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# **Plasma ratio of angiopoietin-2 to angiopoietin-1 is a biomarker of vascular impairment in chronic obstructive pulmonary disease patients**

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## **Keywords**

Angiopoietins · COPD · Pericyte · Vessels · Cardiopulmonary exercise test · V'O<sub>2</sub>max

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## Abstract

Chronic obstructive pulmonary disease (COPD) patients have an increased risk of cardiovascular disease. Muscle biopsies have revealed that the muscle vasculature in COPD patients was characterized by a capillary rarefaction with reduced pericyte coverage. Thus, an imbalance of the plasma Angiopoietin-1 / Angiopoietin-2 (Ang2/Ang1) ratio could constitute a noninvasive marker of the muscle vascular impairment. In 14 COPD patients (65.5±5.1-year-old) and 7 HC (63.3±5.8-year-old), plasma samples were obtained at 3 time-points: before, after 5 weeks (W5), and after 10 weeks (W10) of exercise training. COPD patients showed a muscle capillary rarefaction at baseline with a reduced capillary coverage at W5 and W10. The plasma Ang2/Ang1 ratio was significantly higher in COPD patients vs. HC during the training (Group:  $p=0.01$ ). The plasma Ang2/Ang1 ratio was inversely correlated with the pericyte coverage index regardless of the time period W0 ( $r=-0.51$ ;  $p=0.02$ ), W5 ( $r=-0.48$ ;  $p=0.04$ ), and W10 ( $r=-0.61$ ;  $p<0.01$ ). Last, in ECFC/MSC co-cultures exposed to the W10 serum from COPD patients and HC, the plasma Ang2/Ang1 at W10 were inversely correlated with calponin staining ( $r=-0.64$ .  $p=0.01$  and  $r=0.71$ .  $p<0.01$ , Fig. 1B), in line with a role of this plasma Ang2/Ang1 in the MSC differentiation into pericytes. Altogether, plasma Ang2/Ang1 ratio could constitute a potential marker of the vascular impairment in COPD patients.

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Dear editor,

Chronic obstructive pulmonary disease (COPD) is systematically associated with cardiovascular diseases (coronary, peripheral arterial disease, and hypertension), which increase its burden. We and other have previously reported a capillary rarefaction [1] and a reduced pericyte coverage in COPD patients consistent with an impaired muscle vasculature [2]. Since this vascular impairment is only weakly predicted by classical functional tests such as exercise or muscle function tests [1], and since it requires an invasive procedure (skeletal muscle biopsy), alternative diagnosis tools, such as plasma biomarkers, are highly warranted. Angiopoietin-1 (Ang1) and 2 (Ang2) are major regulators of vascular function with antagonist effects through a competitive binding on the endothelial Tie-2 receptor [3]. While Ang1 promotes vessel maturation, Ang2 induces vessel sprouting and inflammation. Both circulating angiopoietins have been associated with cardiovascular diseases [4, 5], and Ang2 has predicted patients at risk of drug-induced hypertension [6]. Therefore, in COPD patients with an increased risk of cardiovascular disease, an imbalance of the plasma Ang2/Ang1 ratio could constitute a marker of vascular impairment in the muscle.

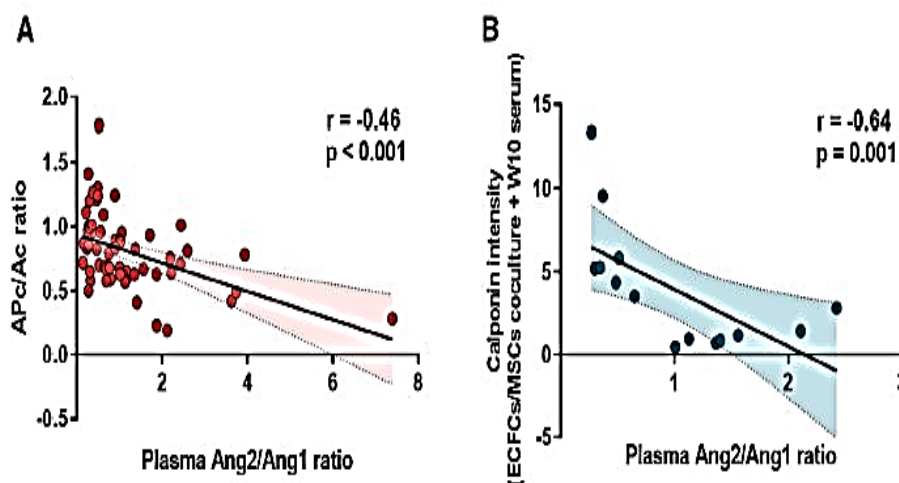
To evaluate Ang2/Ang1 ratio in COPD patients, plasma samples were obtained at 3 time-points in COPD patients and healthy controls (HC): before, after 5 weeks (22 sessions– W5), and after 10 weeks (36 sessions – W10) of exercise training. Patients were recruited in Montpellier University Hospital [2]. Pulmonary function test (Medisoft BodyBox 5500, Belgium), cardiopulmonary exercise test (CPET) (Ergometrics 900, Ergo-line, Germany), and muscle biopsies were performed at W0, W5, and W10. Ang1 and Ang2 concentrations were quantified in platelet-poor plasma with a human magnetic Luminex Assay (R&D systems,

Minneapolis, MN). Data were obtained with the Bio-Plex 200 using the Bio-Plex Manager 5.0 software (Bio-Rad, Marnes-la-Coquette, France). Skeletal muscle biopsies were performed in the *vastus lateralis* of the non-dominant leg [1] and muscle histological staining (CD31+, NG2+) have been assessed as described previously [2]. Finally, the plasma Ang2/Ang1 ratio was related to the intensity of a differentiation marker of mesenchymal stem cells (MSCs) differentiation into mature pericytes (i.e., calponin staining) in contact to endothelial colony-forming cells (ECFCs) [7] and exposed to serum from COPD and HC, as previously described [2]. For each functional or plasma parameter, a linear mixed effect (LME) and post hoc analysis were performed, using the *nlme* R package.

Fourteen COPD patients (65.5±5.1-year-old) and seven HC (63.3±5.8-year-old) were included. COPD patients displayed a mild-to-moderate lung function impairment (FEV1 = 74.6±18.8% pred.). The CPET showed a reduced V'O<sub>2</sub>peak in COPD patients compared with HC patients (83.9 ± 14.7% pred. vs. 108.6 ± 17.1% pred.;  $p < 0.01$ ). The muscle CD31 staining showed a reduced capillary-to-fiber perimeter exchange ratio (3.7±0.6 vs. 4.6±0.5;  $p = 0.002$ ) and capillary density (271±76  $\mu\text{m}^{-2}$  vs. 35 ± 68  $\mu\text{m}^{-2}$ ;  $p = 0.018$ ) in COPD vs. HC patients. In addition, the muscle pericyte area/capillary area index (APc/Ac) – an index of pericyte coverage – was reduced at W5 and at W10 in COPD patients vs. HC ( $p = 0.004$  and  $p < 0.001$ , respectively). At all time period, plasma Ang1 tended to be lower (Group:  $p = 0.06$ ) in COPD patients while Ang2 was significantly higher vs. HC (Group:  $p = 0.05$ ). Consequently, the plasma Ang2/Ang1 ratio was significantly higher in COPD patients vs. HC during the training (Group:  $p = 0.01$ ). In all subjects, the plasma Ang2/Ang1 ratio was inversely correlated with the V'O<sub>2</sub>peak (%pred.) at W0 ( $r = -0.43$ ;  $p = 0.05$ ) and tended to be correlated at W10 ( $r = -0.42$ ;  $p = 0.08$ ), but not in W5 ( $r = -0.32$ ;  $p = 0.20$ ). The plasma Ang2/Ang1 ratio was inversely correlated with the pericyte coverage index APc/Ac regardless of the time period (Fig. 1A), W0, ( $r = -0.51$ ;  $p = 0.02$ ), W5 ( $r = -0.48$ ;  $p = 0.04$ ), and W10 ( $r = -0.61$ ;  $p < 0.01$ ). Specifically, in COPD patients, changes in plasma Ang2/Ang1 ratio after 10 weeks of training were inversely correlated with changes in APc/Ac index ( $r = -0.532$ ;  $p = 0.05$ ). The role of the plasma Ang2/Ang1 balance on pericyte was further investigated using our previously published experiments of ECFCs and MSCs co-cultures in vitro. In ECFC/MSC co-cultures, we observed a reduced calponin staining when exposed to the W10 COPD serum compared to the W10 HC serum [2], reflecting less MSC differentiation in MSC exposed to W10 COPD patients. Additionally, the plasma Ang2/Ang1 at W10 were inversely correlated with calponin staining ( $r = -0.64$ .  $p = 0.01$  and  $r = 0.71$ .  $p < 0.01$ , Fig. 1B).

Our results demonstrated a dysregulation of circulating angiopoietins and their relevance with vascular impairment observed in COPD [1, 2]. In these patients, there were both reduced Ang1 and increased Ang2 and Ang2/Ang1 ratio vs. HC at all time-points. Interestingly, this higher Ang2/Ang1 ratio was associated with the pericyte capillary coverage in the muscle. To our knowledge, this is the first study reporting defaults of circulating angiopoietins in COPD patients. Previous studies in COPD and in exercise training have been limited to the assessment of their muscle expression. Since Ang2 has been involved in many cardiovascular diseases, increased plasma Ang2 and Ang2/Ang1 ratio during the training program could explain the blunted exercise blood pressure response to training in COPD patient, as well as in the onset of other cardiovascular diseases in at risk patients. In addition, we found significant correlations between plasma Ang2/Ang1 ratio and both in vivo histological and in vitro markers of pericyte-to-capillary interactions. Indeed, in line with the role of plasma Ang2 in the vessel destabilization, there was a significant inverse correlation between the pericyte capillary coverage in the muscle biopsy and the plasma Ang2/Ang1 at W5 and W10 in COPD patients and HC. The role of the Ang2/Ang1 balance was further evidenced by the correlation

between plasma Ang2/Ang1 ratio in the serum and the MSC differentiation into pericytes when co-cultured with ECFC and the W10 serum of the subjects. Moreover, our experimental result fits the evidence of vessel dilation associated to pericyte loss in Ang1 deficient mice [3,8] and impairment of tube formation of endothelial cells through Ang1 inhibition [9]. Last, the potential value of the plasma Ang2/Ang1 imbalance in the assessment of the muscle vascular impairment in COPD patients was highlighted by the negative correlation with the  $\dot{V}O_{2peak}$  at W0 ( $r = -0.43$ ;  $p = 0.05$ ) and tendency at W10 ( $r = -0.42$ ;  $p = 0.08$ ). Since the work of Broxterman et al. it is now admitted that the muscle  $\dot{V}O_2$  of COPD patients is related to the vascular oxygen convection to the contracting muscle [10].



**Fig.1** Correlations between the plasma Angiotensin-2/Angiotensin-1 (Ang2/Ang1) ratio and **A** the muscle capillary pericyte coverage assessed by the pericyte area/capillary area index (APC/Ac) and **B** the calponin staining in endothelial colony-forming cells (ECFCs)/mesenchymal stem cells (MSCs) in vitro co-culture exposed to the sera of

chronic obstructive pulmonary disease patients and healthy controls obtained after 10 weeks of exercise training (W10). Data points represent individual measurements. The black bold line indicates line of best fit and the lightly colored area 95% confidence interval.  $r$  value: Pearson's correlation coefficient

All in all, plasma Ang2/Ang1 ratio could constitute a potential marker of the vascular impairment in COPD patients, and its role on the micro- and macro-vascular dysfunction should be assessed in further clinical cohorts of COPD patients.

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## Declarations

**Conflict of interest** All other authors declare they have nothing to disclose. **Ethical approval** This study was conducted in accordance with the Declaration of Helsinki and approved by the ethics committees and the competent authorities of France (No: 2013-A01199-36). **Informed consent** All patients provided written informed consent. [https:// www. clini caltr ials. gov](https://www.clinicaltrials.gov), NCT02040363.

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