Endothelial dysfunction is not a predictor of outcome in chronic obstructive pulmonary disease

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Abstract

Background: Local airway inflammation may cause systemic changes which result in endothelial dysfunction. Only a few studies have used reactive hyperemia peripheral arterial tonometry (RH-PAT) in patients with chronic obstructive pulmonary disease (COPD) in order to measure their endothelial dysfunction.

Objective: To determine the efficacy of endothelial dysfunction, measured by RH-PAT, in assessing disease severity and systemic burden in a cohort of COPD patients.

Methods: In this prospective, monocentric study, 157 patients with moderate to very severe COPD (GOLD class II-IV) were examined for endothelial dysfunction using RH-PAT (Itamar medical Ltd., Caesarea, Israel). In a nested-cohort, examination was repeated at exacerbation. The association between reactive hyperemia index (RHI), augmentation index (AI) and disease severity and outcome parameters was analysed.

Results: 57% of the COPD patients had a dysfunctional endothelium and the median (IQR) RHI was 1.42 (1.27–1.53). Exacerbation of COPD was not associated with a significant change in RHI (p = 0.625) or AI (p = 0.530). None of the diagnostic or clinical outcomes of COPD was associated with RHI or arterial stiffness.

Conclusion: Endothelial dysfunction is common in COPD. However, it does not seem to be a predictor neither of disease severity, nor of outcome and does not change during exacerbations of the disease.

Keywords: Arterial stiffness, COPD, Endothelial dysfunction, Flow-mediated dilation, Reactive hyperemia

Background

Although preventable and treatable, chronic obstructive pulmonary disease (COPD) is one of the leading causes of mortality worldwide [1–3]. It is associated with extrapulmonary comorbid conditions, such as peripheral vascular disease, osteoporosis, skeletal muscle dysfunction, reduced exercise capacity, erectile dysfunction, chronic depression, lung cancer and diabetes [4, 5]. These conditions are responsible for the poor outcome of advanced COPD and may reflect the systemic manifestation of COPD [6]. Cardiovascular comorbidities contribute to the morbidity and mortality of COPD and can be detected independently of traditional risk factors in about one third of COPD patients, accounting for about 50% of deaths [7]. That is because a spill-over of local airway inflammatory mediators into the systemic circulation may result in vascular changes leading to endothelial dysfunction [8]. Endothelial dysfunction may additionally be caused due to a decrease in the availability of nitric oxide (NO) and due to inflammatory-
mediated changes in the structure of the vessel walls, such as the replacement of degraded elastic fibres by collagen, which increases arterial stiffness [8]. Interestingly, endothelial dysfunction is often described as a common feature of COPD and it seems to be positively correlated with the degree of airway obstruction, thus suggesting a mechanistic link between chronic airway obstruction and cardiovascular risk [9–12].

The main method of determining endothelial dysfunction in patients with COPD is by measuring the flow-mediated dilation (FMD) of the brachial artery. Reactive hyperemia-peripheral arterial tonometry (RH-PAT) is a more recent, noninvasive method to evaluate endothelial dysfunction and its use, easier and less operator-dependent, has been rapidly increasing. RH-PAT measures pulsatile volume changes in response to reactive hyperemia, a transient increase in organ blood flow that is dependent on NO synthesis and occurs after a brief period of ischemia [13–15]. RH-PAT assesses endothelial dysfunction in relation to the presence of multiple cardiovascular and metabolic risk factors [16] and can successfully predict cardiovascular events in a population with an intermediate cardiac risk profile [17]. Moreover, abnormal RH-PAT results are correlated with ischemic heart disease diagnosed using an invasive method [18].

Endothelial function assessed by digital RH-PAT is expressed as the reactive hyperemia index (RHI). The RHI describes the ratio of average amplitude during reactive hyperemia compared with the pre-occlusion baseline period [19]. A lower RHI is associated with higher complexity of coronary atherosclerotic plaque, larger systemic atherosclerotic plaque burden and adverse cardiovascular events [20–23]. Tanaka et al. [24] proposed RHI cutoff values 1.67 and 2.10 (< 1.67 for abnormal and ≥ 1.67 and < 2.10 for borderline, ≥ 2.10 for normal). On the other hand, the augmentation index (AI) is a measurement of arterial stiffness, which is calculated from a pulse waveform analysis of the peripheral arterial tonometry (PAT) signal at baseline and normalized to a heart rate of 75 bpm [25]. Lower AI values reflect better arterial elasticity [25].

Minet et al. investigated endothelial dysfunction in patients with COPD using RH-PAT, but their cohort consisted of only 44 patients [26]. Our objective was, therefore, to investigate endothelial dysfunction using RH-PAT in a larger and well-characterised cohort of COPD patients, so that we could determine the importance of endothelial dysfunction in assessing disease severity and systemic burden in COPD.

Methods

The PREVENT study [27] is an investigator-initiated and –driven study compiled to the Declaration of Helsinki and Good Clinical Practice Guidelines. The study was approved by the responsible ethics committee (EKNZ 306/10) and was registered at www.controlled-trials.com (identifier ISRCTN 45572998). All patients were recruited from in- and out-patients referred for clinical evaluation and lung function tests to the Clinic of Pneumology, University Hospital of Basel and provided written consent before any study assessments were initiated.

The current study included a nested cohort of the PREVENT study (n = 136) and 21 COPD patients from a cohort of 365 patients that were admitted for a planned pulmonary workup during a period of 4 months (Fig. 1). The inclusion criteria were as follows: age ≥ 40 years; smoking history ≥10 pack-years; moderate to very severe COPD (GOLD class II–IV); clinically relevant disease, as defined by a history of exacerbations in the previous 12 months; stable disease (free of exacerbation) for ≥4 weeks prior to the start of the study. GOLD grades are based on FEV1% predicted: I ≥ 80%; 50% ≤ II < 80%; 30% ≤ III < 50%; and IV < 30%. The exclusion criteria were as follows: pulmonary condition other than COPD; rapid lethal disease; severe immunosuppression including manifested AIDS, organ transplantation or neutropenia (< 500 × 10⁹/L), pregnancy or breastfeeding and known allergy or intolerance to the study medicine. In addition, 14 patients were re-evaluated within 48 h after an acute exacerbation of COPD (AECOPD).

The assessments for each patient included a detailed history, blood sample collection, a physical examination and a measurement of vital signs and lung function. The 6-min walking test (6MWT) [28], the St. George’s respiratory questionnaire (SGRQ), and the COPD assessment test (CAT) were additionally required. Specifically, the 6MWT is a submaximal exercise test that entails measurement of distance walked over a span of 6 min and assesses the performance ability in a variety of cardiopulmonary diseases [29]. The SGRQ measures the impact of COPD on overall health, daily life and perceived well-being [30]. It scores from 0 to 100, with higher scores indicating more limitations [30]. The CAT is a patient-completed questionnaire, assessing the impact of COPD (cough, sputum, dyspnoea, chest tightness) on health status [31]. It scores from 0 to 40 and higher scores denote a more severe impact of COPD on a patient’s life [31].

Information regarding exacerbations were additionally acquired from the patients for the year preceding the baseline measurements and were classified as mild (requiring medical care and increased dose of short-acting β2 agonists), moderate (requiring either antibiotics and/or parenteral corticosteroids) or severe (requiring hospitalization or resulting in death).

Endothelial function was assessed by a trained and certified study nurse at scheduled and unscheduled visits,
measuring reactive hyperemia with finger plethysmography, according to the recommendations of the manufacturer (Itamar medical Ltd., Caesarea, Israel) [25]. The device also measured AI.

CT-scans were analysed for emphysema and subclassified as centrilobular emphysema and paraseptal emphysema.

For our statistical analysis, the SPSS (Statistical Package for Social Sciences) Release 22.0 software package program (SPPS, Inc., Chicago, IL) was utilized. We based our analysis on the data for FMD obtained by Moro et al. [32]. Correlation of RH-PAT values with other measurable disease-relevant parameters was analysed using the Spearman rho coefficient. A comparison of RH-PAT values during stable disease and AECOPD in the same 14 individuals (paired samples) was performed using Mann-Whitney analysis. The predictive value of RH-PAT for adverse outcome events (AECOPD, hospitalisations, cardiovascular events, death) was analysed by uni- and multivariable cox-regression proportional analysis. Adjustment for confounding variables like age, gender and cardiovascular risk factors was pre-defined according to the literature [26]. All tests were two-tailed and the level of statistical significance was set at 0.05. Results were expressed as mean (standard deviation) or median (interquartile range), unless otherwise stated.

**Results**

In this study, we included 157 patients with stable COPD (Fig. 1), 69% of which were male and 42% were smokers (Table 1). The patients were classified as follows: 68% GOLD II, 22% GOLD III, and 10% GOLD IV. The most common cardiovascular comorbidities were arterial hypertension (64%) and coronary artery disease (28%). Accordingly, 42% were on statin medication and 56% were taking aspirin.

Additionally, 57% of the COPD patients had a dysfunctional endothelium. The median (IQR) RHI score was 1.42 (1.27–1.53) and none of the diagnostic or clinical outcomes of COPD was associated with the RHI or arterial stiffness (Table 2). The RHI had no significant effect on time to exacerbation up to 3.3 years after inclusion in the study (Hazard Ratio = 1.001, 95% CI 0.436–2.300, p = 0.998).

Significantly, more male than female and more patients with diabetes as a comorbidity had a dysfunctional endothelium (Table 3). The COPD patients with dysfunctional endothelium had significantly higher BMI than those with normal endothelial function. There were no other differences between the two groups of patients and no association with other cardiovascular comorbidities. Moreover, there was no association between RHI and aspirin (Beta = −0.052, 95% CI -0.271 – 0.140; p = 0.530) nor between endothelial dysfunction and aspirin (OR = 1.342, p = 0.378).

There was no significant difference in RHI between patients with emphysema and those without (median 1.6 vs. 1.6, p = 0.89), with centrilobular emphysema or without (median 1.6 vs. 1.7; p = 0.87) nor with paraseptal emphysema or without (1.6 vs. 1.7; p = 0.89). Linear
regression adjusting for confounding factors such as age, BMI, gender, statin use, diabetes, arterial hypertension, FEV1, smoking status, and pack years, showed no association between emphysema, any type of emphysema and RHI (Table 4). A chi-squared test showed no association between emphysema (p = 0.85), centrilobular emphysema (p = 0.94), paraseptal emphysema (p = 0.32) and the absence or presence of endothelial dysfunction.

Arterial stiffness, as represented by AI, was 10.50 in COPD patients. The factors associated with arterial stiffness were age (Spearman rho = 0.236; p = 0.001), heart rate (Spearman rho = -0.406; p < 0.001) and systolic blood pressure (Spearman rho = 0.273; p < 0.001). The predictors for arterial stiffness in patients with COPD were heart rate (Beta = -0.409, 95% CI -0.862 - -0.408, p < 0.001), systolic blood pressure (Beta = 0.346, 95% CI 0.187–0.506; p < 0.001), BMI (Beta = -0.175, 95% CI -0.944 - -0.89; p = 0.018) and gender (Beta = 0.264, 95% CI 4.911–16.679; p < 0.001). Arterial stiffness had no effect on time to exacerbation (Hazard ratio = 0.625, Fig. 2a) and AI (p = 0.530, Fig. 2b) when we compared patients during a stable phase of COPD and the same patients during an acute exacerbation of COPD.

Arterial stiffness did not change significantly between patients with emphysema compared to those without emphysema (median 11 vs. 10; p = 0.46), with centrilobular emphysema and those without (10 vs. 13; p = 0.65) and between those with paraseptal emphysema and those without (9 vs. 11; p = 0.88). A significant association was seen in univariate and multivariate analysis between arterial stiffness and paraseptal emphysema (Table 4).

### Discussion

Up-to-date, this is the largest study evaluating endothelial dysfunction in COPD and the first to evaluate...
Table 3 COPD patients with dysfunctional endothelium compared to COPD patients with normal endothelial function

| Parameter                                           | Dysfunctional endothelium, n = 90 | Normal endothelial function, n = 67 | P-value |
|-----------------------------------------------------|-----------------------------------|-------------------------------------|---------|
| Male                                                | 70 (65)                           | 38 (35)                             | 0.005   |
| Age (years)                                         | 67 (61–75)                        | 66 (57–72)                          | 0.169   |
| Current smoker                                      | 38 (58)                           | 27 (42)                             | 0.515   |
| Pack years                                          | 50 (40–63)                        | 50 (40–70)                          | 0.627   |
| BMI (kg/m²)                                         | 28 (25–33)                        | 24 (21–27)                          | < 0.001 |
| 6MWT (m)                                            | 410 (315–490)                     | 425 (340–480)                       | 0.414   |
| Exacerbation rate in the previous year              | 1 (0)                             | 1 (0)                               | 0.473   |
| Heart rate (bpm)                                    | 75 (67–83)                        | 73 (67–84)                          | 0.770   |
| Systolic blood pressure (mmHg)                      | 132 (119–145)                     | 133 (124–147)                       | 0.422   |
| Diastolic blood pressure (mmHg)                     | 74 (63–82)                        | 72 (63–80)                          | 0.295   |
| FeNO (ppm)                                          | 16 (11–26)                        | 19 (13–25)                          | 0.261   |
| Unadjusted Charlson Score                           | 2 (1–3)                           | 2 (1–2)                             | 0.192   |
| CAT Score                                           | 17 (11–21)                        | 15 (11–21)                          | 0.717   |

**Lung Function (post-brd)**

| Parameter                                           | Dysfunctional endothelium, n = 90 | Normal endothelial function, n = 67 | P-value |
|-----------------------------------------------------|-----------------------------------|-------------------------------------|---------|
| FEV₁, in L                                          | 1.5 (1.2–2.1)                     | 1.4 (0.94–1.7)                      | 0.032   |
| FVC, in L                                           | 3.0 (2.5–3.7)                     | 2.9 (2.4–3.4)                       | 0.159   |
| FEV₁, % predicted                                   | 59.0 (44.2–69.6)                  | 55.9 (39.0–69.0)                    | 0.469   |
| FVC, % predicted                                    | 90.1 (73.8–102.4)                 | 91.0 (76.0–105.2)                   | 0.493   |
| FEV₁/FVC %predicted                                 | 47.5 (40.8–60.3)                  | 47.1 (34.4–56.1)                    | 0.237   |
| Reactive hyperemia index                            | 1.42 (1.27–1.53)                  | 2.12 (1.97–2.48)                    | 0.000   |

**BODE Index**

| ≤ 2 | ≥ 3 |
|-----|-----|
| 48  (54) | 35  (51) |
| 41  (46) | 33  (49) |

**GOLD Grade**

| GOLD II | GOLD III | GOLD IV |
|---------|----------|---------|
| 61 (68.5) | 21 (23.6) | 7 (7.9) |
| 45 (66.2) | 15 (22.0) | 8 (11.8) |

**mMRC score**

| ≤ 2 | ≥ 3 |
|-----|-----|
| 51  (56) | 40  (44) |
| 36  (61) | 23  (39) |

**SGRQ**

| Symptoms score | Activity score | Impact score | Total score |
|----------------|---------------|--------------|-------------|
| 47 (25–67)     | 54 (41–72)    | 19 (13–39)   | 34 (25–53)  |
| 41 (23–69)     | 57 (37–66)    | 20 (11–36)   | 33 (23–49)  |

**Comorbidities**

| Diabetes | Pulmonary arterial hypertension | Renal disease | Arterial hypertension | Congestive heart failure | Coronary artery disease | Cerebrovascular disease |
|----------|---------------------------------|---------------|-----------------------|--------------------------|------------------------|------------------------|
| 19 (86)  | 6 (43)                           | 11 (50)       | 61 (60)               | 12 (52)                  | 29 (66)                | 8 (57)                 |
| 3 (14)   | 8 (57)                           | 11 (50)       | 40 (40)               | 11 (48)                  | 15 (34)                | 6 (43)                 |

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potential changes in endothelial dysfunction between stable state and exacerbation of COPD using RH-PAT. We found that 57% of the patients with COPD had endothelial dysfunction. This is comparable to the results of Minet et al. [26], who indicated endothelial dysfunction in 50% of their COPD patients. Malerba et al. [33] reported a higher incidence of endothelial dysfunction (75%) in COPD patients and a lower RHI score (1.31). This might potentially be attributable to the small sample size of their study (n = 16).

Similar to the vast majority of COPD patients, our patients had several cardiovascular comorbidities. Indeed, we found an association between endothelial dysfunction and diabetes mellitus. However, we found no association between dysfunctional endothelium and other cardiovascular comorbidities or aspirin, a medication considered to influence endothelial function [34]. This is surprising and could point to a technical fault, yet, the RHI measured for patients with arterial hypertension (RHI = 1.8 ± 0.7) was similar to that reported by Weisrock et al. (RHI = 1.7 ± 0.4 to 1.8 ± 0.4) [35]. Additionally, we confirmed the finding of van der Heijden et al. [36] that BMI is associated with endothelial dysfunction. On the other hand, more than half of the patients assessed in our study had arterial hypertension. Weisrock et al. [35] indicated that PAT is not a reliable method for measuring endothelial dysfunction in patients with arterial hypertension. This could, therefore, explain why we found no association between RHI and most cardiovascular comorbidities or aspirin in our study.

Unlike Marchetti et al. [37], Ozben et al. [38] and Urban et al. [39], in our study endothelial dysfunction was similar at stable state and exacerbation of COPD. This finding is rather intriguing, as exacerbations of COPD are linked to bacterial and viral infections, increase in local and systemic inflammation, hypoxia,

| Parameter | Dysfunctional endothelium, n = 90 | Normal endothelial function, n = 67 | P-value |
|-----------|---------------------------------|---------------------------------|---------|
| Myocardial infarction | 11 (55) | 9 (45) | 0.870 |
| Medication | | | |
| SHB2AC | 7 (50) | 7 (50) | 0.385 |
| SABA | 31 (65) | 17 (35) | 0.470 |
| LABA/ICS | 81 (60) | 55 (40) | 0.648 |
| LABA | 12 (75) | 4 (25) | 0.209 |
| LAMA | 81 (60) | 54 (40) | 1.000 |
| Mucolytics/Antioxidants | 10 (59) | 7 (41) | 0.863 |
| Statins | 39 (63) | 23 (37) | 0.223 |
| Aspirin | 50 (60) | 33 (40) | 0.377 |
| Ace inhibitor | 46 (64) | 26 (36) | 0.127 |

*Model adjusted for age, gender, body mass index, smoking status, pack years, FEV1, arterial hypertension, diabetes mellitus, and statin use**

**Table 4** Association of emphysema with reactive hyperemia index and arterial stiffness

| RHI | Univariate analysis | | Multivariate analysis* | | |
|-----|---------------------|-----|-----------------------|-----|-----|
| Beta-estimate ± SEM | P-value | Beta-estimate ± SEM | P-value |
| Emphysema | 0.034 ± 0.13 | 0.78 | −0.05 ± 0.15 | 0.74 |
| Centrilobular emphysema | 0.08 ± 0.19 | 0.70 | 0.10 ± 0.19 | 0.60 |
| Paraseptal emphysema | 0.03 ± 0.06 | 0.68 | 0.04 ± 0.06 | 0.48 |

| Arterial stiffness | Univariate analysis | | Multivariate analysis* | | |
|-------------------|---------------------|-----|-----------------------|-----|-----|
| Beta-estimate ± SEM | P-value | Beta-estimate ± SEM | P-value |
| Emphysema | 1.8 ± 3.8 | 0.64 | 3.2 ± 4.3 | 0.457 |
| Centrilobular emphysema | 0.3 ± 5.0 | 0.95 | −6.4 ± 5.1 | 0.210 |
| Paraseptal emphysema | 4.7 ± 1.6 | 0.005 | 4.3 ± 1.5 | 0.006 |
pulmonary arterial hypertension and congestive heart failure; all these conditions are independently related to endothelial dysfunction [40–42]. Furthermore, there is a well-known increase in cardiovascular risk associated with exacerbation of COPD [43, 44]. On the other hand, although Marchetti et al. [37] and Ozben et al. [38] found lower FMD during exacerbation compared to the recovery period, they found no difference between patients with COPD in recovery and controls. They also found no difference in brachial artery diameter during exacerbation and recovery and no association between FMD and lung function [10, 37, 38]. That said, considering the small sample size used for the exacerbation analysis, it would be prudent to look at endothelial dysfunction before and after exacerbation in a larger cohort.

In two previous studies, Minet et al. [26] and Malerba et al. [33] found an association between RHI and FEV1. However, the current results suggest no association between RHI and any COPD parameter i.e. airflow limitation, exercise capacity, BODE index, GOLD status or modified Medical Research Council (mMRC) dyspnoea scale. These differences could lie in the fact that both previous studies had relatively small populations (n = 44 and n = 16, respectively). In addition, the discrepancies in the findings may be attributed to the fact that: 1) our study only excluded COPD patients with other pulmonary conditions, rapid lethal disease and severe immunosuppression and therefore affords a more reasonable representation of the general COPD population, while, on the other hand, Malerba et al. [33] excluded patients with a history of any cardiovascular disease except
arterial hypertension, diabetes mellitus and if the patient had an exacerbation in the previous 6 months; and 2) in the study of Malerba et al. [33], patients had less severe COPD than the patients in our study. In a number of studies, both FMD and RHI are lower in COPD patients than in controls, but the association with COPD parameters is tenuous [45, 46]. Eickhoff et al. [10] found an association between FMD and FEV1% predicted in a univariate analysis, but no significant association between FMD and GOLD status. Moro et al. [32] found an association between FMD and airflow limitation (FEV1/VC), however, they did not measure or adjust for brachial artery diameter. Barr et al [47] found an association between FMD and airflow limitation (FEV1/VC), however, they did not look at the relationship between FMD and FEV1 at baseline, nor did they take COPD medication into account. Importantly, Barr et al. [47] found that COPD medication is associated with large differences in FMD.

Unlike Bhatt et al. [49], we found no association between RHI or AI and centrilobular emphysema. This could be because coronary calcification, which was used in the analysis by Bhatt et al. [49], is an independent process with different pathways compared to endothelial dysfunction [50]. There was, however, an association between paraseptal emphysema and arterial stiffness. Patients with paraseptal emphysema are at higher risk of developing lung cancer [51]. Arterial stiffness is associated with haematological malignancies [52], but its role in lung cancer is unknown.

Our study has several limitations. It was a monocentric study including patients with COPD receiving extensive treatment for their disease. In addition, we did not exclude patients with cardiovascular comorbidities. Strengths included the full characterisation of the study population, the multivariable adjustment for several confounding factors and the inclusion of a large COPD population with commonly encountered comorbidities, which assures the generalizability of our results.

Conclusions
In conclusion, we found that 57% of patients with COPD had an incidence of endothelial dysfunction with a median (IQR) RHI of 1.42 (1.27–1.53). However, RHI was not associated to any COPD-related outcome and endothelial dysfunction was similar at stable state and at exacerbation of COPD. In addition, endothelial dysfunction, as measured using RH-PAT, is neither a predictor of disease severity nor outcome in COPD.
