Increased Platelet-Monocyte Interaction in Stable COPD in the Absence of Platelet Hyper-Reactivity

Floor E. Aleva^{a,c} Godfrey Temba^b Quirijn de Mast^{b,c} Sami O. Simons^a
Philip G. de Groot^{b,c} Yvonne F. Heijdra^a André J.A.M. van der Ven^{b,c}

Departments of ^aRespiratory Medicine and ^bInternal Medicine, Radboud University Medical Center, and
^cRadboud Center for Infectious Diseases, Radboud University Medical Center, Nijmegen, The Netherlands

Keywords

Blood platelets · Inflammation · Cardiovascular diseases

Abstract

Background: Chronic obstructive pulmonary disease (COPD) is well known for its cardiovascular co-morbidities. Increased platelet-monocyte interaction is found in COPD and may reflect altered platelet function and a potential role for anti-platelet therapy. **Objectives:** The objectives were to investigate platelet-monocyte interaction, platelet activation and reactivity and plasmatic coagulation in stable COPD. **Methods:** Platelet-monocyte interaction and platelet activation were determined by flow cytometry in 30 stable COPD patients and 25 controls. Platelet activation was measured by binding of fibrinogen to the activated fibrinogen receptor and platelet P-selectin expression at baseline and after platelet stimulation with platelet agonists. Plasmatic coagulation was measured by D-dimer and thrombin generation. **Results:** Platelet-monocyte interaction was increased in stable COPD (median fluorescence intensity of platelet CD61 was 19.8 [IQR 14.0–33.2] vs. 10.0 [IQR 8.7–16.7], $p = 0.002$). In con-

trast, platelet activation and reactivity, reflected by fibrinogen binding and P-selectin expression, were the same in both groups. Plasma P-selectin and interleukin-6 were increased in COPD ($p = 0.01$ and $p = 0.02$, respectively), whereas soluble fibrinogen, D-dimer and thrombin generation were similar. **Conclusions:** Increased platelet-monocyte interaction was found in the absence of platelet hyper-reactivity and activation of plasmatic coagulation in stable COPD. Future clinical evaluation of the effects of different anti-platelet drugs in COPD is warranted, as anti-platelet therapy may interfere with platelet-monocyte interaction.

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Introduction

Chronic obstructive pulmonary disease (COPD) is not only recognized as the third leading cause of death worldwide, but is also associated with the development of cardiovascular co-morbidities [1–5]. Most cardiovascular diseases (CVD) in COPD result from arterial thrombosis [2–4]. However, the risk for venous thrombosis should

not be underestimated [6, 7]. A further increase in thrombotic complications is seen during or shortly after episodes of acute exacerbations of COPD (AE-COPD) [8, 9]. A recent meta-analysis indicates a higher risk of myocardial infarction during AE-COPD compared to stable disease [9], and similar results have been reported for stroke [10]. Low-grade systemic inflammation in COPD is commonly thought to play an important role both in COPD pathophysiology and in the development of its cardiovascular complications [4, 11, 12].

Extensive cross-talk between inflammation and coagulation exists, and platelets, monocytes and their interaction play a pivotal role in chronic inflammation and development of CVD [13, 14]. When activated, platelets secrete a variety of biologically active substances, including cytokines and chemokines. Platelet degranulation leads to increased expression of P-selectin on the platelet membrane, whereas a conformational change in the integrin $\alpha\text{IIb}\beta 3$ results in increased fibrinogen binding [15]. P-selectin facilitates binding to P-selectin glycoprotein ligand-1 (PSGL-1) expressed on monocytes, resulting in platelet-monocyte complexes (PMCs), an early process in atherothrombosis [13, 16]. Fibrinogen binding results in platelet aggregation, clot formation and adhesion of platelets to the vascular endothelial surface [13, 14]. Compelling evidence shows that platelet hyper-reactivity and increased PMC formation contribute to the development of CVD [13, 17, 18]. Anti-platelet therapy is the cornerstone of treatment to prevent thrombotic cardiovascular morbidity.

Surprisingly, platelet function is scarcely studied in COPD. Some evidence indicates that platelet function may be altered [19–22]. Maclay et al. [21] showed that stable COPD patients have increased circulating PMCs that increase further during AE-COPD. Increased PMC formation is often considered to be a result of platelet hyper-reactivity; however, platelets and monocytes can interact in various ways [15]. To the best of our knowledge, no direct assessment of platelet function in stable COPD has been performed to date. These data add to the current debate on whether COPD patients may benefit from anti-platelet therapy in order to prevent CVD [20, 21, 23].

We hypothesized that COPD patients have increased platelet-monocyte interaction due to increased platelet activation and hyper-reactivity upon platelet stimulation. Therefore, we determined (1) platelet-monocyte interaction; (2) platelet activation and reactivity and (3) plas-matic coagulation in stable COPD patients and control subjects.

Materials and Methods

Patient Selection

Thirty stable COPD patients and 25 controls were included in the study. The inclusion criteria for COPD patients were irreversible expiratory airway obstruction according to the ATS/ERS criteria [24] (FEV_1/FVC ratio $<70\%$ and post-bronchodilator $\text{FEV}_1 <80\%$ [COPD GOLD II–IV]) and clinical diagnosis of COPD confirmed by a pulmonologist. General exclusion criteria were use of platelet function inhibitors, asthma, malignancies or chronic inflammatory diseases, such as rheumatoid arthritis and inflammatory bowel disease. Patients were considered stable when they had not experienced an AE-COPD, as defined by the Anthonisen criteria [25], in the preceding 6 months. Patients were recruited between October 2015 and February 2016 at the outpatient clinic of our respiratory department, and controls were recruited among employees at Radboud University Medical Center. Spirometry was performed in the control group to exclude underlying lung disease. This study was approved by the Ethical Committee of Radboud University Nijmegen and was conducted according to the principles of the Declaration of Helsinki (version October 2008) and in accordance with the Dutch Medical Research Council working under the Human Subjects Act. All participants gave written informed consent. Blood was collected in EDTA and 3.2% sodium citrate vacutainer tubes (Becton Dickinson, Plymouth, UK) and processed immediately. Blood counts were measured with a haematology analyser (Sysmex XN-450, Kobe, Japan).

Platelet Reactivity and Platelet-Monocyte Interaction

Platelet activation and reactivity were measured by a flow cytometry-based assay that has previously been described and that is able to detect both platelet hyper-reactivity and thrombopathy in different clinical settings [26–28]. In short, whole-blood samples were stained with antibodies for the platelet identification marker CD61 and for 2 platelet activation markers. The platelet activation markers used were the binding of fibrinogen to the activated fibrinogen receptor $\alpha\text{IIb}\beta 3$ (GPIIb/IIIa complex) and the expression of α -granule protein P-selectin (CD62P) on the platelet surface, representing platelet aggregation and degranulation, respectively. Platelet reactivity was defined as the expression of these markers after stimulation of the samples with platelet agonists [27]. The samples were measured unstimulated (at baseline) and after *ex vivo* stimulation with the following agonists: adenosine 5'-diphosphate (ADP) (Sigma-Aldrich, Saint Louis, MO, USA), cross-linked collagen-related peptide (CRP-XL) (kind gift from Prof. Dr. R. Farndale, Cambridge, UK) and thrombin receptor-activating peptide-6 (TRAP) (Sigma-Aldrich). The staining of the samples was performed with antibodies for CD61 (PC7-conjugated) (Beckman Coulter, Brea, CA, USA), anti-human fibrinogen (fluorescein isothiocyanate-conjugated) (Dako, Santa Clara, CA, USA) and P-selectin (CD62P, phycoerythrin-conjugated) (BioLegend, San Diego, CA, USA), and the samples were co-incubated with the platelet agonists or medium control for 20 min. After stimulation, the samples were fixated with 0.2% paraformaldehyde and measured by flow cytometry (FC500 flow cytometer, Beckman Coulter). Gating of platelets was performed based on forward and sideward scatter and additionally for CD61 positivity. The mean fluorescence intensity (MFI) of the human fibrinogen and human P-selectin antibodies on the platelet surface was used as a quantification of

the platelet activation marker signal. The MFI is a measure for the mean intensity of the fluorescence signal of the labelled antibody that is detected in the corresponding flow cytometry fluorescence channel.

For measurement of platelet-monocyte interaction, samples were incubated with monoclonal antibodies to CD14 (phycoerythrin-conjugated) (Becton Dickinson, Franklin Lakes, NJ, USA) and CD61 (PC7-conjugated) (Beckman Coulter) for 20 min. Erythrocytes were lysed with OptiLyse B (Beckman Coulter), and the reaction was stopped by dilution with distilled water. Gating of monocytes was performed based on forward and sideward scatter and additionally for CD14 positivity. The degree of platelet-monocyte interaction was quantified by the MFI of platelet marker CD61 on monocytes (CD14-positive cells).

Plasmatic Coagulation

Tissue factor-triggered thrombin generation was measured in platelet-poor plasma using the automated thrombogram method as described previously [29]. In this assay, the thrombin generation curve was evaluated based on parameters that describe the initiation, propagation and termination phases of thrombin generation expressed as lag time, endogenous thrombin potential and thrombin peak, respectively. Plasma was obtained after centrifugation of blood tubes at 3,800 rpm for 10 min. Samples were measured unstimulated and after stimulation with tissue factor (1 pM).

Soluble Markers of Platelet Activation, Coagulation and Inflammation

Soluble fibrinogen and soluble P-selectin levels were determined in plasma using the human fibrinogen ELISA kit and human P-selectin/CD62P DuoSet ELISA kit (R&D Systems, Minneapolis, MN, USA), respectively. D-dimer, high-sensitivity interleukin-6 (hsIL-6) and C-reactive protein were determined with the human D-dimer ELISA kit (Abcam, Cambridge, UK), human hsIL-6 Quantikine ELISA kit (R&D Systems) and human C-reactive protein ELISA kit (R&D Systems), respectively. For platelet content, whole blood was centrifuged for 15 min at 156 g without break to obtain platelet-rich plasma. Platelet concentration was adjusted to $300 \times 10^9/L$ by addition of autologous plasma. Samples were freeze-thawed for 3 cycles to fragment the platelets, followed by ultra-centrifugation for 5 min at 5,000 rpm to spin down large particles, and supernatants were taken for ELISA. Platelet fibrinogen and P-selectin were determined with the ELISA kits described above.

Data Analyses

Statistical analysis was performed with GraphPad Prism 5.0 (San Diego, CA, USA) and IBM SPSS Statistics 22.0 (New York, NY, USA). Non-normally distributed data were analysed with Mann-Whitney U tests, and results are presented as medians with interquartile ranges (IQR). Normally distributed data were analysed by Student *t* tests (2-sided), and results are presented as means with standard errors of the mean. Two-way ANOVA analyses were performed for repeated measures. Haematological data were analysed using the Student *t* test for continuous variables and presented as means with standard deviations. For expected counts <5, a Fisher exact test was performed. Associations between inflammation and platelet markers were determined using the Spearman rank correlation coefficient. The differences were considered significant if $p < 0.05$.

Table 1. Demographics

	COPD patients	Control subjects
Participants, <i>n</i>	30	25
Age, years	61.6±10.0	53.0±11.7
Male	14 (46.7)	9 (36.0)
Current smokers	6 (20.0)	2 (8.0)
Smoking history, pack-years	50.7±27.8	1.6±4.3
GOLD classification		na
I	1 (3.3)	
II	11 (36.7)	
III	15 (50.0)	
IV	3 (10.0)	
Respiratory function		
FEV ₁ , L	1.36±0.6	3.12±0.89
FEV ₁ % predicted	48.9±17.5	98.0±13.9
FVC, L	3.31±0.73	3.94±1.08
FEV ₁ /FVC ratio	42.9±12.9	80.4±5.5

Values are means ± SD or *n* (%). COPD, chronic obstructive pulmonary disease; FEV₁, forced expiratory volume in 1 s; FVC, forced vital capacity; GOLD, Global Initiative for Chronic Obstructive Lung Disease; na, not applicable; SD, standard deviation.

Table 2. Haematological parameters

	COPD patients	Control subjects	<i>p</i> value
Leukocytes, $\times 10^9/L$	7.0±2.3	6.5±1.2	0.399
Platelets, $\times 10^9/L$	273±103	261±66	0.876
Mean platelet volume, fL	10.2±0.8	10.2±0.7	0.958
Platelet distribution width, fL	11.7±1.8	12.0±1.6	0.526
Platelet-large cell ratio, %	26.6±6.7	26.8±5.9	0.931

Values are means ± SD. COPD, chronic obstructive pulmonary disease; SD, standard deviation.

Results

The study population demographics can be found in Table 1. Control subjects were slightly younger and had less tobacco smoke exposure, as reflected by pack-years. Haematological parameters were not different, including platelet counts and platelet characteristics (Table 2). Notably, mean platelet volume, a commonly used measure for platelet activation, was not different between the groups.

Fig. 1. Platelet-monocyte interaction. **a** Patients with stable COPD. Increased platelet-monocyte complexes compared to controls; median MFI for platelet marker CD61 on CD14-positive cells 19.8 (IQR 14.0–33.2) in stable COPD patients versus 10.0 (IQR 8.7–16.7) in controls, $p = 0.002$. **b** In addition, in CD14/CD61-double-positive cells, CD61 expression was higher in COPD patients than in controls; median MFI 24.6 (IQR 19.6–37.7) versus 19.3 (IQR 16.6–24.2), $p = 0.006$. Lines represent medians with IQR. COPD, chronic obstructive pulmonary disease; IQR, interquartile range; MFI, mean fluorescence intensity.

Fig. 2. Platelet activation in COPD patients and controls. Flow cytometric analyses of platelet fibrinogen binding and platelet P-selectin expression. **a** The median MFI of fibrinogen binding in COPD was 5.11 (IQR 3.8–6.2) versus 5.68 (IQR 5.2–6.3) in controls, $p = 0.20$. **b** The mean MFI of P-selectin expression in COPD was 7.018 ± 0.26 versus 7.194 ± 0.24 in controls, $p = 0.71$. Normally distributed data are presented as means $\pm$ SEM. Lines represent medians with IQR (**a**) and means with SEM (**b**). COPD, chronic obstructive pulmonary disease; IQR, interquartile range; MFI, mean fluorescence intensity; SEM, standard error of the mean.

Interaction between platelet and monocytes was increased in stable COPD patients compared to controls; the median MFI for platelet marker CD61 on CD14-positive cells was 19.8 (IQR 14.0–33.2) in COPD patients versus 10.0 (IQR 8.7–16.7) in controls ($p = 0.002$; Fig. 1a). In addition, in the population of CD14/CD61-double-positive cells, the intensity of the CD61 fluorescence signal was significantly higher in COPD patients; the median MFI was 24.6 (IQR 19.6–37.7) versus 19.3 (IQR 16.6–24.2) ($p = 0.006$; Fig. 1b), suggesting that more platelets were bound to the monocyte surface. After exclusion of outliers, both parameters were still statistically significant ($p = 0.005$ and $p = 0.01$, respectively).

Next, we investigated platelet activation by measurement of fibrinogen binding to the activated fibrinogen receptor and expression of P-selectin both in unstimulated samples and after ex vivo stimulation by ADP, CRP-XL and TRAP. In unstimulated samples, no differences in these 2 parameters were observed between stable

COPD patients and controls (the median MFI for fibrinogen was 5.11 [IQR 3.8–6.2] vs. 5.68 [IQR 5.2–6.3], $p = 0.20$, and the mean MFI for P-selectin was 7.018 ± 0.26 vs. 7.194 ± 0.24 , $p = 0.71$) (Fig. 2). In stimulated samples, platelet reactivity was similar for ADP, CRP-XL and TRAP in COPD patients and controls for both markers (Fig. 3).

Soluble P-selectin and soluble fibrinogen, markers for platelet degranulation, were measured in plasma. Plasma P-selectin concentrations were found to be increased in COPD patients (median 31.07 ng/mL [IQR 26.3–40.5] vs. 25.25 ng/mL [IQR 24.3–32.3] in controls, $p = 0.03$), whereas plasma fibrinogen levels were similar (mean $3,314 \pm 259$ vs. $3,051 \pm 245$ μ g/mL, $p = 0.53$) (Fig. 4).

Since fibrinogen is rapidly stored intracellularly after binding to the receptor, we measured platelet fibrinogen content to investigate fibrinogen internalization. However, no differences in both platelet fibrinogen and platelet P-selectin content could be detected (data not shown).

Fig. 3. Platelet reactivity in COPD patients and controls. Platelet reactivity was measured after stimulation with 2 concentrations of different platelet agonists. Data are presented as means $\pm$ SD. **a** Reactivity after stimulation with ADP was not different; fibrinogen binding $p = 0.91$, P-selectin expression $p = 0.56$. **b** The same applies to stimulation with CRP-XL; fibrinogen binding $p = 0.39$,

P-selectin expression $p = 0.95$. **c** Stimulation with TRAP; fibrinogen binding $p = 0.39$, P-selectin expression $p = 0.26$. ADP, adenosine 5'-diphosphate; COPD, chronic obstructive pulmonary disease; CRP-XL, cross-linked collagen-related peptide; SD, standard deviation; TRAP, thrombin receptor-activating peptide.

Fig. 4. Soluble markers for platelet activation in COPD patients and controls. Soluble markers for platelet activation were measured in platelet-poor plasma. **a** Soluble P-selectin was higher in COPD patients, median 31.07 ng/mL (IQR 26.3–40.5) versus 25.25 ng/mL (IQR 24.3–32.3) in controls, $p = 0.03$. **b** Fibrinogen was not statistically different, mean $3,314 \pm 259$ versus $3,051 \pm 245$ μ g/mL, $p = 0.53$. Normally distributed data are presented as means $\pm$ SEM. Lines represent medians with IQR (**a**) and means with SEM (**b**). COPD, chronic obstructive pulmonary disease; IQR, interquartile range; SEM, standard error of the mean.

In summary, increased platelet-monocyte interaction was observed in the absence of increased platelet activation and platelet hyper-reactivity. Since considerable variation in platelet fibrinogen binding (Fig. 2a) and soluble P-selectin (Fig. 4) was observed between patients, a correlation with systemic inflammation was explored.

Plasma levels of hsIL-6 were determined and were found to be higher overall in stable COPD patients than in controls (median 313.5 pg/mL [IQR 265–465] vs. 258.3 pg/mL [IQR 310–316], $p = 0.02$) (Fig. 5). No correlations could be detected between hsIL-6 and platelet activation and platelet reactivity, nor for platelet-monocyte interac-

Fig. 5. Systemic inflammation in stable COPD patients and controls. hsIL-6 was higher in COPD patients; however, only a subgroup shows increased systemic inflammation; median 313.5 pg/mL (IQR 265–465) in stable COPD versus 258.3 pg/mL (IQR 310–316) in controls, $p = 0.02$. Lines represent medians with IQR. COPD, chronic obstructive pulmonary disease; hsIL-6, high-sensitivity interleukin-6; IQR, interquartile range.

Table 3. Thrombin-generating capacity of stable COPD patients and control subjects

	Lag time, min	ETP, nM×min	Peak, nM
<i>0 pM TF</i>			
Stable COPD	5.24±1.72	1,110±334	216.9±99.8
Control subjects	5.59±3.37	1,068±423	192.2±100.2
<i>p</i> value	0.79	0.96	0.55
<i>1 pM TF</i>			
Stable COPD	13.39±5.64	1,327±392	298.3±75.8
Control subjects	14.90±6.77	1,276±402	285.3±94.6
<i>p</i> value	0.33	0.85	0.68

Values are means ± SD. No statistically significant differences were seen in terms of clotting time (lag time), total amount of thrombin generated (ETP), and maximum thrombin concentration (peak). COPD, chronic obstructive pulmonary disease; ETP, endogenous thrombin potential; SD, standard deviation; TF, tissue factor.

tion, in stable COPD patients (data not shown). Additionally, we determined C-reactive protein, and similarly, no significant correlations could be detected with the platelet parameters. A trend towards a positive correlation was seen for C-reactive protein and platelet-monocyte interaction, although it was not statistically significant (Spearman rho 0.264, $p = 0.095$) when examining the

entire cohort (online suppl. Fig. 1; for all online suppl. material, see www.karger.com/doi/10.1159/000480457).

To further investigate the underlying mechanisms, we determined activation of plasmatic coagulation. D-dimer, a measure for fibrinolysis, was not different between stable COPD patients and controls (median 636.8 ng/mL [IQR 293–535] vs. 294.3 ng/mL [IQR 232–455], $p = 0.16$). An automated thrombin generation assay was used to investigate hypercoagulable phenotypes. Thrombin generation was measured in the presence and absence of tissue factor. Clotting time (lag time), total amount of thrombin generated (endogenous thrombin potential) and maximum thrombin concentration (thrombin peak) were not different between stable COPD patients and controls (Table 3).

Discussion

The present study shows that stable COPD patients have increased platelet-monocyte interaction in the absence of increased platelet activation, platelet hyper-reactivity and activation of plasmatic coagulation. Increased expression of platelet marker CD61 was observed on monocytes in stable COPD patients compared to controls. The lack of platelet hyper-reactivity was reflected by platelet fibrinogen binding and platelet P-selectin expression in unstimulated samples and after stimulation with 3 different platelet agonists. Though a minority of COPD patients had a certain degree of systemic inflammation, this could not be related to platelet-monocyte interaction and platelet activation. Also, no differences in D-dimer and thrombin generation were detected between stable COPD patients and control subjects.

COPD patients are at increased risk for CVD, and studies that improve insight into the underlying pathophysiological mechanisms are warranted [3, 30–32]. We hypothesized that platelet-monocyte interaction would be increased in COPD in concurrence with increased platelet activation and platelet hyper-reactivity. In contrast to our hypothesis, no alterations in platelet function were found. We confirm previous findings by Maclay et al. [21] showing that COPD patients have increased platelet-monocyte interaction, however, in the absence of platelet hyper-reactivity. PMC formation is often used as a surrogate marker for platelet activation, as platelets and monocytes can interact via platelet P-selectin and PSGL-1 on monocytes and through platelet GPIIb/IIIa and monocyte-activating complex-1 (MAC-1) via binding of fibrinogen, interactions that are facilitated by

platelet activation [33]. These interactions seem less relevant in COPD, as platelet activation and response to stimulation was not different.

Platelets and monocytes can interact in multiple ways [13, 15, 16], and monocyte-related factors, such as monocyte activation, may play an important role. Platelets constitutively express GPIIb α , a receptor that directly interacts with MAC-1 on monocytes [34]. MAC-1 expression increases with monocyte activation, thereby promoting this interaction [34, 35]. Our data suggest that not primarily platelet activation but monocyte activation may be a key factor in their interaction. This is supported by the trend that was observed between platelet-monocyte interaction and plasma C-reactive protein levels. C-reactive protein is thought to play an important role in atherosclerosis [36], and studies have shown that C-reactive protein induces monocyte-endothelial cell adhesion, an early event in atherosclerosis [37]. Moreover, increased plasma C-reactive protein levels are linked in plaque instability, which may be explained by monocyte activation [36, 38, 39].

These findings add to the current debate on whether COPD patients would benefit from anti-platelet therapy. At first sight, the benefits of anti-platelet therapy in COPD patients seem limited. Increased platelet-monocyte interaction, however, is thought to play a key role in CVD pathogenesis, as it augments the development of atherosclerotic lesions and is involved in plaque instability [13, 40, 41]. P2Y₁₂ receptor blockers, such as clopidogrel, prasugrel and ticagrelor, are known to decrease platelet-monocyte interaction and systemic inflammation, partially via reduced monocyte activation [42–45], whereas these effects are less clear for the COX inhibitor aspirin [44, 45]. Our data suggest that P2Y₁₂ receptors may have a beneficial effect in COPD, and clinical confirmation is warranted.

Interestingly, a small increase in soluble plasma P-selectin levels was observed in COPD patients. P-selectin can be released by endothelial cells during endothelial inflammation, a mechanism that has been previously described for COPD [46, 47]. This may explain our observation. In addition to platelet function, we assessed markers for plasmatic coagulation. Risk for venous thrombosis, especially pulmonary embolism, is increased in COPD [2, 3, 6, 7]. Pulmonary embolism often results from thrombus dislocation from deep venous thrombosis, a mechanism dependent on blood stasis and plasmatic coagulation [48]. We found that COPD patients did not show a hypercoagulable phenotype in stable disease, as reflected by D-dimer and thrombin generation.

An important, yet unresolved, question to address is whether platelet function is affected during and after acute exacerbations. Harrison et al. [20] showed that thrombocytosis during AE-COPD is associated with increased 1-year mortality (OR 1.53, 95% CI 1.03–2.29, $p = 0.030$), and patients on anti-platelet therapy showed improved survival, although no explanation was given. Data on platelet function and platelet-monocyte interaction during and after AE-COPD are crucial to assess future implications for specific anti-platelet therapy in COPD.

This study has several limitations; the first limitation is the heterogeneity in COPD patients, which makes our findings less robust and adds to the difficulty to extrapolate our findings. Tremendous research efforts focus on the identification of different COPD “phenotypes”; however, today we are not able to differentiate clinically relevant subgroups from an immunological perspective. A second limitation is the difference between COPD patients and controls in terms of tobacco smoke exposure and age. Tobacco smoke exposure and ageing may increase platelet hyper-reactivity. However, these factors would have resulted in platelet hyper-reactivity in the COPD group, which was not shown by our data. Lastly, data on risk factors for CVD were not collected, and, therefore, no stratification for cardiovascular risk factors could be performed. It was recently shown that co-morbidities in COPD tend to cluster in different phenotypes [49], and, therefore, it would have been of great interest to investigate the effects of these factors on our findings from a mechanistic point of view.

In summary, we demonstrated that stable COPD patients have increased platelet-monocyte interaction in the absence of increased platelet activation, platelet hyper-reactivity and activation of plasmatic coagulation. The mechanisms underlying cardiovascular risk in COPD may be more dependent on platelet-monocyte interaction than platelet function alterations. The benefits of common anti-platelet therapy may differ for the COX inhibitor aspirin compared to P2Y₁₂ receptor blockers, as the latter interfere with platelet-monocyte interaction. Further confirmation of the effects of anti-platelet therapy in COPD is warranted.

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