Enhanced circulating levels of CD3 cells-derived extracellular vesicles in different forms of pulmonary hypertension

Djuro Kosanovic1,2, Ujjwal Deo1, Henning Gall1, Balachandar Selvakumar1, Susanne Herold1, Astrid Weiss1, Aleksandar Petrovic1, Akylbek Sydykov1, Hossein Ardeschir Ghofrani1 and Ralph Theo Schermuly1

1Universities of Giessen and Marburg Lung Center (UGMLC); Member of the German Center for Lung Research (DZL), Giessen, Germany; 2Sechenov First Moscow State Medical University (Sechenov University), Moscow, Russia

Abstract

It has been shown previously that increased circulating endothelial cells-derived extracellular vesicles represent an important pathological attribute of pulmonary hypertension. Although it is a well-known fact that inflammatory cells may also release extracellular vesicles, and pulmonary hypertension is a disease associated with abnormal inflammation, there is no profound knowledge with regard to the role of inflammatory cells-derived extracellular vesicles. Therefore, our study demonstrated that circulating levels of extracellular vesicles derived from T-cells are enhanced in various pulmonary hypertension forms and that endothelial cells-derived extracellular vesicles may have distinctive profiles in different clinical subgroups of pulmonary hypertension, which still remains as a poorly treatable and life-threatening disorder.

Keywords
extracellular vesicles, pulmonary hypertension, biomarkers, T-cells, endothelial cells

Date received: 14 March 2019; accepted: 23 June 2019

Pulmonary Circulation 2019; 9(3) 1–4
DOI: 10.1177/2045894019864357

To the Editor,

Microparticles (MPs) are a type of extracellular vesicles (EVs) and represent shed membrane structures, mostly found in the blood circulation, which originate from different cellular sources during apoptosis or/and activation. In the previous years, some studies described the altered circulating profiles of endothelial cells-derived and pro-coagulant MPs in the context of pulmonary hypertension (PH). Amabile et al. have demonstrated that patients with precapillary PH had significantly higher levels of circulating endothelial cells-derived MPs and some MPs correlated with increased mean pulmonary arterial pressure (mPAP). In addition, EVs may also be active pathological players in pulmonary vascular disease development/progression, considering the fact that they represent carriers for various micro-RNAs. Although there are evidences about the potential involvement of endothelial cells-derived MPs in the PH pathology, there is insufficient knowledge with regard to the inflammatory cells-derived MPs. It is well known that massive accumulation of inflammatory/immune cells is a characteristic of PH, and inflammatory cells-derived MPs were found to play a pathogenic role in some lung disorders. Finally, the levels of circulating endothelial cells-derived MPs in different clinical forms of PH are still not analyzed in detail.

Therefore, our study aimed to investigate the circulating profiles of different inflammatory (CD3 (T-cells), CD14

Corresponding author: Ralph Theo Schermuly, Universities of Giessen and Marburg Lung Center (UGMLC), Aulweg 130, 35392 Giessen, Germany.
Email: ralph.schermuly@innere.med.uni-giessen.de

Creative Commons Non Commercial CC BY-NC: This article is distributed under the terms of the Creative Commons Attribution-NonCommercial 4.0 License (http://www.creativecommons.org/licenses/by-nc/4.0/) which permits non-commercial use, reproduction and distribution of the work without further permission provided the original work is attributed as specified on the SAGE and Open Access pages (https://us.sagepub.com/en-us/nam/open-access-at-sage).
(macrophages/monocytes), CD68 (macrophages) and endothelial (CD62E (E-selectin) and CD144 (VE-cadherin)) cell-derived EVs in various clinical subgroups of PH.

Human blood samples were prospectively collected during right heart catheterization from non-PH subjects as controls and patients with different forms of PH: idiopathic/heritable PAH (i,hPAH), associated PAH (connective tissue disease, portal hypertension and congenital heart disease), PH due to left heart disease (LHD-PH), PH due to chronic obstructive pulmonary disease (COPD-PH), PH associated with lung fibrosis (fibrosis-PH) and chronic thromboembolic PH (CTEPH). As a limitation of the study, it is important to mention that non-PH control is based on patients that were excluded from any form of PH, but still can suffer from other health disorders which may be associated with altered EVs as well. Different available clinical parameters of the patients (age, gender ratio, New York Heart Association Functional Classification (NYHA) and hemodynamics) are summarized in Table 1. The study was approved by the local ethical committee of the Justus-Liebig University in Giessen. Three milliliters of the platelet-free plasma (PFP) was obtained from the blood taken from each patient and drawn into citrated tubes, by successive centrifugation (500 g/15 min, followed by 10,000 g/5 min at room temperature) as previously reported. PFP samples were initially stored at −80°C and used later for flow cytometry (BD LSRFortessa™) quantification of different inflammatory (CD3, CD14 and CD68) and endothelial (CD144 and CD62E) cell-derived EVs, similarly as described in the literature. Briefly, for each analysis, 50 µl of freshly thawed PFP samples were incubated for 20 min in the dark at room temperature with different fluorochrome-labeled antibodies or corresponding isotype-matched IgG, including: anti-CD3-PE (Phycoerythrin) (BD Pharmingen), anti-CD14-PE (R&D Systems), anti-CD68-PE (R&D Systems), anti-CD144-PE (BD Pharmingen) and anti-CD62E-Allophycocyanin (BD Pharmingen). EVs were identified as events with a 0.5–3 µm diameter on forward light scatter and side-angle light scatter intensity dot-plot representation, by comparison to flow cytometry calibration beads and analyzed for their specific fluorescence. Due to this size determination, we have used the term EVs, which is also considered to be the preferred generic term, as indicated in the literature. Results were expressed as events per microliter of plasma (events/µl) and presented as mean±SEM in percentage, considering the average values of each non-PH group for all analyzed EVs as 100%. Due to the technical reasons, not all values for all analyzed targets and for all enrolled patients are available. ROUT test was used for identification of outliers. Further, unpaired T-test with Welch’s correction in the case of normally distributed values or Mann–Whitney test when values were not normally distributed were performed to compare non-PH control with respective PH groups. Finally, Spearman test was used for analyses of the correlations.

Our results revealed that there was a prominent increase in the levels of circulating CD3 (T-cell)-EVs in all analyzed clinical forms of PH compared to the non-PH control, with the profiles for i,hPAH and CTEPH being statistically significant (Table 2). In contrast to the T-cells-derived EVs, there was no convincing change in the circulating profiles for macrophages/monocytes, as evident from the comparable levels of CD14-EVs and CD68-EVs in the most of clinical PH forms in comparison to the non-PH control, with regard to the endothelial cells-derived EVs, there was no substantial alteration in the levels of circulating CD144-EVs, except slight tendencies to increase in most of the PH subgroups, as compared to the non-PH control (Table 2). But CD62E-EVs demonstrated more conclusive information about the endothelial cells-derived EVs. There were enhanced levels of circulating CD62E-EVs in associated PAH, COPD-PH and CTEPH

**Table 1. Available clinical data of the patients with different forms of pulmonary hypertension (PH).**

| PH group          | Age (years) | Gender ratio (f/m) % | mPAP (mmHg) | PVR (dyn × s × cm⁻⁵) | NYHA |
|-------------------|-------------|----------------------|-------------|----------------------|------|
| Non-PH (n=8)      | 62±5        | 50/50                | 17.0±1.0    | 162±38               | na   |
| i,hPAH (n=6–11)   | 47±5        | 82/18                | 54.7±4.2    | 1033±232             | I–IV |
| Associated PAH (n=4–6) | 45±9   | 50/50                | 38.5±6.9    | 450±159              | II–IV|
| LHD-PH (n=11–14)  | 69±3        | 57/43                | 33.8±3.7    | 326±71               | II–IV|
| COPD-PH (n=7)     | 66±4        | 29/71                | 37.0±2.9    | 470±38               | III–IV|
| Fibrosis-PH (n=9–13) | 67±2   | 8/92                 | 32.7±3.5    | 430±57               | III–IV|
| CTEPH (n=4–12)    | 67±4        | 75/25                | 37.7±6.3    | 487±125              | II–IV|

Note: The patients’ characteristics/clinical parameters, such as age, gender ratio, mean pulmonary arterial pressure (mPAP), pulmonary vascular resistance (PVR) and New York Heart Association Functional Classification (NYHA) classes are given. Available values with the numbers of patients for each PH group are presented as mean±SEM. f: female; m: male; non-PH: control (excluded PH); i,hPAH: idiopathic/heritable pulmonary arterial hypertension; LHD-PH: PH due to left heart disease; COPD-PH: PH due to chronic obstructive pulmonary disease; CTEPH: chronic thromboembolic pulmonary hypertension; na: not available.
some data from this study have been previously reported in the form of abstract during the ATS conference in 2018.

**Conflict of interest**

The author(s) declare that there is no conflict of interest.

**Funding**

This study was supported by the Universities of Giessen and Marburg Lung Center (UGMLC).

**ORCID iD**

Henning Gall https://orcid.org/0000-0001-7016-7373

**References**

1. Ling ZL, Combes V, Grau GE, et al. Microparticles as immune regulators in infectious disease – an opinion. *Front Immunol* 2011; 2: 67.
2. Amabile N, Heiss C, Real WM, et al. Circulating endothelial microparticle levels predict hemodynamic severity of pulmonary hypertension. *Am J Respir Crit Care Med* 2008; 177: 1268–1275.
3. Bakouboula B, Morel O, Faure A, et al. Procoagulant membrane microparticles correlate with the severity of pulmonary arterial hypertension. *Am J Respir Crit Care Med* 2008; 177: 536–543.
4. Tual-Chalot S, Guibert C, Muller B, et al. Circulating microparticles from pulmonary hypertensive rats induce endothelial dysfunction. *Am J Respir Crit Care Med* 2010; 182: 261–268.
5. Amabile N, Heiss C, Chang V, et al. Increased CD62e(+) endothelial microparticle levels predict poor outcome in pulmonary hypertension patients. *J Heart Lung Transplant* 2009; 28: 1081–1086.
6. Diehl P, Fricke A, Sander L, et al. Microparticles: major transport vehicles for distinct microRNAs in circulation. *Cardiovasc Res* 2012; 93: 633–644.
7. Cerri C, Chimenti D, Conti I, et al. Monocyte/macrophage-derived microparticles up-regulate inflammatory mediator synthesis by human airway epithelial cells. *J Immunol* 2006; 177: 1975–1980.
8. Savai R, Pullamsetti SS, Kolbe J, et al. Immune and inflammatory cell involvement in the pathology of idiopathic pulmonary arterial hypertension. *Am J Respir Crit Care Med* 2012; 186: 897–908.

9. Amabile N, Guerin AP, Leroyer A, et al. Circulating endothelial microparticles are associated with vascular dysfunction in patients with end-stage renal failure. *J Am Soc Nephrol* 2005; 16: 3381–3388.

10. Ayers L, Kohler M, Harrison P, et al. Measurement of circulating cell-derived microparticles by flow cytometry: sources of variability within the assay. *Thromb Res* 2011; 127: 370–377.

11. Thery C, Witwer KW, Aikawa E, et al. Minimal information for studies of extracellular vesicles 2018 (MISEV2018): a position statement of the International Society for Extracellular Vesicles and update of the MISEV2014 guidelines. *J Extracell Vesicles* 2018; 7: 1535750.