±1.21	0.451
Platelet (×10^9/l)	285.6±77.1	301.9±71.5	265.4±71.4	0.346
MPV (fl)	9.9±0.8	8.7±0.9	10.9±1.5	<0.001
WBC (×10^9/l)	7.99±2.14	11.8±2.31	6.33±1.91	<0.001
CRP (mg/l)	9.7±4.3	20.9±3.1	1.9±2.4	<0.001

COPD, chronic obstructive pulmonary disease; CRP, C-reactive protein; FEV_1, forced expiratory volume in 1 s; FVC, forced vital capacity; HCT, hematocrit; MPV, mean platelet volume; $PaCO_2$, partial pressure of carbon dioxide in arterial blood; PaO_2, partial pressure of oxygen in arterial blood; RBC, red blood cells; WBC, white blood cells.

Table 3 Laboratory characteristics during the stable period and during COPD exacerbation in group B patients (6 weeks after recovery)

Variables	Mean±SD		*P* value
	Group B with exacerbation (during exacerbation)	Group B with exacerbation (2 weeks after being stable)	
HCT	49.84±11.63	48.7±10.5	0.342
Hemoglobin (g/dl)	16.78±1.45	15.6±2.1	0.151
Platelet ($\times10^9$/l)	301.9±71.5	289.1±69.4	0.251
MPV (fl)	8.7±0.9	9.9±0.4	<0.011
WBC ($\times10^9$/l)	11.8±2.31	7.2±1.45	<0.001
CRP (mg/l)	20.9±3.1	12.7±2.4	<0.001

COPD, chronic obstructive pulmonary disease; CRP, C-reactive protein; HCT, hematocrit; MPV, mean platelet volume; RBC, red blood cells; WBC, white blood cells.

Table 4 Correlation between MPV and grade of severity of stable COPD

Variable	Mean±SD				*P* value
	Stage I	Stage II	Stage III	Stage IV	
Number of patients	10	30	22	18	
Platelet ($\times10^9$/l)	259±36	295±71	340±81	377±45	0.52
MPV (fl)	8.2	9.3	9.9	10.2	0.011

Stage I (mild): forced expiratory volume in 1 s to forced vital capacity ratio ($FEV_1/FVC<70\%$ and $FEV_1\geq80\%$ predicted; stage II (moderate): $FEV_1/FVC<70\%$ and $50\%\leq FEV_1<80\%$ predicted; stage III (severe): $FEV_1/FVC<70\%$ and $30\%\leq FEV_1<50\%$ predicted; stage IV (very severe): $FEV_1/FVC<70\%$ and $FEV_1<30\%$ predicted or $FEV_1<50\%$ predicted plus chronic respiratory failure. COPD, chronic obstructive pulmonary disease; FEV_1, forced expiratory volume in 1 s; FVC, forced vital capacity; MPV, mean platelet volume.

Table 5 Prevalence of pulmonary arterial hypertension among the studied stable COPD patients (80 patients)

Variables	Patients with pulmonary arterial hypertension [*n* (%)]	Patients without pulmonary arterial hypertension [*n* (%)]
Stable COPD patients (*N*=80)	48 (60)	32 (40)

follow-up visit 2 weeks later than during exacerbation (*P*=0.011), as shown in Table 3.

After re-evaluation and assessment of group B patients when they were clinically stable, they were added to group A to obtain 80 stable COPD patients to get a larger sample of stable patients for better valuable results. There was increased PLT count with increased COPD severity but it was statistically nonsignificant (*P*=0.52). On the other hand, there was a statistically significant positive correlation between MPV and COPD severity (MPV increased with increased grade of severity of COPD) (*P*=0.011), as presented in Table 4.

There were 48 patients (60%) among the studied 80 stable COPD patients with pulmonary arterial hypertension (PAH) diagnosed by echocardiographic examination, whereas the remaining 32 had no PAH (Table 5).

Among patients diagnosed by echocardiography to have PAH (48 patients) when in a stable state of COPD, 10 patients (20.8%) were found to have mild PAH [pulmonary artery systolic pressure (PASP)=35–50], whereas 26/48 (54.2%) had moderate PAH (PASP=50–70), and the remaining 12/48 (25%) patients had severe PAH (Table 6).

There was a statistically significant higher MPV among patients with PAH (9.6±0.8) than those without PAH (8.9±0.8) (*P*<0.0001) as shown in Table 7.

There was a statistically nonsignificant positive correlation between PLT count and increased severity of PAH (*P*=0.438); although PLT count increased with increased severity of PH, it was 285±18.13 in patients with mild PH and increased up to 335±12.29 among those with moderate PH and to 360±17.6 in severe cases, but such increases did not reach a significant value. On the other hand, there was a statistically significant positive correlation between MPV and increased severity of PAH (*P*=0.011), whereas MPV was 8.3±27.12 among patients with mild PH and it increased to 9.1±22.23 in those with moderate PH and up to 9.9±20.19 in severe PH (Table 8).

Discussion

COPD should be considered a complex systemic disease involving several organs and systems (musculoskeletal, cardiovascular, and endocrine) as well as metabolic

Table 6 Severity of pulmonary arterial hypertension among patients with pulmonary hypertension (48 patients)

Variables	Mild pulmonary arterial hypertension [*n* (%)]	Moderate pulmonary arterial hypertension [*n* (%)]	Severe pulmonary arterial hypertension [*n* (%)]
Patients with pulmonary hypertension (*N*=48)	10 (20.8)	26 (54.2)	12 (25)

Patients are said to have pulmonary hypertension when pulmonary artery systolic pressure (PASP) is more than or equal to 35 mmHg. Patients were categorized into mild (PASP=35–50), moderate (PASP=50–70), and severe PH (PASP>70). PASP, pulmonary artery systolic pressure; PH, pulmonary hypertension.

Table 7 Evaluation of mean platelet volume between patients with and without pulmonary arterial hypertension

Variables	Patients with pulmonary arterial hypertension [48/80 (60%)]	Patients without pulmonary arterial hypertension [32/80 (40%)]	*P* value
MPV (mean ±SD) (fl)	9.6±0.8	8.9±0.8	<0.0001

MPV, mean platelet volume.

abnormalities leading to weight loss [28]. Recent studies have shown that COPD is associated with low-grade systemic inflammation including systemic oxidative stress, activation of circulating inflammatory cells, and increased levels of inflammatory cytokines. The levels of inflammatory proteins such as CRP, fibrinogen, and proinflammatory cytokines are increased in the systemic circulation of patients with stable COPD [29]. CRP and tumor necrosis factor are important mediators linking disease severity with systemic inflammation. Increased proinflammatory cytokines such as CRP, tumor necrosis factor, and interleukin-6 in COPD are involved in enhanced oxidative stress, which contribute to PLT activation [30]. It is suggested that PLT activation represents a novel mechanism linking COPD, inflammation, and cardiovascular disease [31]. During exacerbation periods, this inflammatory state becomes worse and higher levels of interleukin-6, CRP, fibrinogen, and lipopolysaccharide-binding protein are released, which decrease again during recovery (32). Overproduction of proinflammatory cytokines and acute-phase reactants can suppress the size of PLTs by interfering with megakaryopoiesis and a subsequent release of small-sized PLTs from the bone marrow [33]. Recent studies indicated that low levels of MPV are associated with high-grade inflammatory diseases and reverse during the course of anti-inflammatory therapy [34]. There are various studies that show PLT dysfunction in patients of chronic obstructive airway disease. These studies showed local PLT activation in pulmonary vessels, increased PLT adhesiveness, and increased PLT aggregation and volume in such patients [17], which could possibly lead to PH and pulmonary thrombosis. The reasons and implications of this increased PLT volume in these patients are still not clear. Increased MPV reflects heightened PLT activity. Large PLTs are more reactive, probably younger, and produce more prothrombotic compounds. They also contain more dense granules and 5′ hydroxytriptamine [35]. Previous studies have suggested that COPD is associated with a prothrombotic and hypercoagulable state. A few studies have measured PLT activation and none has used a direct measure of PLT function. This study included a total of 80 COPD patients: group A included 40 patients in a stable state and group B included the remaining 40 selected from patients who sought medical advice in the outpatient clinic or were admitted to our outpatient and emergency clinics at the Pulmonary Diseases Department of Ain Shams Hospitals for exacerbation of COPD and were matched with 40 healthy, nonsmoking controls for age, sex, and BMI. The stable disease group (group A) (no exacerbation of COPD within the previous 6 weeks) were not prescribed regular oral steroid therapy or long-term oxygen therapy. An exacerbation of COPD was defined as sustained (48 h or more) worsening of dyspnea, cough, or sputum production leading to an increase in the use of maintenance medications and/or supplementation with additional medications [22]. They were studied within 24 h of admission and for at least 2 weeks following discharge from the hospital when their condition was considered to be clinically stable. We recorded the follow-up data of the same patients in the stable period 2 weeks after an acute exacerbation. The participants examined during exacerbation (group B) were further re-evaluated and assessed when in the stable phase of COPD during their follow-up visit. Spirometry, CBC including MPV, CRP, and arterial blood gases were performed for all the included patients. Transthoracic echocardiography was performed in group A and in group B after the patients were clinically stable during their follow-up visit. In this study, there was no significant correlation regarding age, sex, or smoking habits among the three studied groups (P=0.564, 0.743, and 0.451, respectively) as shown in Table 1. CRP and WBC counts tended to increase in a significant way with disease progression, and thus they were higher in patients with exacerbation than stable ones who had higher significant values compared with control healthy participants as well (P<0.001 for each). These results are in agreement with

Table 8 Correlation between mean platelet volume and severity of pulmonary arterial hypertension

Variables	Mean±SD			*P* value
	Mild pulmonary hypertension (10 patients)	Moderate pulmonary hypertension (26 patients)	Severe pulmonary hypertension (12 patients)	
Platelet ($\times 10^9$/l)	285±18.13	335±12.29	360±17.6	0.438
MPV (fl)	8.3±27.12	9.1±22.23	9.9±20.19	0.011

Mild (PASP=35–50), moderate (PASP=50–70), and severe PH (PASP>70). MPV, mean platelet volume; PASP, pulmonary artery systolic pressure; PH, pulmonary hypertension.

Freedman *et al.* [36] who observed that the median total leukocyte count was 36% higher in current smokers as compared with nonsmokers, and with Muhammad *et al.* [37] who suggested that the high WBC count in their study showed that male participants who smoked might be at a greater risk for developing atherosclerosis and cardiovascular diseases than nonsmokers. The mechanism for smoking-induced increase in WBC count is not clear. It has been suggested that inflammatory stimulation of the bronchial tract induces an increase in inflammatory markers in the blood, but it has also been suggested that nicotine may induce an increase in blood lymphocyte counts [38]. In fact, several studies have shown that WBC count is an independent predictor of atherosclerosis and cardiovascular disease [39]. In the present study, although PLT count and Hb levels were increased among exacerbated patients than in stable COPD patients, who showed higher ratios compared with controls, this was not significant (where *P*=0.346 and 0.451, respectively); these values are not in agreement with Muhammad *et al.* [37] who found Hb values to be significantly higher in smokers than in nonsmokers, but they did not study patients during exacerbation. In the present study, there was a significant correlation among the studied groups regarding RBC count and HCT, results that are in agreement with Muhammad *et al.* [37] who stated that RBC values were significantly high in smokers than in nonsmokers. High levels of RBC, termed as polycythemia, and very high RBC mass slow down blood velocity and increase the risk of intravascular clotting, coronary vascular resistance, decreased coronary blood flow, and a predisposition to thrombosis [40]. In the present study, MPV showed a highly significant statistical correlation among the studied groups, showing a significant decrease within the exacerbated patient group compared with stable patients who had lower values than the controls as shown in Table 2. This was not in agreement with Muhammad *et al.* [37] who did not find any significant changes in PLT, MPV, or platelet distribution width between smokers and nonsmokers. Moreover, Butkiewicz *et al.* [41] studied the effect of smoking on PLT activation and some morphological parameters including MPV, and they did not find any effect of smoking on MPV. Similarly, Arslan *et al.* [42] investigated the effects of smoking on MPV in a young, healthy, male population (smokers 56 and nonsmokers 46), and they found no significant difference in MPV between the smoking and nonsmoking healthy, male participants. However, this may be explained by the fact that they did not evaluate MPV among patients with COPD exacerbation, but only compared MPV between smokers and nonsmokers, which may have yielded nonsignificant results, and differences in the technique of PLT examination during the biochemical analysis regarding the tube into which the blood sample is collected for the whole blood count can cause PLTs to exhibit a time-dependent swelling when blood samples are anticoagulated with EDTA; however, this swelling does not occur in the presence of citrate. The time interval between blood sampling and blood analysis will yield completely different results, and they may have not excluded patients who may affect the outcome of PLT examination. However, these results were in agreement with the previous reports by Varol *et al.* [43] and Caponnetto *et al.* [44], who have shown that chronic smoking causes PLT activation and smoking cessation improves PLT function. The results of the present study are in agreement with Bansal *et al.* [45], who reported that MPV values were significantly higher in 100 patients with COPD compared with 100 healthy participants, which could be due to hypoxia causing bone marrow stimulation or increased sequestration of smaller PLTs with larger PLTs remaining in the circulation. Onder *et al.* [46] reported the effect of hypoxia on thrombocytes in COPD patients and found that MPV values were significantly increased in hypoxic patients with COPD compared with nonhypoxic participants and controls. In addition, there was agreement with the results of Biljak *et al.* [47], who found that 109 patients with COPD in the stable period had significantly increased PLT count and reduced MPV compared with 51 healthy controls, but they did not evaluate MPV values during acute COPD exacerbation. In the present study, follow-up data of the patients included in group B in a clinically stable period after an acute exacerbation 6 weeks from hospital discharge were recorded and further re-evaluated and assessed during their follow-up visit. There was a nonsignificant decrease in hematological parameters

after the exacerbated patient group became clinically stable, regarding RBC, HCT, Hb, and PLTs (P=0.451, 0.342, 0.151, and 0.251, respectively). On the other hand, there was statistically highly significant decrease in both CRP and WBCs during the stable period than during exacerbation for the same included group of patients (group B), when P values were less than 0.001 for both. MPV was significantly increased during the stable follow-up visit after 2 weeks than during exacerbation (P=0.011), as shown in Table 3. These results are in agreement with Ulasli *et al.* [48] who observed that MPV decreased during COPD exacerbation compared with the stable period and the control group, whereas serum leukocyte count and neutrophil percentage were increased. Moreover, a negative correlation between MPV and neutrophil percentage was observed indicating increased systemic inflammation during COPD exacerbation than in a stable state. Therefore, they suggested that decreased MPV values could predict acute exacerbation of COPD similar to other inflammatory markers. Moreover, these results are in accordance with John *et al.* [49] who found that during acute exacerbations all clinical parameters that were consistent with an acute exacerbation returned to normal values by the follow-up visit: PLT–monocyte aggregates were increased during acute exacerbation compared with follow-up; PLT expression of P-selectin was higher during acute exacerbation than during follow-up; MPV was increased in stable patients than during exacerbation, but not in a significant manner, which may have been due to a shorter follow-up period (only after 2 weeks), causing an underestimation of an effect of exacerbation on PLT activation; and also increases in the number of peripheral blood leukocytes and neutrophils as well as higher serum concentrations of CRP in patients with COPD exacerbation compared with stable participants. Circulating leukocyte and neutrophil concentrations were associated with PLT–monocyte aggregates, supporting the hypothesis that systemic inflammation in COPD may contribute to PLT activation in this condition. In the present study, after re-evaluation and assessment of group B, when being clinically stable, they were added to group A to obtain 80 stable COPD patients to get larger sample of stable patients for better valuable results. There was increased PLT count with increased COPD severity but it was statistically nonsignificant (P=0.52). On the other hand, there was a statistically significant positive correlation between MPV and COPD severity (MPV increased with increased grade of severity of COPD) (P=0.011), as presented in Table 4. These results are in agreement with Ulasli *et al.* [48]. MPV values of patients with stage IV COPD during the stable period were the highest; however, these results did not achieve statistical significance. This might be because of the small sample size and lower number of patients at stage IV included in their study. PAH and cor pulmonale are important complications in the natural history of COPD. Their presence is associated with decreased survival and increased use of health personnel and economic resources. The two major factors implicated in mortality due to COPD are severity of PH and development of cor pulmonale [50]. There were 48 patients (60%) among the studied 80 stable COPD patients with PAH determined by echocardiographic examination, whereas the remaining 32 had no PAH, in the present study (Table 5). These results were confirmed by Fazli *et al.* [51], who found the prevalence of PAH in their study to be 65.4%, which closely resembled those of the international study by Chaouat *et al.* [52] and Chatila *et al.* [53]. This was not in agreement with the results obtained by Naeiji *et al.* [54] who found the prevalence of PAH and cor pulmonale to be less than 10%. This may be because of the inclusion of all COPD patients regardless of severity in the present study. In the present study, among patients diagnosed by echocardiography to have PAH (48 patients) when in a stable state of COPD, 10 patients (20.8%) were found to have mild PAH (PASP=35–50), whereas 26/48 (54.2%) had moderate PAH (PASP=50–70) and the remaining 12/48 (25%) patient had severe PAH (Table 6). These results are in accordance with Sertogullarindan *et al.* [55] and Andersen *et al.* [56] who found the same frequencies of PH severity among the studied clinically stable COPD patients, but were not in agreement with Gajanan [57] who found the frequencies of mild PAH, moderate PAH, and severe PAH to be 23, 18, and 12%, respectively – a fact that may be explained by a small number of clinically stable patients included in his study. PAH constitutes a group of disorders that are characterized by hypertrophic and thrombotic obstructive changes in the pulmonary arterial tree [58]. PLT abnormalities and abnormal PLT activation are important mediators of disease progression, as they both contribute to thrombotic obstruction of pulmonary arteries and induce vessel remodeling [59]. The role of PLTs in the development and progression of PAH is well established. PLTs are an integral part of the coagulation system that contributes to thrombotic pulmonary arteriopathy. PLTs appear to be activated during pulmonary transit, and they may contribute to thrombin and clot generation [60]. Moreover, mediators liberated from thrombocytes, such as seratonin and PLT-derived growth factor, stimulate smooth muscle cell proliferation and medial hypertrophy. In the present study, there was a statistically significant higher MPV among patients with PAH (9.6±0.8) than those without

PAH (8.9±0.8) ($P<0.0001$) as shown in Table 7. These results are in agreement with Tolga *et al.* [61] who showed that patients with PH had higher MPV compared with normal controls and that patients with PH but not PAH had higher MPV compared with normal controls (9.06±1.05, 8.83±1.27, and 7.89±0.53, respectively). Can *et al.* [62] showed MPV values in patients with PAH, along with an enhanced aggregation in response to adenosine diphosphate. Similarly, we found increased MPV values in patients with PAH, and this increase was only weakly related to total thrombocyte count, thereby suggesting a primary abnormality rather than a response to total thrombocyte number. These results were also confirmed by a study performed by Perros *et al.* [63] who showed that MPV was significantly higher among adult patients with PAH than in a control group. Moreover, Mustafa *et al.* [64] found that MPV was significantly higher among patients with PAH when compared with controls (8.68±0.87 vs. 8.02±0.68 fl, respectively; P=0.006). These results were not in agreement with Derya *et al.* [65] who evaluated MPV among Group who had congenital heart disease (CHD) in left-to-right shunt patients with PAH, and another group who had CHD without PAH; there was a significant decrease in MPV ($P<0.0001$) was noted in the group patients (with PAH) than those without PAH. This may be explained by a different mechanism among patients with congenital heart disease than patients with thrombo-embolic or hypoxic PH. In the present study, there was a statistically nonsignificant positive correlation between PLT count with increased severity of PAH ($P=0.438$), although PLT count increased with increased severity of PH, and it was 285±18.13 in patients with mild PH, increased up to 335±12.29 among those with moderate PH, and increased to 360±17.6 in severe cases, but such increases did not reach significance. On the other hand, there was a statistically significant positive correlation between MPV and increased severity of PAH ($P=0.011$) – MPV was 8.3±27.12 among patients with mild PH, increased to 9.1±22.23 in those with moderate PH, and increased up to 9.9±20.19 in patients with severe PH (Table 8). These results are not in agreement with Can *et al.* [62] and Perros *et al.* [63] who found increased MPV but no increase in PLT count with increased severity of PAH, but not in significant values, a fact that may be explained by a small number of patients included in their study (only 30 patients); however, they showed that MPV was significantly higher among adult patients with PAH compared with a control group. The results of the present study were confirmed by Chu *et al.* [15], Peinado *et al.* [66], and Alper *et al.* [67] who found a significant positive correlation between PLT count and MPV with increased severity of PH among their studied patients.

Conclusion

It is suggested that decreased MPV values might indicate increased systemic inflammation during COPD exacerbation. Therefore, MPV could be used as a negative acute-phase reactant in acute COPD exacerbation. Measurement of changes in MPV values during follow-up may be considered as a quick and reliable tool for the assessment of inflammatory response. To conclude, this study shows that significantly increased PLT volume in patients with COPD could possibly contribute to increased pulmonary thromboembolism and PH in these patients; therefore, PLT activation as an important prothrombotic manifestation of the disease may be a useful therapeutic target in COPD.

Financial support and sponsorship

Nil.

Conflicts of interest

There are no conflicts of interest.

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