



Sputum inflammatory cells in COPD patients classified according to GOLD 2011 guidelines

To the Editor:

Recently the definition of chronic obstructive pulmonary disease (COPD) severity, stated in 2001 by Global Initiative for Chronic Obstructive Lung Disease (GOLD) guidelines and based on forced expiratory volume in 1 s (FEV₁) evaluation, has been considered inadequate to recognise all the multifaceted aspects of the disease. To overcome this limitation, 2011 GOLD recommendations suggested a new approach, which stratified COPD patients in four groups of severity (A, B, C, D) according to the level of symptoms (as assessed by either the modified Medical Research Council (mMRC) score or the COPD Assessment Test), the degree of airflow limitation (expressed as percentage of predicted forced expiratory volume in 1 s (FEV₁) value) and the number of exacerbations in the previous year [1]. It is still matter of debate if this new classification helps to better understand the disease and improve COPD treatment and prognosis. Studies on the existing database have been published comparing the old with the new GOLD classifications, showing that GOLD 2011 classification adds new details on the phenotyping and on the prognosis of COPD [2–5]. Despite the many *post hoc* analyses performed on the large databases of the most important recent observational studies (ECLIPSE, CCLS, COPDgene), the demonstration that these different groups of GOLD 2011 classification are different for physiological and biological features is controversial.

We performed a detailed examination of a group of 83 patients with COPD of different severity who attended to a pulmonary rehabilitation programme. COPD was defined according to GOLD guidelines [1], and patients were referred for a pulmonary rehabilitation programme by pulmonary specialists in our area.

As a part of the baseline evaluation of the pulmonary rehabilitation programme, all patients underwent clinical (symptoms, exacerbation rate, comorbidities), nutritional (body mass index (BMI), fat-free mass index (FFMI) and functional (pre- and post-bronchodilator static and dynamic lung volumes, diffusing capacity, resting blood gas measurement, 6-min walking distance) assessment, according to the standard procedures. Furthermore, each patient collected a sputum sample induced by hypertonic/isotonic saline inhalation, which was processed for sputum cell analysis [6], and also an exhaled breath condensate sample for the measurement of malondialdehyde as a marker of oxidative stress [7]. Informed consent for participation to the study was obtained from all patients. The local ethics committee was advised of this observational study. All patients were examined in a stable phase of the disease and had not had an acute exacerbation for at least 4 weeks. Regular treatment was withdrawn 24 h before each examination.

Comparisons among groups were performed with ANOVA, with Student–Newman–Keuls multiple comparison test, for normally distributed variables, and with Kruskal–Wallis one-way analysis of variance, followed by *post hoc* Bonferroni Dunn's test, for non-normally distributed variables.

Patients were stratified in the four GOLD 2011 categories according to the three parameters: level of dyspnoea on exercise (mMRC scale), FEV₁ % predicted, and number of exacerbations in the last year (requiring oral corticosteroids and/or antibiotics) (table 1). The mean±SD age of the enrolled patients was 68.8±7.8 years, 65 were males, 75 were smokers or ex-smokers; post-bronchodilator FEV₁ % pred was 54.1±19.0 and FEV₁/forced vital capacity was 48.0±12.3% of predicted values. There was no significant difference among the four groups for demographic data, except for BMI and FFMI which were higher in group A. Diffusing capacity was significantly lower, and total lung capacity higher, in B and D compared with A and C. These data were associated with a poorer quality of life, as assessed by St George's Respiratory Questionnaire total score, in B and D. Group D showed the lowest mean value of arterial PO₂. Patients were under regular treatment with long-acting bronchodilators (LABA and/or LAMA, n=77), inhaled corticosteroids (ICS, n=66) and oral theophylline (n=15); no patient was under regular oral corticosteroid regimen. Groups B and D showed higher sputum neutrophil percentages than A and C groups, while sputum eosinophil percentages were significantly higher in A and C in comparison with B and D groups. Neutrophilia (neutrophils >64%) was present in 62 (75%) patients and eosinophilia (eosinophils >2%) was present in 45 (54%) patients [8, 9]. No relationship was observed between sputum cell counts and pharmacological treatment. No significant difference among the four groups was observed for malondialdehyde levels in exhaled breath condensate.

TABLE 1 Main characteristics of patients divided according to the GOLD 2011 classification

	GOLD 2011 categories				Anova/Chi-squared/Kruskall Wallis + post hoc analysis
	A	B	C	D	
Patients N=83	15 [18.1]	10 [12.0]	20 [24.1]	38 [45.8]	
Gold 2011 assessment variables					
mMRC dyspnoea score	0.7±0.5	2.5±0.7	0.8±0.4	2.9±0.7	<0.0001; A,C versus B,D
FEV ₁ % predicted	66.8±11.7	69.0±15.6	54.7±16.3	44.8±18.6	<0.0001; A,B versus C,D
Exacerbations last year n	0.5±0.5	0.4±0.5	2.4±1.9	2.8±1.8	<0.0001; A,B versus C,D
Demographics					
Age years	69.5±6.8	73.8±6.8	68.6±7.4	69.5±8.5	NS; p=0.08
Male %	13 (87)	5 (50)	18 (90)	29 (76)	NS; p=0.33
Smoking status %					
Ex-smoker	12 (80)	8 (80)	14 (70)	25 (66)	NS; p=0.27
Current	2 (13)	0 (0)	4 (20)	10 (26)	
Pack-years	48.3±26.7	60.2±28.8	39.3±22.9	51.2±23.3	p<0.05; B versus C
Body mass index kg·m ⁻²	29.7±5.1	26.7±4.8	26.0±4.7	27.1±4.8	p<0.05; A versus C
Fat free mass index kg·m ⁻²	20.8±2.7	17.9±5.4	16.9±2.2	17.8±2.8	p<0.01; A versus C,D
Charlson index	1.7±1.6	2.2±0.7	1.6±1.3	1.5±1.2	NS; p=0.08
Physiology					
TLC %	105.4±9.4	123.7±15.8	119.7±25.2	132.3±29.2	p<0.01; A versus B,D
DLC ₀ %	86.3±16.3	58.4±19.4	91.3±27.6	58.6±24.5	p<0.01; A,C versus B,D
DLC ₀ /VA %	113.5±20.0	80.1±32.2	110.9±24.7	87.4±27.7	p<0.05; A,C versus B,D
P _{O₂}	78.3±15.0	77.1±7.7	74.6±9.9	69.7±9.0	p<0.05; D versus A,B,C
P _{CO₂}	38.4±4.8	36.4±5.6	40.9±4.7	42.1±6.5	p<0.05; B versus D
SGRQ total %	25.4±9.7	43.7±14.7	35.7±11.0	58.7±16.6	p<0.01; A versus B,C,D C versus D
Pharmacological treatment					
CS therapy	6 (40)	7 (70)	17 (85)	36 (95)	p<0.001
LAMA/LABA therapy	11 (73)	9 (90)	19 (95)	38 (100)	NS; p=0.25
Biomarkers					
IS neutrophils 10 ⁶ cells·mL ⁻¹	2.2 [0.1–7]	4.9 [1–60]	2.7 [0.1–15]	2.4 [0.1–14]	p=0.026; B versus A,C,D
IS neutrophils %	66.5 [15–91]	84.2 [58–99]	72.1 [18–88]	78.7 [44–97]	p=0.028; A,C versus B,D
IS neutrophilia	7 (46)	9 (90)	12 (60)	34 (89)	p=0.03
IS eosinophils 10 ⁶ cells·mL ⁻¹	0.06 [0–1.1]	0.07 [0–0.4]	0.3 [0–5]	0.03 [0–0.7]	NS; p=0.08
IS eosinophils %	2.6 [0.4–21]	1.4 [0–9]	2.9 [0–60]	1.1 [0–23]	p=0.048; A,C versus B,D
IS eosinophilia	11 (73)	4 (40)	15 (75)	15 (39)	p=0.02
EBC MDA nM	17.2 [4–68]	17.1 [4–33]	25.5 [4–81]	26.3 [6–126]	NS; p=0.54

Data are reported as n (%), mean±SD or median (range), unless otherwise stated. mMRC: modified Medical Research Council; FEV₁: forced expiratory volume in 1 s; TLC: total lung capacity; DLC₀: single-breath diffusing capacity of the lung for CO; AV: alveolar volume; SGRQ: Saint George Respiratory Questionnaire; ICS: inhaled corticosteroids; LAMA/LABA: Long acting bronchodilator; N: number; IS: induced sputum; EBC: exhaled breath condensate; MDA: malondialdehyde. NS: not significant. A value of p<0.05 was considered as significant.

Our observation, although performed on a small group of COPD patients attending to an outpatient Pulmonary Rehabilitation Programme, confirms that other physiologic and biologic differences, apart from those used for the new GOLD classification, can be observed among the A, B, C and D groups. As expected, markers of emphysema (like tendency to have pulmonary hyperinflation, and reduction in diffusing capacity) were higher in B and D groups with higher dyspnoea score, confirming that dyspnoea in COPD patients is mainly related to the level of mechanical abnormalities due to the emphysema component of COPD [10]. In the much larger cohort of the ECLIPSE study, emphysema as assessed by CT evaluation, was more evident in C and D categories, while there was no difference between A and B categories. The higher dyspnoea score in group B in comparison with group A of the ECLIPSE study was explained by the higher number of comorbidities in group B (mainly cardiovascular comorbidities) which may increase the level of dyspnoea for other non-pulmonary limitations. Our study examined a limited number of patients, and furthermore they had been selected from the possibility to attend to an outpatient Pulmonary Rehabilitation Programme; therefore severe cardiovascular diseases, which might represent an exclusion criteria for the exercise training, were excluded.

In our experience, Group B and D showed the higher neutrophil percentage in the induced sputum, while eosinophil percentages were higher in A and C groups in comparison with B and D groups. This observation might suggest that sputum neutrophilia is really associated with a higher level of COPD severity. While sputum neutrophilia is a characteristic feature of all COPD patients, independently from

the specific clinical phenotype, sputum eosinophilia seems to be more frequent in a “bronchitic phenotype” with lower level of symptoms and markers of emphysema. At our knowledge, this is new information, not reported in the GOLD 2011 classification. Sputum and blood neutrophilia has been reported as a marker of severity of COPD, according to FEV₁ or quality of life measurement [11], while sputum eosinophilia has been demonstrated in subgroups of COPD patients [12] and it has been used as a biomarker potentially useful in the therapeutic decision (e.g., use or not of inhaled corticosteroids) [13].

In summary, our observation, although performed on a small group of selected COPD patients, suggests that the A, B, C and D groups of the new GOLD classification are at least partly related to a different COPD phenotype (prevailing emphysema in B and D groups with greater neutrophilic inflammation, prevailing chronic bronchitis A and C groups with greater eosinophilic inflammation). This information may contribute to a better characterisation and possibly management of the wide range of categories included now in the COPD definition.



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The A, B, C and D groups of the new GOLD classification are at least partly related to different COPD phenotypes <http://ow.ly/Xgr8q>

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