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ORIGINAL ARTICLE



PLATELET ACTIVATION AND CARDIOVASCULAR CO-MORBIDITIES IN PATIENTS WITH CHRONIC OBSTRUCTIVE PULMONARY DISEASE

Mario Malerba, Alessia Olivini, Alessandro Radaeli, Fabio Luigi Massimo Ricciardolo, Enrico Clini

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Abstract

Objective: Platelet activation in COPD patients is associated with an increased risk of cardiovascular events. We aimed at assessing the mean platelet volume (MPV), as an index of platelet activation, in COPD patients both when stable or during acute exacerbation (AE).

Re*search design and methods*: 478 patients (75 with AE) and 72 age-matched healthy controls were enrolled. Medical history, co-morbidities, medications, pulmonary function tests, MPV and blood cell count, erythrocyte sedimentation rate (ERS) and C reactive protein (CRP) were recorded.

Results: MPV was higher in COPD than in controls (8.7 ± 1.1 fL and 8.4 ± 0.8 fL respectively, p = 0.025) and increased across the severity of the diseases as assessed by post bronchodilator FEV_1 categorized I to IV ($p > 0.05$). MPV was higher in COPD patients during AE as compared with stable condition (8.7 ± 1.0 fL and 8.9 ± 1.0 fL, p = 0.021).

MPV ≥ 10.5 fL correlated with the presence of at least one co-existing cardiovascular disease (p = 0.008) . No correlation was observed between MPV and CRP or ERS in patients or in controls. A negative correlation was found between platelet count and MPV in COPD patients taken together.

Limitations: The retrospective design did not allow to assess a clear cause-effect relationship between MPV and all the pathophysiological factors considered

Conclusions: Elevated MPV is associated with lower platelet count and with cardiovascular co-morbidity in COPD patients. MPV value is higher in the more severe COPD and during AE. Present findings warrant future studies to confirm a possible clinically relevant role for platelet activation on cardiovascular risk in the population of COPD.

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ORIGINAL ARTICLE

PLATELET ACTIVATION AND CARDIOVASCULAR CO-MORBIDITIES IN PATIENTS WITH CHRONIC OBSTRUCTIVE PULMONARY DISEASE

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Key words: mean platelet volume, COPD, cardiovascular co-morbidities, platelet, inflammation.

[Short title: MPV and cardiovascular co-morbidity in COPD**]**

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ABSTRACT

Objective: Platelet activation in COPD patients is associated with an increased risk of cardiovascular events. We aimed at assessing the mean platelet volume (MPV), as an index of platelet activation, in COPD patients both when stable or during acute exacerbation (AE).

Re*search design and methods*: 478 patients (75 with AE) and 72 age-matched healthy controls were enrolled. Medical history, co-morbidities, medications, pulmonary function tests, MPV and blood cell count, erythrocyte sedimentation rate (ERS) and C reactive protein (CRP) were recorded.

Results: MPV was higher in COPD than in controls (8.7 ± 1.1 fL and 8.4 ± 0.8 fL respectively, p = 0.025) and increased across the severity of the diseases as assessed by post bronchodilator FEV_1 categorized I to IV (p>0.05). MPV was higher in COPD patients during AE as compared with stable condition (8.7 ± 1.0 fL and 8.9 ± 1.0 fL, p = 0.021).

MPV ≥ 10.5 fL correlated with the presence of at least one co-existing cardiovascular disease (p = 0.008) . No correlation was observed between MPV and CRP or ERS in patients or in controls. A negative correlation was found between platelet count and MPV in COPD patients taken together.

Limitations: The retrospective design did not allow to assess a clear cause-effect relationship between MPV and all the pathophysiological factors considered

Conclusions: Elevated MPV is associated with lower platelet count and with cardiovascular co-morbidity in COPD patients. MPV value is higher in the more severe COPD and during AE. Present findings warrant future studies to confirm a possible clinically relevant role for platelet activation on cardiovascular risk in the population of COPD.

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INTRODUCTION

An important association was observed between chronic obstructive pulmonary disease (COPD) and cardiovascular diseases [1,2], being events such as myocardial infarction [3] and stroke [4] more frequent in these patients than in the healthy population. Indeed, both COPD and cardiovascular diseases share common risk factors, smoking habit as most important [5].

A large body of research aimed at finding the explanation of this enhanced risk in the COPD population, focusing in particular on systemic inflammation as a driving mechanism for atherothrombosis [6, 7]. Recent studies have shown the presence of increased platelet activation in patients with COPD [8-10], which may be correlated with the presence of circulating inflammatory mediators and with the hypoxemia which is typical in the advanced stages of the disease [11]. The increased mean platelet volume (MPV) is an index of accelerated platelet activation, derives from the change of the platelets' shape, and leads to enhanced thrombosis [12-13]. Very recently, it has been demonstrated that high MPV is correlated with higher risk of developing atherosclerotic thrombotic cardiovascular events, mainly in chronic patients suffering from diabetes, hypertension and obesity, where platelet activation is enhanced [14]. Moreover, in elderly male patients with COPD, the MVP was inversely correlated with the level of FEV_1, and the impairment of cardiopulmonary function [15].

In the present study we aimed at assessing the MPV value in a large population of COPD patients both when stable or during an episode of acute exacerbation (AE) and to determine whether correlation exists between MPV and the stage of disease severity, the systemic inflammation and the increased prevalence of atherothrombotic cardiovascular events in the studied population.

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METHODS

Study protocol

Four-hundred and seventy eight COPD patients admitted between April 2012 and December 2013, for different purposes (poorly controlled arterial hypertension, syncope, pleuritic chest pain, dizziness and disequilibrium, associated-disease disability), at both the Unit of Internal Medicine-Spedali Civili (Brescia), Rehabilitation Unit-Ospedale Villa Pineta (Pavullo n/F, MO), and Pneumology Unit- Ospedale San Luigi (Orbassano, TO) were retrospectively studied. Seventy-five of them were admitted due to an AE of the disease.

Diagnosis of COPD was based on the medical history and confirmed by spirometry performed during the first day following hospitalization [16], or when reaching stability at discharge in those subjects having exacerbation.

Patients were classified by severity in four stages according to the post bronchodilator FEV_1 as follows: Stage I= $FEV_1 \geq 80\%$ pred, Stage II FEV_1= 50-79% pred, Stage III FEV_1= 30-49%pred, Stage IV $FEV_1 < 30\%$pred [16] (Table 1). AE has been defined according to the international consensus [17]. Clinical stability was defined as the absence of significant changes in symptoms beyond the expected daily variation, along with no requirements for any increase in usual treatments [18].

Control population consisted of 72 subjects matched for age, sex and smoking history, but with normal pulmonary functions, similarly referred to our units.

Subjects with bronchial asthma, acute and/or chronic pulmonary thrombo-embolism, obstructive sleep apnea, connective tissue and inflammatory bowel diseases, fever of any origin, chronic renal failure, chronic liver diseases, haematological abnormalities, or unable to perform spirometry were excluded.

This study complied with the Declaration of Helsinki and was approved by the local Review Board at each centre. A waiver of consent was granted, and patient identity was protected.

Measurements

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Medical history, respiratory function tests, and blood analysis was recorded in each participant. Active smoking was defined if a current use of >2 cigarettes/day was reported; the number of pack/year was also recorded.

Respiratory Function Tests- Lung spirometry was performed in accordance with the standard procedure [19]. Forced Vital Capacity (FVC) and Forced Expiratory Volume in the first second (FEV_1) were recorded.

Peripheral Blood analysis- Blood sample was taken at admission. Ten millilitres of fasting bloods were drawn from each subject enrolled, tubes containing the dipotassium salt of thylenediamine tetraacetic acid (EDTA) were used for measurement of MPV [20]. Samples were immediately taken to the Hospital central laboratory to be analysed.

Total count on platelets, red and white cell, the MPV, and the level of both erythrocyte sedimentation rate (ESR) and C-reactive protein (CRP) were recorded for study purposes. The inter- and intra-assays coefficients of variation of these assays were below 5%.

Co-morbidities- Co-existing chronic diseases were identified by examining the clinical documentation provided by each subject at admission and/or by the results from diagnostic tests performed during hospitalization.

Diabetes mellitus was diagnosed if plasma glucose > 126 mg/dL, and/or treatment with anti-diabetic drugs, and/or presence of haemoglobin A1c > 6.5% [21, 22].

Systemic arterial hypertension was defined if assuming antihypertensive treatment, in the presence of a medical history with documented high blood pressure (BP), if systolic BP >140 mm Hg and/or diastolic BP > 90 mm Hg were recorded on 3 separate occasions [23].

Dyslipidemia was defined in the presence of hypolipidemic treatment, and if total cholesterol >200 mg/dL, and/or triglycerides >200 mg/dL, high-density lipoprotein cholesterol >40 mg/dL, low-density lipoprotein cholesterol >130 mg/dL [24].

Coronary artery disease was based on the history of previously documented myocardial infarction and/or suggestive changes at the electrocardiogram (ECG) [25].

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Heart failure was defined according to the medical history and the presence of documented left ventricular ejection fraction <55% (Simpson method) together with some degree of exertional dyspnea [26].

Obesity was defined when BMI was greater than or equal to 30.

The presence of cerebrovascular disease was based on the past medical history of cerebral infarction, cerebral thrombosis, transient ischemic attack, artery occlusion at the carotid or vertebral vessels.

Statistical Analysis

Data were presented as mean ± standard deviation (SD). Subjects were analysed according to different groups (stable or exacerbated COPD and Controls) or categories (presence of at least one cardiovascular co-morbidity, MPV value ≥10.5 fL as the median cut-off of distribution).

After verifying that data were normally distributed, group comparisons were performed using t-test for independent variables. Analysis of correlation among variables was performed by linear regression with a straight trend and through the calculation of Pearson coefficient. Categorical variables were compared by the chi-square test method.

A p value <0.05 was considered to be significant. All analysed were performed with a dedicated software (SPSS 21.0 for Windows; SPSS, Chicago, IL).

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RESULTS

Characteristics of subjects in study are displayed in Table 1. Most of the COPD patients were in stage II or III according to the post bronchodilator FEV_1. Seventy-five patients were under AE, being twenty-five (33%) of them in stage IV of the disease.

Chronic co-morbidities were similar between COPD and controls, as well as the use of antiaggregants and/or anticoagulants was similar.

Two-hundred twenty seven (47.4%) out of the 478 COPD patients (192/403 stable and 35/75 during AE) had at least one cardiovascular disease (hypertension, acute myocardial infarction, exertional angina, coronary artery disease, cerebral vascular disease, arterial disease of the lower limbs, thromboembolic disease). The proportion of patients with at least one cardiovascular co-morbidity was also similar (48.6%) in the control group.

Two-hundred fifty eight COPD were taking acetylsalicylic acid (ASA) as an antiplatelet therapy (11 subjects were under dual antiplatelet therapy with ASA and clopidogrel); 89 patients were receiving anticoagulation therapy with warfarin (n=86) or enoxaparin (n=3). Forty-one out of 72 controls were receiving ASA (one subject was under dual therapy with ASA and clopidogrel), while 11 were assuming warfarin. No difference was noted in the medication taken in the group of COPD patients under AE compared to the group of COPD patients in stable condition.

Table 2 resumes data of both lung function and peripheral blood in the study groups. In particular, the mean MPV differed significantly between COPD patients taken together and controls (respectively 8.7 ± 1.1 fL and 8.4 ± 0.8 fL in COPD patients and in controls $p = 0.025$), despite platelet count was similar. The mean values of ESR and PCR were similar in patients and in controls (see Table 2).

Noteworthy, we observed an inverse correlation between the platelets count and the MPV in patients with COPD (Pearson = -0.115, $p < 0.05$, Figure 1) but not in controls (Pearson = -0.009, ns). Mean MPV did not correlate with ESR, PCR, the total number of co-morbidities and the use of

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antiplatelet/anticoagulant therapies in the whole population in study, nor in the COPD group either when stable or during AE.

Sub-group analysis: COPD patients without or with AE.

Table 3 and Figure 2 show comparison of mean MPV value between study groups including COPD with or without AE. In COPD patients, MPV level was lower when stable as compared during AE (8.7 ± 1.0 fL, and 8.9 ± 1.0 fL respectively, p = 0.021). Moreover, MPV was higher in COPD stage II to IV when compared with stage I (see Table 3 and Figure3). Mean MPV was also compared within each post bronchodilator category in stable patients and in those under AE, but no significant differences were reported. Among the COPD patients, neither ESR nor PCR differed according to the different clinical condition (stable state or AE).

Platelet count was lower during AE than in stable state (211.2 ± 96.5 and 218.7 ± 64.2 x 103 / mm3 respectively, $p<0.05$).

Finally, the presence of a MPV ≥10.5 fL significantly correlated with the presence of at least one cardiovascular co-morbidity in COPD patients taken together (but not in controls) (see Table 4).

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DISCUSSION

Our study shows that MPV values are higher in COPD patients than in healthy controls and increases with the degree of severity and during AE. Moreover, high level of MPV is associated with at least one cardiovascular co-morbidity in the population of COPD but not in comparable controls.

Data of mean MPV in COPD are still inconsistent and contradictory. Onder et al. [27] observed for the first time higher MPV values in hypoxic COPD patients when compared with controls. Additionally, Bansal et al. [28] as well as Ulasli et al [29] reported higher MPV in patients with COPD. The latter study also measured a reduced MPV during AE, however the smoking status of patients was not reported and COPD patients had a significantly lower BMI, at difference with our study.

A recent paper [30] has evaluated 269 COPD patients and finding have shown that, in disagreement with our data, lower MPV during AE negatively correlated with PCR values. This discrepancy is probably due to different characteristics between the two populations studied, furthermore neither BMI nor smoking history were reported and lung function (FEV1 and FEV1/FVC) was surprisingly similar both in stable and exacerbated COPD patients.

Cui et al [15] was the first to observe that increased MPV in very old male patients with COPD was associated with impaired cardiopulmonary function, linking the concept of platelet activation with the prevalence of cardiovascular co-morbidities in this population. Recent awareness on the enhanced risk of cardiovascular co-morbidities in COPD patients has stimulated research to study the possible pathophysiological mechanisms that stand behind this phenomenon [31]. Several mechanisms including hypoxia, systemic inflammation and imbalance of the proteases to antiproteases ratio (with an increased expression of neutrophil elastase) have been considered able to promote platelet activation and hyperaggregability and to stimulate atheromasic plaque progression [8-11]. The mechanism of platelet activation involves different phenomena such as change in the shape of platelets, increase in the MPV, expression of P-selectin in the cell's

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membrane [32], formation of aggregates with platelets and monocytes [33], thus favouring the endothelial activation and the atherosclerotic plaque formation.

The increased values of MPV could follow the hypoxemia that is typical in patients with COPD, in particular at peripheral tissue level, which may trigger bone marrow stimulation or increase the sequestration of smaller platelets [28]. COPD is associated with systemic inflammation, oxidative stress, activation of circulating inflammatory cells and increased levels of inflammatory mediators, mechanisms that may be partly responsible of platelet activation as observed in COPD [29,31], where the presence of a pro-thrombotic state with high production of thrombin and pro-coagulative mediators was reported [34].

By confirming previous data on increased MPV in the COPD population, present study is the first to show that it is present in the more severe stages of the disease and/or during AE. Therefore, the severity of COPD is possibly associated with a progressive increase of MPV, despite the pathophysiological mechanism(s) involved, among all those mentioned above, has not yet been completely clarified. Indeed, our data do not suggest a direct relationship between hypoxemia and elevation of MPV, neither demonstrated a significant difference of inflammatory serum markers (ESR and CRP) between COPD and controls. Notwithstanding, values of both CRP and ESR are higher in the two groups compared to the normal range.

Interestingly, we have found a significant negative correlation between platelet count and MPV value only among the COPD population. This could be explained by the increased proportion of activated platelets sequestered at the peripheral level and by the precipitation of the latter in the form of aggregates of platelets and monocytes, which are typically seen during the process of platelet activation [35,36]. One of the most interesting and novel aspect of our study is the observation of a direct association between high MPV level (above the pre-defined limit of ≥10.5 fL) and the at least one cardiovascular co-morbidity in patients with COPD but not in the controls. This finding could support the above mentioned hypothesis about the presence of a tight relationship between platelet activation, systemic atherosclerosis, plaque rupture and cardiovascular

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events in COPD patients. In a large population cohort (the “Rotterdam Study”) it has been recently demonstrated a higher prevalence of carotid atherosclerosis in elderly COPD compared with healthy controls, which is independent on the stage of the disease [37]. This study also advanced the concrete hypothesis that COPD may be an independent predictor for the presence of carotid atherosclerosis.

Despite our findings in a consistent population of COPD confirm the role of platelet activation in the disease and also clarify the concept that this happens according to the degree of severity and during AE, present study has some limitations that deserves to be outlined. A first potential limit is the retrospective design, thus a selection bias (e.g. the similar systemic inflammation level in both groups) may have occurred. Second, we were not able to report a patent cause-effect relationship between variable of interest (MPV) and all the pathophysiological factors behind (such as arterial oxygen pressure level). Notwithstanding, the strong point of our study is primarily the large sample size of COPD patients and the objective recording of co-morbidities [38].

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CONCLUSION

Our observational study underlines the presence of platelet activation (i.e. elevated MPV and reduce platelet count) in COPD patients, being patients with higher degree of severity an/or those under AE at higher risk of developing cardiovascular co-morbidity. This indirectly suggests a clear relationship between platelet activation and the excess of cardiovascular events commonly observed in the COPD population. On the basis of the present data it would be possible to hypothesize the prophylactic use of antiplatelets or anticoagulants in order to reduce the future risk of cardiovascular events [39] in patients with COPD, especially when severe or frequent exacerbators. To test this hypothesis, however, further prospective studies on larger populations are needed.

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Transparency

Declaration of funding:

There was no funding for this study.

Declaration of financial/other relationship:

MM, AO, AR, FLMRA, ECR have disclosed that they have no significant relationships with or financial interests in any commercial companies related to this study or article. CMRO Peer Reviewers on this manuscript have no relevant financial relationships to disclose.

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FIGURE LEGENDS

Figure 1: Relationship between MPV and Platelet count in COPD patients.

Figure 2: Comparison between MPV in controls vs stable COPD vs COPD with exacerbation. *(horizontal black lines show the means, boxes show values between 25 and 75%, vertical black lines show maximum and minimum values).*

Figure 3: Comparison between MPV in COPD Stage I vs COPD Stage II, III and IV. *(horizontal black lines show the means, boxes show values between 25 and 75%, vertical black lines show maximum and minimum values).*

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Table 1: Demographic and clinical characteristics of the subjects studied.

	COPD	COPD stable	COPD exacerbation	Controls	p
Total number	478	403	75	72	
Age years, mean ± SD	74.26 ± 7.8	74.26 ± 7.8	74.26 ± 7.8	75.83 ± 9.19	N.S.
SEX, % of Male	62%	64%	60%	60%	N. S.
BMI, kg/m²	27.17 ± 4.51	28.47 ± 3.61	26.87 ± 5.51	27.75 ± 5.2	N. S.
Smoking status, %					
Yes	32%	30%	32%	31%	N.S.
No	18%	20%	18%	20%	N.S.
Former	50%	51%	50%	49%	N.S.
Packs/year, mean ± SD	40.82 ± 25.23	38.43 ± 33.21	40.23 ± 43.21	39.72 ± 18.58	N.S.
Patients with acute exacerbation	75 (18.6%)	0	75	-	
Disease Severity, n/%					
Stage I (FEV_1≥80%pred)	50 (10.5%)	36 (8,9%)	14 (18,7)	-	
Stage II (FEV_1=50-79% pred)	175 (36.6%)	156 (38,7%)	19 (25,3%)	-	
Stage III (FEV_1=30-49% pred)	165 (34.5%)	148 (36,7%)	17 (22,7%)	-	
Stage IV (FEV_1<30% pred)	88 (18.4%)	63 (15,6%)	25 (33,3%)	-	
Co-morbidities, n/%					
Diabetes	143 (30%)	118 (29%)	25(33%)	23 (32%)	N.S.
Dyslipidemia	86 (18%)	72 (18%)	14 (19%)	15 (21%)	N.S.
Arterial Hypertension	282 (59%)	231 (57%)	51 (68%)	45 (63%)	N.S.
Obesity	52 (11%)	44 (11%)	8 (10%)	5 (7%)	N.S.
Myocardial infarction, angina, coronaroscl.	162 (34%)	135 (33%)	27 (36%)	20 (29%)	N.S.
Cerebrovascular disease	158 (33%)	132 (33%)	26 (34%)	21 (30%)	N.S.
Heart failure	258 (54%)	219 (54%)	39 (52%)	34 (47%)	N.S.
Other cardiovascular diseases	124 (26%)	105 (26%)	19 (25%)	17 (24%)	N.S.
Medication, n/%					
Antiaggregation					
ASA	258 (54%)	219(54%)	39(52%)	41 (57%)	N.S.
Clopidogrel	11 (2%)	9(2%)	2(2%)	1 (1%)	N.S.
Anticoagulation					
Warfarin sodico	86 (18%)	73(18)	13(17)	11 (15%)	N.S.
Enoxaparina	3 (1%)	3(1%)	0	0 (0%)	N.S.
Inhaled drugs					
Anticholinergics	224 (47%)	190(47%)	34(45%)	-	
β_2-agonists	200 (42%)	170(42%)	30(40%)	-	
Corticosteroids	230 (48%)	195(48%)	35(46%)	-	
Oxygen	33 (7%)	28(7%)	5(6%)	-	

COPD: chronic obstructive pulmonary disease. BMI: body mass index. FEV_1: Forced expiratory volume at first second. ASA: acid acetylsalicylic.

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Table 2. Lung function and peripheral blood analysis of the subjects studied.

	COPD	COPD stable	COPD exacerbation	Controls	p
Functional data					
FEV_1 (% pred.)	47.4 ± 18.32	48.6±16.9	26.3±16.9	98 ± 1.2	p < 0.001
FVC (% pred.)	71.6±21	73.0±19.0	37.4±33	90.2 ± 14.3	p < 0.001
Tiffenau index	51.0±10.1	51.2±10.3	56.2±18.3	93.2± 4.5	p < 0.001
Laboratory data					
White Blood Cells ($x10^3/mm^3$)	9.05 ± 21.32	9.6 ± 26.2	10.6 ± 31.2	7.55 ± 14.58	p < 0.01
Red Blood Cells ($x10^6/mm^3$)	3.98 ± 0.23	3.7 ± 0.33	3.99 ± 0.26	3.81 ± 0.48	N.S.
Platelets ($x10^3/mm^3$)	215.2 ± 80.6	218.7 ± 64.2	211.2 ± 96.5	229.5 ± 61.6	p<0.05
Mean Platelet Volume (MPV) (fL)	8.76 ± 1.08	8.7 ± 1.0	8.9 ± 1.0	8.44 ± 0.78	p < 0.05
ESR (mm/h)	31.3 ± 67.1	32.3 ± 66.1	29.3 ± 50.3	28.4 ± 83.44	N.S.
CRP (mg/l)	43.2 ± 153.2	46.2 ± 144	42.2 ± 156	46.5 ± 145.8	N.S.

COPD: chronic obstructive pulmonary disease, FEV_1: forced exhaled volume at first second, FVC: forced vital capacity, ESR: Erythrocyte Sedimentation Rate, CRP: C Reactive Protein.

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Table 3. Statistical comparison of MPV in the study groups and within the COPD population.

	MPV (fL)	p
COPD	8.7 ± 1.1	0.025
Controls	8.4 ± 0.8	
COPD stable	8.7 ± 1.0	0.021
COPD with exacerbation	8.9 ± 1.0	
Stage I(FEV_1≥80% pred)	8.3 ± 0.9	0.011*
Stage II (FEV_1=50-79% pred)	8.7 ± 0.8	
Stage III(FEV_1=30-49% pred)	9.1 ± 1.5	
Stage IV(FEV_1<30% pred)	8.8 ± 1.0	

Data expressed as mean ± standard deviation
COPD: chronic obstructive pulmonary disease, MPV: mean platelet volume
*ANOVA

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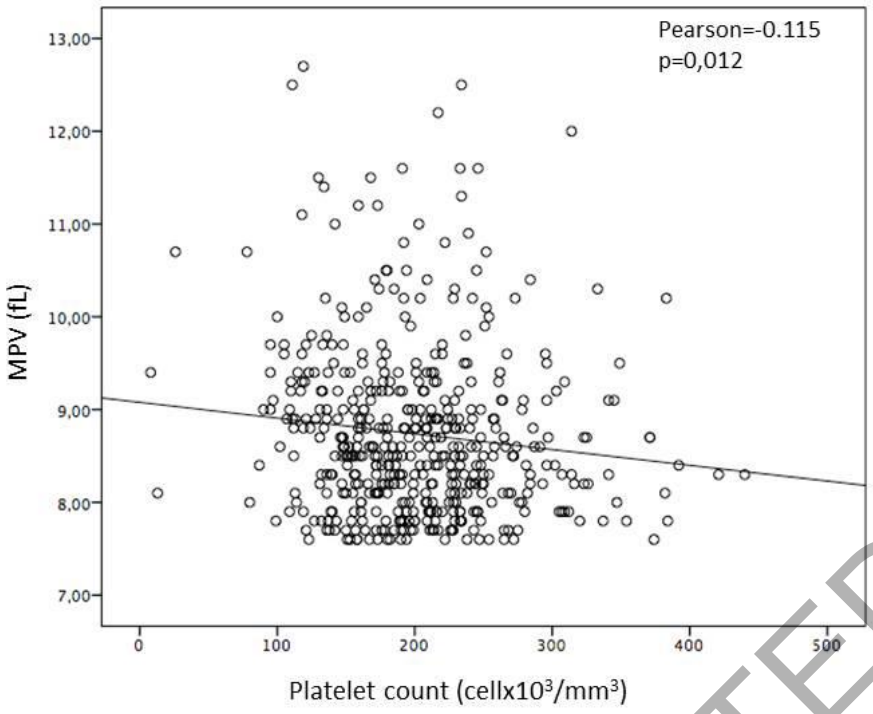

Figure 1: Relationship between MPV and Platelet count in COPD patients

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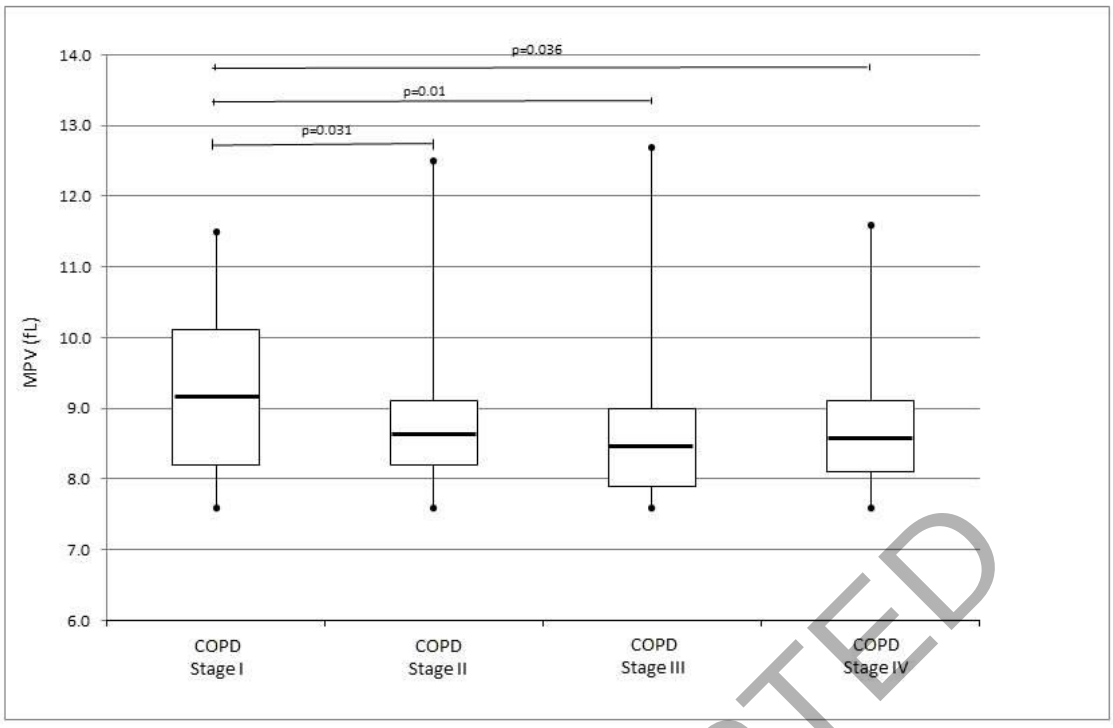

Figure 3: comparison between MPV in COPD Stage I vs COPD Stage II, III and IV. *(horizontal black lines show the means, boxes show values between 25 and 75%, vertical black lines show maximum and minimum values)*

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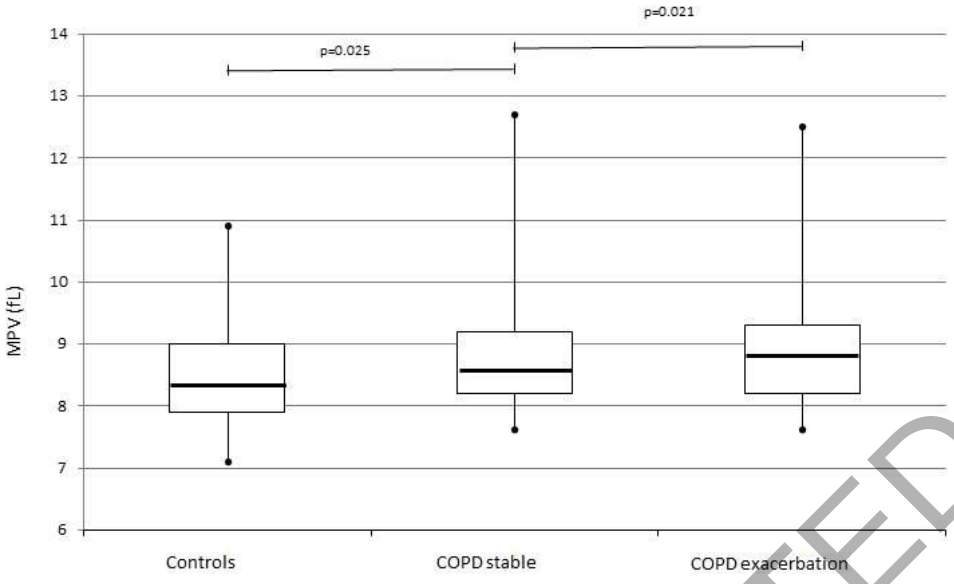

Figure 2: comparison between MPV in controls vs stable COPD vs COPD with exacerbation. *(horizontal black lines show the means, boxes show values between 25 and 75%, vertical black lines show maximum and minimum values)*

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