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Predictors of short-term LAMA ineffectiveness in treatment naïve patients with moderate to severe COPD

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Summary

Background No specific (only subgroup) recommendations for the use of long-acting muscarinic antagonists in chronic obstructive pulmonary disease (COPD) exist. The aim of this exploratory hypothesis generating study was to assess whether different phenotypic/endotypic characteristics could be determinants of the short-term ineffectiveness of the initial tiotropium bromide monotherapy in treatment naïve moderate to severe COPD patients.

Methods A total of 51 consecutively recruited COPD patients were followed for 3 months after the initial evaluation and prescribed initial treatment (tiotropium). Short-term treatment ineffectiveness was assessed as a composite measure comprising COPD exacerbations, need for additional treatment, and no improvement in functional parameters, e.g. 6-min walking test (6MWT), body-mass index, airflow obstruction, dyspnea, and exercise (BODE) index and forced expiratory volume in 1 s (FEV_1), and as single components.

Results Treatment ineffectiveness was significantly associated with baseline hemoglobin level, COPD assessment test (CAT) score, modified Medical Research Council (mMRC) scale and BODE index ($p=0.002$). Incident exacerbation during the follow-up was associated with baseline bronchoalveolar lavage fluid (BALF) alpha-amylase level and CAT score ($p<0.001$), and change in treatment with leukocyte count, 6MWT desaturation and fatigue ($p<0.001$). No improvement in 6MWT was associated with baseline CAT score, body mass index, mMRC, fatigue, 6MWT and BODE index ($p=0.002$). No improvement in BODE index was associated with leukocyte count, serum interleukin 8 (IL-8) and BALF albumin levels ($p<0.001$); and no improvement in FEV_1 with CAT score, baseline vital capacity and BALF tumor necrosis factor alpha (TNF-alpha) level ($p<0.001$).

Conclusion Our results suggest that there is a possibility to identify predictors of short-term tiotropium ineffectiveness in patients with moderate to severe COPD.

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Keywords Cholinergic antagonists · Chronic obstructive pulmonary disease · Endophenotypes · Treatment failure · Outcome assessments

Abbreviations

ACE	Angiotensin-converting enzyme
ALP	Alkaline phosphatase
AT1T	Alpha1-antitrypsin
ATS	American Thoracic Society
AUC	Area under the curve
BALF	Bronchoalveolar lavage fluid
BMI	Body mass index
BODE	Body-mass index, airflow obstruction, dyspnea, and exercise
CAT	COPD assessment test
CBC	Complete blood cell
CD	Cluster of differentiation
CI	Confidence interval
COPD	Chronic obstructive pulmonary disease
CRP	C-reactive protein
DL_{CO}	Diffusing capacity of the lungs for carbon monoxide
ERS	European Respiratory Society
FAS	Tilburg fatigue assessment scale
FeNO	Fraction of exhaled nitric oxide
FEV_1	Forced expiratory volume in 1 s
FVC	Forced vital capacity
GCP	Good clinical practice
GOLD	Global Initiative for Chronic Obstructive Lung Disease
HRQoL	Health related quality of life
ICS	Inhaled corticosteroids
IL	Interleukin
LAMA	Long-acting muscarinic antagonists
LDH	Lactate dehydrogenase
mMRC	Modified Medical Research Council
NPV	Negative predictive value
OR	Odds ratio
PEF	Peak expiratory flow
pCO2	Partial pressure of carbon dioxide
pO2	Partial pressure of oxygen
PPV	Positive predictive value
RV	Residual volume
SD	Standard deviation
SpO2	Arterial oxygen saturation
6MWT	6 min walking test
TGF-beta	Transforming growth factor beta
TLC	Total lung capacity
TNF-alpha	Tumor necrosis factor alpha

Introduction

Chronic obstructive pulmonary disease (COPD) is the fourth leading cause of death worldwide and is characterized by chronic and progressive airway limitation caused by chronic airway inflammation due to, but not restricted to, airway exposure to cigarette smoke and pollutants [1]. The course of disease is often complicated by exacerbations and comorbidities [2]. Chronic obstructive pulmonary disease is classified by disease specific symptoms, grade of airflow limitation, comorbidities and exacerbation risk using instruments, such as the modified Medical Research Council (mMRC) dyspnea scale [3] and/or the COPD assessment test (CAT) [4]. Based on this assessment preferential initial treatment recommendations are unspecific [2]. Besides the usual contraindications for a specific treatment, there are no specific (only subgroup) recommendations for the primary choice of treatment, except for inhaled corticosteroids (ICS) suggested in eosinophilic inflammation endotypes. Pathologic changes characteristic for COPD can be found in airways, lung parenchyma and pulmonary vascular structures [5]. Aforementioned changes (endotype) include the infiltration of pulmonary interstitial tissue with neutrophils [6], macrophages [7], T and B lymphocytes [8], eosinophils during exacerbations [9], as well as the elevated concentrations of several inflammatory mediators [10] and pro-inflammatory cytokines, such as tumor necrosis factor alpha (TNF-alpha), interleukin 1 (IL-1), IL-6, IL-8, transforming growth factor beta (TGF-beta), markers of oxidative stress [11] and protease inhibitors. On the other hand, clinical markers (phenotype), such as the grade of dyspnea (mMRC), CAT and body mass index (BMI), airflow obstruction, dyspnea, and exercise (body-mass index, airflow obstruction, dyspnea, and exercise [BODE]) index can be valuable in predicting the prognosis, exacerbations, treatment efficacy/effectiveness and mortality risk [12].

Inhaled long-acting muscarinic antagonists (LAMA) represent a standard treatment as the alternative or combination choice (with long-acting beta agonists, LABA) in patients with moderately severe to severe COPD and an add on treatment for very severe COPD [2, 13–15]. Tiotropium bromide as LAMA has a very good proven bronchodilator effect [16] with a long-term treatment having improvements in lung function, functional status, exercise tolerance, reduces dyspnea and acute exacerbations rate [17] and improvement in general well-being and health-related quality of life (HRQoL) [18]. As known from other drug classes used in respiratory disorders, such as ICS or leukotriene modifiers in asthma, these drugs have a wide response profile, ranging from non-responders (up to 40%) to extreme responders (up to 15%) [19]. There are still no specific recommendations on which COPD patients would mostly benefit from its usage and in which patients it would not be effective (personalized medicine advice).

Bearing in mind the enormous heterogeneity of COPD clinical presentations and disease progression, there are many phenotypic/endotypic characteristics that can help in predicting the therapeutic outcome and the prognosis but there is a need to form an integrative scale of phenotype characteristics to enable the subgrouping of patients according to the prognosis and therapeutic effectiveness. The aim of our hy-

pothesis generating study was to assess whether different cytokine profiles from serum and bronchoalveolar lavage fluid (BALF) together with other biomarkers and phenotypic characteristics could be used as determinants of the short-term ineffectiveness of the initial monotherapy with tiotropium bromide in treatment naïve moderate to severe COPD patients.

Patients and methods

Study design

The study was a hypothesis generating study designed as a prospective non-randomized follow-up intervention study of a previously treatment naïve moderate to severe COPD patients. The study started with initial phenotype/endotype characterization of patients. This was followed by initial treatment with LAMA (tiotropium bromide) as a monotherapy, as recommended by the Global Initiative for Chronic Obstructive Lung Disease (GOLD) [1]. Patients were prescribed tiotropium bromide once a day at a dose of 18 mg via HandiHaler® (Boehringer Ingelheim GmbH, Ingelheim, Germany) and were instructed on the use of the device. They were followed for 3 months during 3 visits: initial/baseline visit (Visit 1), second visit (Visit 2) 1 month after baseline visit, and the third one (Visit 3) 3 months after baseline visit. At Visit 1 a comprehensive work-up was done in all patients. At both follow-up visits (Visits 2 and 3) patients were evaluated for study outcomes. At Visit 1, besides medical history and physical examination, the work-up included CAT, mMRC scale and Tilburg fatigue assessment scale (FAS), lung function assessments, e.g. spirometry with bronchodilator test, fraction of exhaled nitric oxide (FeNO), diffusing capacity of the lungs for carbon monoxide (DL_{CO}), arterial blood gases analysis, hematology and biochemistry, 6 min walking test (6MWT), and fiber bronchoscopy with BALF analysis. At follow-up visits history was taken with specifics on exacerbations, regular use of tiotropium, use of salbutamol, intensity of symptoms, vital signs were measured, and dyspnea assessed (mMRC). Adherence to tiotropium treatment was encouraged but the number of capsules used was not checked. Additional to spirometry and DL_{CO}, arterial blood gas analysis was done. Additional treatment according to GOLD was provided if symptoms worsened or an exacerbation occurred in between visits [1].

Study outcomes (treatment ineffectiveness) were assessed as a composite measure, comprising COPD exacerbations, need for additional treatment, and no significant improvement in functional parameters, e.g. 6MWT, BODE index and forced expiratory volume in 1 s (FEV_1); and as single components. As no significant improvements consecutive criteria were used: (1) for 6MWT the improvement <54 m, (2) for BODE index, no change/deteriorated score and (3) for FEV_1, no change/deteriorated value.

The study protocol and the informed consent form were approved by the institutional review board/local ethics committee and done in accordance with the Declaration of Helsinki, good clinical practice (GCP), and all relevant international and national legislations. Eligible patients were given the informed consent form with enough time to read it and to discuss any relevant issues regarding the study before consenting. All participants signed a written consent before entering the study and before any procedure was done.

Subjects

A total of 51 newly diagnosed, treatment naïve COPD patients with moderately severe ($50\% \le FEV_1 < 80\%$), GOLD 2 ($n = 30$) and severe ($30\% \le FEV_1 < 50\%$), GOLD 3 ($n = 21$) airway limitation, postbronchodilator FEV_1/forced vital capacity (FVC) <0.70, were recruited into the study. The patients were additionally categorized into subgroups A–D based on CAT scores and the level of airway limitation. The patients had to be exacerbation free for at least 1 month prior to recruitment. Patients with other severe lung, cardiac or gastrointestinal disorders were excluded as well as patients with cancer. An enrolment flow diagram is presented in Fig. 1.

Material and methods

The chronic obstructive pulmonary disease assessment test (CAT) is a validated, short (8-item) and simple patient completed questionnaire, with good discriminant properties, developed for use in routine clinical practice to measure the health status of patients with COPD. Every item has a scale of 0–5 so the scoring range is from zero (no impairment) to 40 (maximum impairment; [20]). The modified Medical Research Council (mMRC) scale of dyspnea [3] is a quantitative assessment tool only for breathlessness, simple to administer as it allows the patients to indicate the extent to which their breathlessness affects their mobility. The Tilburg fatigue assessment scale [21], a 10-item, unidimensional fatigue scale was used to assess fatigue connected with COPD. Spirometry was done using computerized pneumotachograph MasterScreenTM PFT (Jaeger, CareFusion, CA) according to the American Thoracic Society/European Respiratory Society (ATS/ERS) guidelines [22]. The best of three technically satisfactory efforts was recorded. Bronchodilator test was done with the repeated spirometry 30 min after the inhalation of 400 µg of salbutamol with the use of spacer. Spirometric indexes, e.g. FVC, FEV_1, FEV_1/FVC, peak expiratory flow (PEF) at baseline and after bronchodilator were recorded as absolute values and as percentage of predicted according to Quanjer et al.

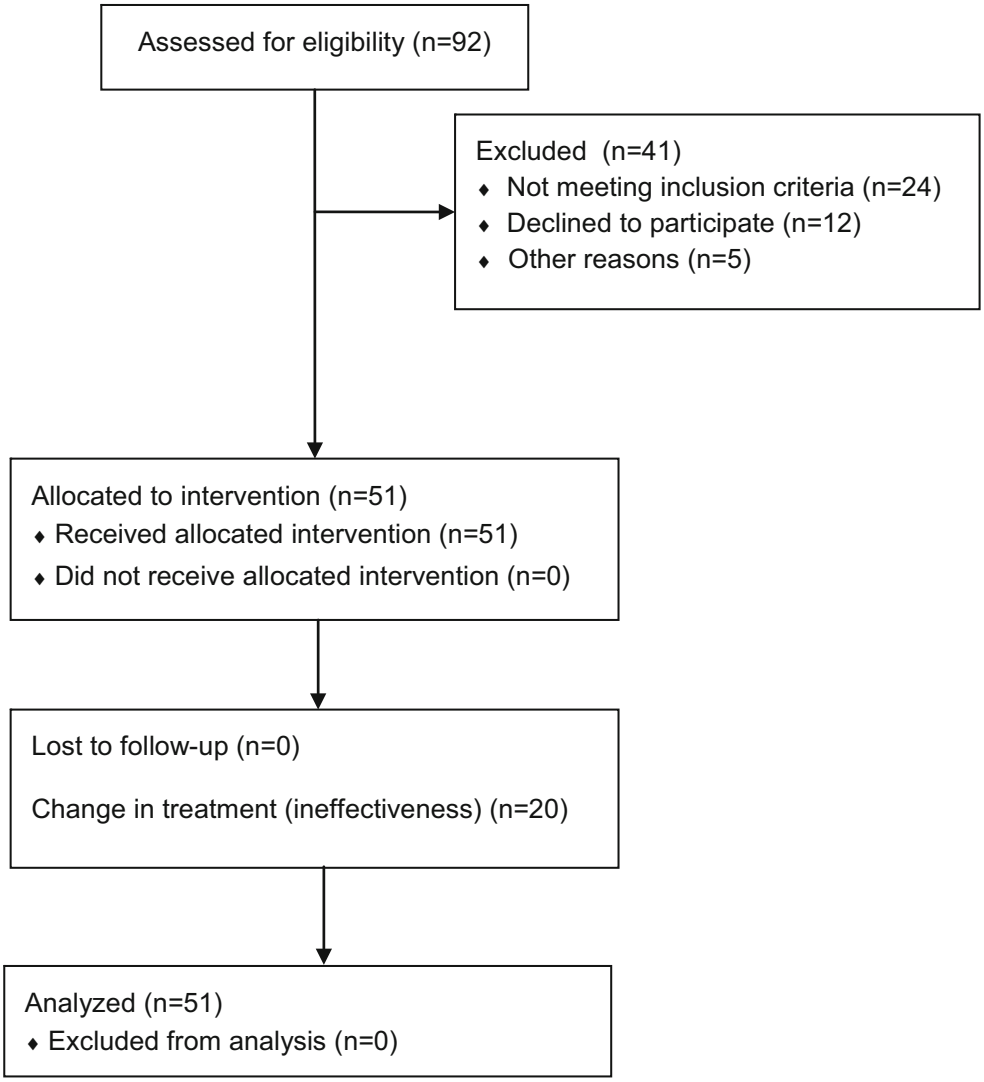

Fig. 1 Enrolment flow diagram

[23]. The DL_{CO} was measured using a single breath method on a MasterScreenTM PFT (Jaeger) according to ATS/ERS recommendations and expressed as % predicted according to Crapo et al. [24]. The fraction of exhaled nitric oxide (FeNO) was measured using a HypAirFeNO measuring system (Medisoft, Sorinnes, Belgium) during a single-breath exhalation according to the ATS/ERS recommendations at a flow rate of 50 ml/s [25]. Functional exercise capacity was assessed by the 6MWT according to the ATS guidelines and expressed as walked distance in meters [26]. Arterial blood gases analysis was done immediately after arterial blood was drawn from the radial artery on a GEM Premier 3000 blood gas analyzer (IL Werfen, Lexington, MA).

Fasting morning peripheral blood samples were taken using venous puncture for complete blood cell (CBC) count and differentials and for C-reactive protein (CRP) and analyzed using standard clinical laboratory procedures. Serum samples after centrifugation at 1500 g for 15 min were divided in two aliquots. The first one was used immediately for biochemical analyses using standard clinical laboratory procedures, and the second one was stored at –80 °C for subsequent cytokine analyses. The bronchoalveolar lavage was sampled from the middle lung lobe using a flexible bronchoscope Pentax FB-15 (Pentax Medical, Montvale, NJ), rinsing with a 4 × 50 ml isotonic fluid (NaCl 0.9%) and recovery of fluid (BALF) into a silicon container [27]. The bronchoalveolar lavage fluid (BALF) was immediately sent for analysis where the sample was filtered through nylon mesh and centrifuged for 10 min at 450 g at 4 °C. If there were macroscopically visible erythrocytes, cell were resuspended in a lysis buffer for 5 min and centrifuged again to remove red blood cells. The BALF lymphocytes were instantly analyzed using direct double staining immunofluorescence method on the BD FACSCalibur™ flow cytometer (BD Bioscience, San Jose, CA). Surface cluster of differentiation (CD)3, CD4 and CD8 markers were determined and results expressed as the % of positive lymphocytes from the total lymphocyte population. The BALF aliquots were stored at –80 °C for further immunocytochemistry.

Table 1 Baseline characteristics of participants according to COPD GOLD severity stage ($n = 51$)

	All ($n = 51$)	COPD GOLD 2 ($n = 30$)	COPD GOLD 3 ($n = 21$)	Statistics	*p*-value
Age, years	64.2 ± 9.7	63.3 ± 8.7	65.5 ± 11.0	t = 0.791	0.433
Sex, male (%)	42 (82.4)	23 (76.7)	19 (90.5)	$\chi 2 = 1.621$	0.203
Smoking (%)					
Non-smokers	8 (15.7)	5 (16.7)	3 (14.3)	–	–
Ex-smokers	30 (58.8)	19 (63.3)	11 (52.4)	$\chi 2 = 1.158$	0.56
Current smokers	13 (25.5)	6 (20.0)	7 (33.3)	–	–
COPD duration, years	11.5 ± 7.3	9.3 ± 6.0	14.8 ± 8.0	t = 2.305	0.026
Body mass index, kgm^{-2}	28.4 ± 5.2	29.89 ± 4.84	26.24 ± 5.09	t = 2.573	0.013
GOLD groups (%)					
A	5 (9.8)	5 (16.7)	0	–	–
B	25 (49.0)	25 (83.3)	0	Fisher	<0.001
C	3 (5.9)	0	3 (14.3)	–	–
D	18 (35.3)	0	18 (85.7%)	–	–
mMRC	2.3 ± 0.8	2.0 ± 0.8	2.8 ± 0.7	t = 3.555	0.001
CAT	18.1 ± 7.6	16.7 ± 7.4	20.1 ± 7.7	t = 1.613	0.113
FVC, % predicted	73.22 ± 14.46	81.07 ± 10.00	62.01 ± 12.38	t = 6.073	<0.001
FEV_1,% predicted	54.03 ± 15.35	65.01 ± 7.34	38.35 ± 8.47	t = 11.981	<0.001
FEV_1/FVC	0.57 ± 0.11	0.64 ± 0.07	0.47 ± 0.08	t = 8.198	<0.001
BODE index	3.3 ± 2.2	1.9 ± 1.5	5.1 ± 1.6	t = 7.195	<0.001
DL_{CO}, % predicted	66.35 ± 22.84	78.78 ± 15.89	49.19 ± 19.79	t = 5.862	<0.001
RV, % predicted	110.87 ± 27.13	109.50 ± 23.91	112.87 ± 31.79	t = 0.423	0.674
RV/TLC	49.22 ± 9.54	0.45 ± 0.07	0.54 ± 0.10	t = 3.619	0.001
RV/TLC, % predicted	127.34 ± 25.71	118.09 ± 17.02	140.12 ± 30.29	t = 3.275	0.002
FeNO, ppb	20.4 ± 16.7	22.2 ± 17.8	17.9 ± 15.0	t = 0.414	0.681
pO2, kPa	9.14 ± 1.27	9.38 ± 1.34	8.79 ± 1.11	t = 1.668	0.102
pCO2, kPa	5.74 ± 0.83	5.72 ± 0.92	5.76 ± 0.70	t = 0.184	0.855
6MWT, m	370 ± 125	405 ± 116	320 ± 122	t = 2.539	0.014
SpO2 before 6MWT, %	95.8 ± 3.0	96.2 ± 2.0	95.1 ± 4.0	t = 1.373	0.176
SpO2 after 6MWT, %	94.1 ± 6.9	96.1 ± 2.0	91.1 ± 9.8	t = 2.560	0.014
Fall in SpO2 after 6MWT, %	–1.7 ± 4.9	–0.1 ± 1.9	–4.0 ± 6.7	t = 2.959	0.005
Fatigue Assessment Scale (FAS)	27.0 ± 6.5	26.1 ± 6.5	28.2 ± 6.4	t = 1.138	0.261
+ Bronchial aspirate bacteriology (%)	13 (25.5)	6 (19.4)	7 (33.3)	$\chi 2 = 9.334$	0.156
+ Bronchial aspirate cytology (%)	0 (0)	0 (0)	0 (0)	Fisher	1

Data are presented as mean±SD or as number of observations and percentage (%)
GOLD Global initiative for Obstructive Lung Disease; *mMRC* modified Medical Research Council scale; *CAT* COPD Assessment Test; *FVC* forced vital capacity; *FEV1* forced expiratory volume in 1 s; *DL_{CO}* diffusing capacity of the lungs for CO; *RV* residual volume; *TLC* total lung capacity; *FeNO* fraction of exhaled NO; *pO2* partial pressure of oxygen; *pCO2* partial pressure of CO_2; *6MWT* 6-minute walking test; *SpO2* saturation of oxygen

Frozen serum and BALF samples were defrosted at the same time for detection of IL-6, IL-8 and TNF-alpha using commercial ELISA kits (eBioscience, San Diego, CA) on an automated absorbance microplate reader ETI-Max 3000 (Diasorin S.p.A., Saluggia, Italy). Lower limit of detection for IL-6 was 0.92 pg/ml, for IL-8 2.0 pg/ml and for TNF-alpha 5.0 pg/ml.

Data analysis

Data analysis was conducted using STATISTICA version 12 (StatSoft, Tulsa, OK). Numerical data were presented as mean and standard deviation (SD) or median and interquartile range, and categorical data were presented as numbers and %. Normality of distribution was tested using the Kolmogorov-Smirnov test. Variables not having normal distribution were normalized using a method dependent on the distribution. Differences between groups were tested using χ^2-test or Fisher's exact test as appropriate for categorical data, and using Student's t-test or Mann-Whitney U test for numerical data. Association with outcomes was tested using generalized linear/nonlinear regression analysis using a stepwise approach and expressed as area under the curve (AUC) with 95% confidence intervals (CIs) separately for each of the outcome measures. Independent variables for the model included baseline characteristics, e.g. age, sex, smok-

Table 2 Baseline values of serum and BALF inflammatory markers according to COPD GOLD severity stage ($n = 51$)

		All ($n = 51$)	COPD GOLD 2 ($n = 30$)	COPD GOLD 3 ($n = 21$)	Z	p-value
SERUM	A1AT (g/l)	1.45 (1.23–1.71)	1.42 (1.22–1.53)	1.56 (1.28–1.93)	1.397	0.162
	IL-6 (pg/ml)	3.00 (2.30–5.00)	2.75 (2.30–4.70)	4.0 (2.4–7.8)	1.388	0.165
	IL-8 (pg/ml)	14 (11–17)	13 (10–20)	14 (12–17)	0.957	0.339
	TNF-alpha (pg/ml)	35 (32–37)	35 (32–38)	34 (32–37)	0.182	0.856
	CRP (mg/l)	3.4 (1.6–8.4)	2.4 (1.6–5.6)	5.4 (1.5–14.2)	1.494	0.135
	Leukocytes (10^9/l)	7.00 (5.60–8.40)	6.85 (5.30–7.90)	8.20 (6.80–9.00)	2.048	0.041
	Hemoglobin (g/L)	143 (136–151)	143 (137–150)	143 (135–151)	0.096	0.924
BALF	ACE (U/ml)	1.20 (0.50–2.00)	1.20 (0.50–1.95)	1.20 (0.50–2.30)	0.087	0.931
	CRP (mg/l)	0.0 (0.0–0.1)	0.0 (0.0–0.1)	0.0 (0.0–0.1)	0.455	0.649
	Albumin (g/l)	56.90 (26.90–91.50)	57.90 (30.90–89.55)	52.00 (26.90–96.00)	0.043	0.965
	IL-6 (pg/ml)	7.05 (4.15–9.25)	6.30 (3.80–8.10)	7.90 (5.30–11.00)	1.033	0.302
	IL-8 (pg/ml)	209 (56–645)	95 (30–220)	404 (223–1864)	3.837	<0.001
	TNF-alpha (pg/ml)	59 (53–68)	59 (54–66)	58 (53–71)	0.074	0.941
	ALP (IU/l)	29.0 (17.0–40.0)	29.5 (17.0–45.5)	28.0 (18.0–40.0)	0.249	0.803
	Alpha-amylase (U/L)	741 (52–1459)	436 (25–1280)	940 (179–1617)	1.171	0.242
	LDH (U/L)	78.0 (53.0–128.0)	66.5 (46.0–83.5)	127.0 (93.0–176.0)	3.588	<0.001
	Total proteins (g/l)	213.0 (127.0–316.0)	196.0 (111.5–292.0)	272.0 (167.0–719.0)	1.507	0.132
	Lymphocytes (10^9/l)	4.0 (2.0–7.5)	6.0 (3.0–12.0)	2.0 (0.5–4.0)	3.637	<0.001
	CD3+ (%)	74.5 (60.0–85.0)	81.0 (64.0–88.0)	66.5 (54.0–75.0)	2.615	0.009
	CD4+ (%)	36.0 (20.0–48.0)	41.0 (31.5–56.5)	21.5 (17.0–37.0)	2.935	0.003
	CD8+ (%)	30.0 (22.0–42.0)	29.0 (22.5–40.0)	31.0 (22.0–52.0)	0.587	0.557
	CD4+/CD8+	1.13 (0.66–1.93)	1.58 (0.77–2.25)	0.75 (0.35–1.30)	2.228	0.026

Data is presented as median and interquartile range. z calculated using Mann-Whitney U test; *GOLD* Global initiative for Obstructive Lung Disease; *BALF* bronchoalveolar lavage fluid; *AT1T* alpha1-antitrypsin; *IL* interleukin; *TNF* tumor necrosis factor; *CRP C*-reactive protein; *ACE* angiotensin-converting enzyme; *ALP* alkaline phosphatase; *LDH* lactate dehydrogenase; *CD* cluster of differentiation.

ing habit, body mass index (BMI), duration and severity of COPD, lung function, blood gases, dyspnea and fatigue scale scores, 6MWT, CAT, BODE and serum and BALF inflammatory markers. After the best model was defined for each of the outcomes using an automated procedure, only a set of statistically significant independent predictive variables was used to test the validity of each model by conducting the confirmatory regression analysis. These validated models were then used to calculate the predictive power expressed as odds ratio (OR) with 95% CIs and as sensitivity, specificity, positive and negative predictive values (PPV and NPV). All tests were two-sided with statistical significance set at $p < 0.05$ with corrections for multiple comparisons.

Results

Baseline characteristics

Baseline characteristics of the 51 treatment naïve COPD patients recruited into the trial are presented in Table 1. Of the patients 30 (58.8%) were assessed as being of severity stage GOLD 2 and 21 (41.2%) of severity stage GOLD 3. The average (SD) age of patients was 64.2 (9.7) years, most being men (82.4%) with no significant differences for age and sex between severity subgroups ($p > 0.05$ for both) (Table 1). Most patients were ex-smokers comparable between severity subgroups ($p = 0.560$) with significantly longer duration of previous COPD symptoms in GOLD 3 subgroup ($p = 0.026$) and significantly lower BMI ($p = 0.013$) (Table 1). The dyspnea score (mMRC) and BODE index were as expected significantly worse in GOLD 3 subgroup ($p = 0.001$, $p < 0.001$, respectively) but the CAT score and FAS score although worse in GOLD 3 subgroup did not reached statistical significance ($p > 0.05$ for both) (Table 1). Also, as expected spirometry indexes were worse in GOLD 3 subgroup ($p < 0.001$ for all three, Table 1) together with DL_{CO} ($p < 0.001$) and markers of hyperinflation (residual volume RV/total lung capacity TLC, $p = 0.001$; RV/TLC%, $p = 0.002$), but not for RV ($p = 0.674$) (Table 1). The fraction of exhaled NO (FeNO), partial pressure of oxygen (pO2) and partial pressure of carbon dioxide (pCO2) of blood and arterial oxygen saturation (SpO2) were not significantly different between severity subgroups ($p > 0.05$ for all); however, 6MWT results, SpO2 after 6MWT and desaturation after 6MWT were all significantly worse in GOLD 3 subgroup (6MWT, $p = 0.014$; SpO2, $p = 0.014$; desaturation, $p = 0.005$) (Table 1).

Parameters and inflammatory markers from blood/serum and BALF at baseline are presented in Table 2. The only blood/serum inflammatory marker significantly different between severity subgroups was the number of leukocytes showing a higher level in

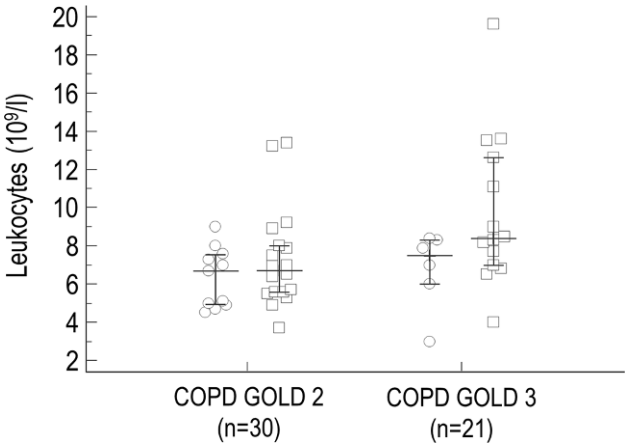

Fig. 2 Plot of the number of leukocytes according to COPD severity; $p = 0.041$, Mann-Whitney U-test, *circles* and *squares* represent individual data with *squares* representing individuals with the positive composite outcome, longer *horizontal line* median value and shorter horizontal lines 25th and 75th percentiles (interquartile range)

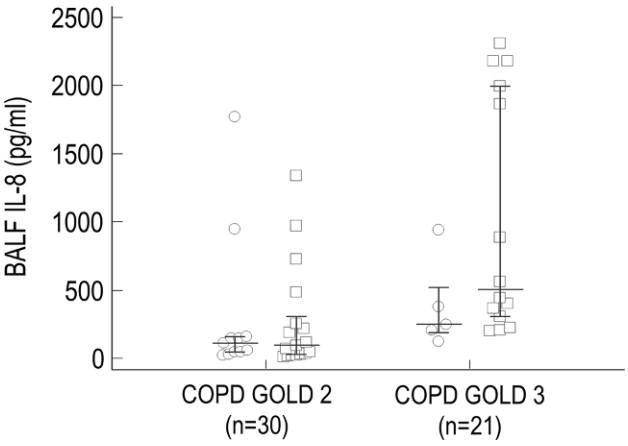

Fig. 3 Plot of bronchoalveolar lavage fluid (BALF) IL-8 values according to COPD severity; $p < 0.001$, Mann-Whitney U-test, *circles* and *squares* represent individual data with *squares* representing individuals with the positive composite outcome, longer *horizontal line* median value and shorter *horizontal lines* 25th and 75th percentiles (interquartile range)

GOLD 3 subgroup ($p = 0.041$) (Table 2 and Fig. 2). All other markers did not reach significance although some of them were higher in GOLD 3 subgroup: alpha1-antitrypsin ($p = 0.162$), IL-6 ($p = 0.165$), and CRP ($p = 0.135$) (Table 2). On the other hand several BALF inflammatory markers were significantly different between the severity subgroups (GOLD 2 vs. GOLD 3): IL-8 ($p < 0.001$, Table 2 and Fig. 3), lactate dehydrogenase (LDH) ($p < 0.001$, Table 2 and Fig. 4), lymphocytes ($p < 0.001$, Table 2), and lymphocyte subpopulations CD3+ cells, CD4+ cells, and CD4+/CD8+ ratio ($p = 0.009$, $p = 0.003$ and $p = 0.026$, respectively) (Table 2 and Fig. 5).

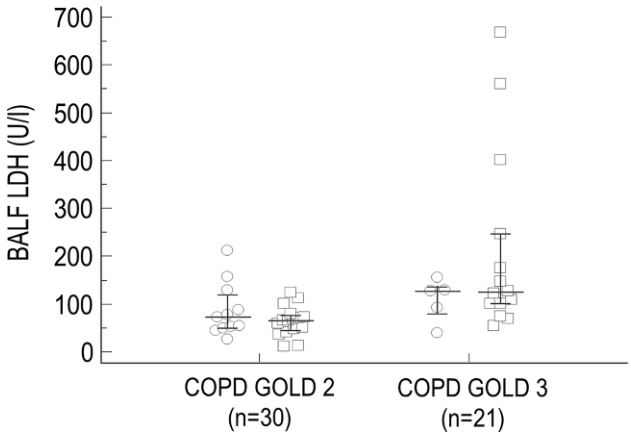

Fig. 4 Plot of bronchoalveolar lavage fluid (BALF) LDH values according to COPD severity; $p < 0.001$, Mann-Whitney U-test, *circles* and *squares* represent individual data with *squares* representing individuals with the positive composite outcome, longer *horizontal line* median value and shorter horizontal lines 25th and 75th percentiles (interquartile range)

Outcomes

The number of single and composite outcome measures according to severity subgroups is presented in Table 3. Of the patients 32 (62.7%) had a positive primary outcome measure (treatment ineffectiveness) with no significant difference between the severity subgroups ($p = 0.566$). Significant predictors for the primary outcome measure were baseline hemoglobin, CAT, BODE and mMRC scores with an OR of 9.41, AUC of 0.818, and sensitivity of 88.5%, specificity of 81.8%, PPV 92.0% and NPV of 75% for the model ($p = 0.002$, Table 4).

Out of five secondary outcome measures two were significantly more common in GOLD 3 subgroup: exacerbations ($p = 0.033$) and treatment change ($p = 0.004$) (Table 3). Other secondary outcome measures (no improvements for 6MWT, BODE index and FEV_1) were more common in GOLD 2 subgroup but did not reach significance ($p > 0.05$ for all, Table 3). Predictors and model characteristics for secondary outcomes are presented in Table 4. Significant predictors for exacerbations were baseline BALF alpha-amylase and CAT score with an OR of 8.31, AUC of 0.883, sensitivity of 85.7%, specificity of 93.3%, PPV 75.0% and NPV of 96.6% for the model ($p < 0.001$, Table 4). Significant predictors for treatment change were baseline number of leukocytes, desaturation after 6MWT and FAS score with an OR of 22.6, AUC of 0.926, sensitivity of 86.7%, specificity of 90.9%, PPV 86.7% and NPV of 90.9% for the model ($p < 0.001$, Table 4). Significant predictors for no improvement in 6MWT result were baseline 6MWT result, CAT, FAS and mMRC scores, BMI, and BODE index with an OR of 14.3, AUC of 0.876, sensitivity of 71.4%, specificity of 82.4%, PPV 80.0% and NPV of 89.3% for the model ($p = 0.002$, Table 4). Significant predictors for

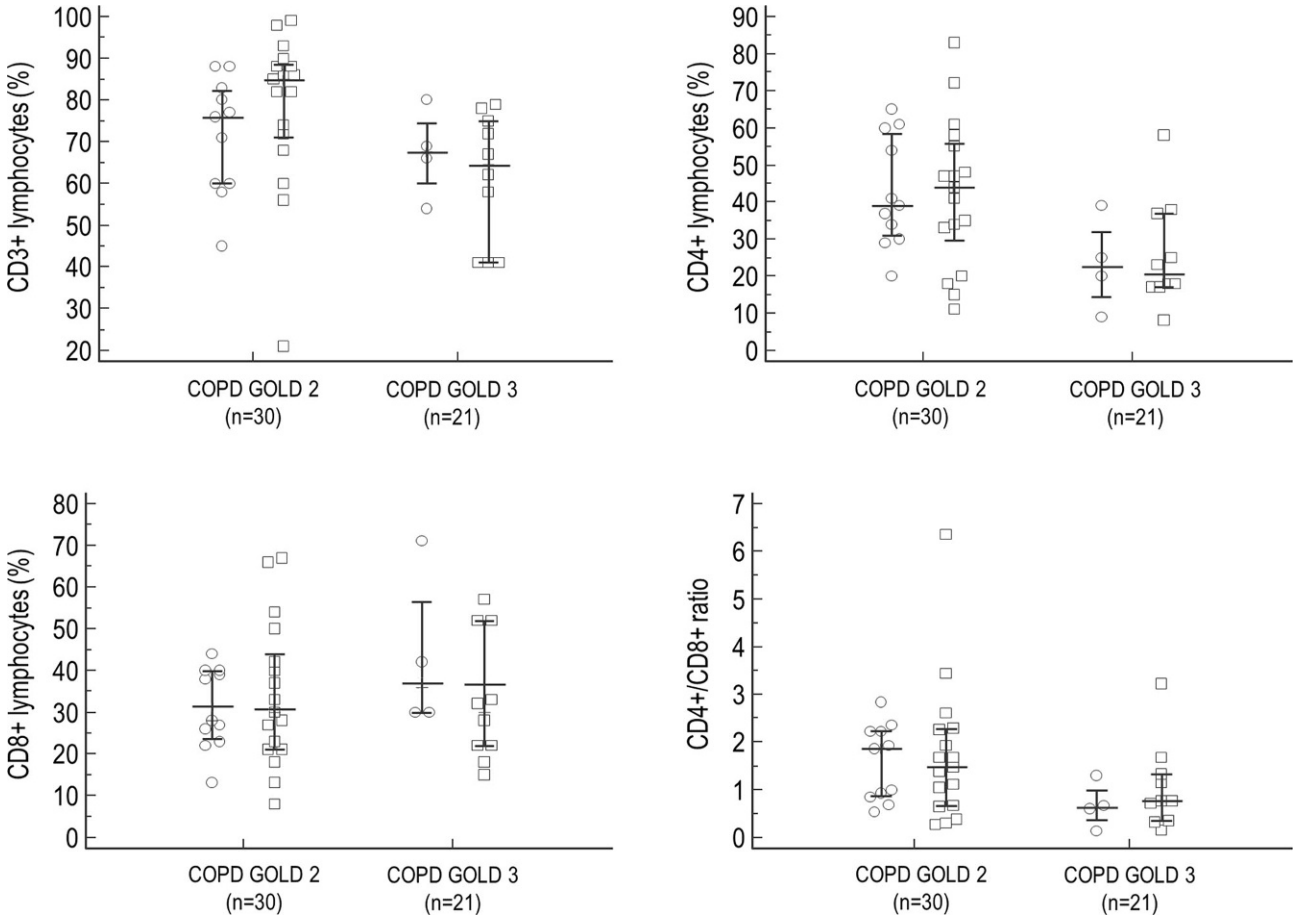

Fig. 5 Plot of bronchoalveolar lavage fluid (BALF) lymphocyte subpopulations (CD3+, CD4+, CD8+ and CD4+/CD8+ ratio) according to COPD severity; $p = 0.009$, $p = 0.003$, $p = 0.557$, $p = 0.026$, respectively, Mann-Whitney U-test, *circles* and *squares* represent individual data with squares representing individuals with the positive composite outcome, longer horizontal line median value and shorter horizontal lines 25th and 75th percentiles (interquartile range)

no improvement in BODE index were baseline serum IL-8 level, BALF albumin level and leukocytes with an OR of 13.4, AUC of 0.922, sensitivity of 80.0%, specificity of 89.3%, PPV 57.1% and NPV of 96.2% for the model ($p < 0.001$, Table 4). Significant predictors for no improvement in lung function (FEV_1) were baseline BALF TNF-alpha level, CAT score and FVC (as % predicted) with an OR of 7.21, AUC of 0.870, sensitivity of 70.7%, specificity of 90.9%, PPV 88.5% and NPV of 81.8% for the model ($p < 0.001$, Table 4).

Discussion

This study identified some of the possible potential phenotypic/endotypic traits associated with the short-term ineffectiveness of LAMA monotherapy (tiotropium bromide) in patients with moderate to severe COPD. As expected GOLD 2 and GOLD 3 subgroups showed significant differences regarding many baseline characteristics, but the COPD severity stage was not predictive for any of the outcome measures. This has also been shown in the post hoc analysis of the UPLIFT trial [28]. The most represented predictor for outcomes of short-term ineffectiveness of tiotropium was the CAT score, which together with hemoglobin, BODE index, and mMRC score was identified as significant predictors for the composite measure of outcome, showing the full complexity of COPD. The CAT score has been identified by several authors as a good predictor of poor treatment outcomes as well as previous and future exacerbations, health status deterioration, depression, and mortality [29–31]. The CAT score assesses 8 items, not only dyspnea as mMRC scale, but also other symptoms and health status. When CAT was compared with mMRC [32] a difference in classification of COPD patients was identified yielding a difference in treatment decisions. In our study both questionnaires were used and were shown to be independent predictors of need for treatment change, ineffectiveness in improving lung function and future exacerbations. As shown in the ECLIPSE study, symptoms, airflow limitation, number of reported exacerbations and the extent of emphysema increased in proportion to BODE scores, confirming the BODE index as a good predictive tool to assess the severity of COPD [33, 34]. Our study

Table 3 Outcome measures according to COPD GOLD severity stage ($n = 51$)

Outcomes	All ($n = 51$)	COPD GOLD 2 ($n = 30$)	COPD GOLD 3 ($n = 21$)	Statistics	p-value
Treatment failure composite	32 (62.7)	18 (60.0)	14 (66.7)	$\chi 2 = 0.329$	0.566
Exacerbations	11 (21.6)	3 (10.0)	8 (38.1)	Fisher	0.033
Change in treatment	20 (39.2)	7 (23.3)	13 (61.9)	$\chi 2 = 8.182$	0.004
No improvement of 6MWT	18 (35.3)	13 (43.3)	5 (23.8)	$\chi 2 = 0.229$	0.632
No improvement of BODE index	8 (15.7)	6 (20.0)	2 (9.5)	Fisher	1.000
No improvement of FEV_1	15 (29.4)	11 (36.7)	4 (19.0)	$\chi 2 = 0.364$	0.546

Data is presented as number of observations and percentage (%)
6MWT 6-minute walking test; *FEV_1* forced expiratory volume in 1 s

Table 4 Predictor variables, odds ratios (95% CI), diagnostic characteristics and AUC (95% CI) for outcome measures ($n = 51$)

Outcomes	Predictors	OR (95% CI) for the model	AUC (95% CI) for the model	Sensitivity (%), specificity (%) PPV (%), NPV (%)
Treatment ineffectiveness composite	Hemoglobin, CAT, BODE, mMRC	9.41 (2.33–44.4)	0.818 (0.682–0.914)	88.5, 81.8 92.0, 75.0
Exacerbations	BALF alpha-amylase, CAT	8.31 (1.53–52.8)	0.883 (0.754–0.959)	85.7, 93.3 75, 96.6
Change in treatment	Leukocytes, desaturation after 6MWT, FAS	22.6 (5.30–120)	0.926 (0.814–0.981)	86.7, 90.9 86.7, 90.9
No improvement of 6MWT	CAT, BMI, mMRC, FAS, 6MWT, BODE	14.3 (3.22–78.5)	0.876 (0.734–0.959)	76.9, 77.8 71.4, 82.4
No improvement of BODE index	Serum IL-8, BALF albumin, leukocytes	13.4 (1.83–134)	0.922 (0.792–0.983)	80.0, 89.3 57.1, 96.2
No improvement of FEV_1	BALF TNF-alpha, CAT, FVC%	7.21 (1.64–36.5)	0.870 (0.740–0.950)	80.0, 87.0 70.7, 90.9

Predictors for outcomes were calculated using generalized linear/nonlinear regression analysis using stepwise approach using baseline characteristics (age, sex, smoking, BMI, duration and severity of COPD, lung function, blood gases, dyspnea, fatigue, 6MWT, CAT, BODE) and serum and BAL inflammatory markers to build the models
OR odds ratio, *CI* confidence interval; *AUC* area under the curve; *PPV* positive predictive value; *NPV* negative predictive value; *CAT* COPD Assessment Test; *6MWT* 6-minutes walking test; *FEV_1* forced expiratory volume in 1 s; *mMRC* modified Medical Research Council scale; *BALF* bronchoalveolar lavage fluid; *FAS* Fatigue Assessment Scale; *BMI* body mass index; *FVC* forced vital capacity

confirmed the predictive value of BODE index. High values of hemoglobin, as well as polyglobulia (a secondary event due to chronic hypoxemia) have been confirmed in COPD patients in several analyses, although anemia could also be present in patients with severe COPD (anemia of chronic disease) [35]. Our study also confirmed hemoglobin as valuable measure, being predictive for treatment ineffectiveness.

Serum and BALF inflammatory markers were poorly represented as predictors for outcome measures. Exceptions were BALF alpha-amylase (with CAT score) for exacerbations; leukocytes, serum IL-8 and BALF albumin for no improvement of BODE index and BALF TNF-alpha (with CAT and FVC%) for no improvement of FEV_1. The immune inflammatory changes associated with COPD cause a tissue repair and remodelling process that increases mucus production and causes emphysematous destruction of the gas exchanging surface of the lungs [36]. This might also explain the higher concentration of LDH in BALF of COPD patients in GOLD 3 subgroup as compared to GOLD 2 subgroup in our study, namely cytoplasmic enzymes, such as LDH when present in the extracellular space are indicators of cell damage or cell death [37]. We also found that values of CRP, TNF-alpha and IL-6 were higher in the COPD group but still not significantly higher than those in the control group (historical control, data on file), whereas the ECLIPSE study reported significantly higher serum values of IL-6 in COPD patients than in the control group [38]. Of note is that the ECLIPSE study had significantly higher number of patients. As opposed to our findings, ECLIPSE biomarker cohort sub-analysis detected 10% of patients with serum TNF-alpha values above the detection limit but serum CRP values were also increased [39].

The BALF markers of inflammatory response on the other hand were significantly different between GOLD 2 and GOLD 3 subgroups. The GOLD 3 subgroup had significantly higher values of BALF IL-8 and LDH, whereas GOLD 2 subgroup had higher values of lymphocytes, CD3+, CD4+ and CD4+/CD8+ ratio. High IL-8 values are due to inflammatory nature of the disease and the chemoattractant role of IL-8 for neutrophils and monocytes/macrophages [38].

Cytotoxic T-lymphocytes (CD8+) dominate in the bronchial mucosa and lymph nodes in COPD whereas T-helper cells (CD4+) are more abundant in the air spaces. There are data indicating that emphysema is related to the number of CD8+ T-cells in the lung

tissue, whereas the proportion of circulating cytotoxic T-cells (CD8+) is lower in COPD patients with impaired diffusion capacity [39] which could explain the higher BALF CD4+/CD8+ ratio in the less severe GOLD 2 subgroup of COPD patients.

The limitations of this hypothesis generating study are the relatively small number of patients and a relatively short follow-up time. This is somewhat limiting for the assessment of the outcome measure predictors and becomes clear from wider confidence intervals of both ORs and AUCs for the constructed models. Also, entering many variables into the regression models having a limited sample, could possibly yield an imprecise estimates and random variable selection. This was in part overcome by checking the resulting regression models produced by the automatic stepwise approach with regression analyses using only the selected variables. The small sample and a short follow-up time (3 months) could produce the small cumulative number of outcomes, which was not the case in our study (Table 3). There were almost two thirds of patients with a composite outcome and at least 15% for each of the single outcome measures, allowing comparisons and associations analyses. Also, outcome measures were defined in a way (no improvement) allowing their evaluation during a represented period of time. This can be seen from the data of several pharmacokinetics and pharmacodynamics studies of tiotropium bromide and the UPLIFT trial where the peak of tiotropium efficacy is seen already after several weeks thus allowing that ineffectiveness could be assessed during 3 months of follow-up [17, 40–42]. These limitations most probably limit the generalizability of our results and further confirmation studies are warranted. As this was a hypothesis generating study, further intervention studies would need to compare the outcomes between groups defined by identified models using a larger sample and longer treatment period, also comparing other long-acting bronchodilators.

Conclusion

Tiotropium has been proven to have a very good bronchodilator effect, effectively suppressing many features of COPD (airway limitation, exacerbations, deterioration in activity and HRQoL), but if the inflammatory component is significantly expressed in COPD it seems that the efficacy/effectiveness and the overall treatment outcome could be impaired. The results of our hypothesis generating study suggest that there is a possibility to predict tiotropium short-term ineffectiveness in patients with moderate to severe COPD by phenotyping/endotyping patients before starting the treatment by using mostly simple measures, thus showing a potential for a personalized medicine approach.

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Author contributions V. Fijačko conceived the idea for the study, together with M. Labor, S. Škrinjarić-Cincar, S. Labor, T. Bačun, A. Včev and M. Fijačko collected data. D. Plavec and S. Popović-Grle were responsible together with V. Fijačko for the design of the research and for data analysis. I. Dumbović Dubravčić contributed by literature research, writing and editing the data. All authors contributed by editing and approving the final version of the manuscript. All authors have read and approved the final version of the manuscript.

Compliance with ethical guidelines

Conflict of interest V. Fijačko has received honoraria for lectures from Novartis, Sandoz, AstraZeneca, Berlin Chemie, Pliva, and Boehringer Ingelheim. S. Škrinjarić-Cincar has received honoraria for lectures from Novartis, Sandoz, AstraZeneca, Berlin Chemie, Pliva, and Boehringer Ingelheim. S. Labor has received honoraria for lectures from Novartis, and Boehringer Ingelheim. T. Bačun has received honoraria for lectures from Novo Nordisk, Eli Lilly, Novartis, Sandoz, AstraZeneca, Berlin Chemie, Pliva, and Boehringer Ingelheim. S. Popović-Grle has received honoraria for advisory boards and/or lectures from Boehringer Ingelheim, Novartis, AstraZeneca, Pliva-Teva, Takeda, GlaxoSmithKline, Meda Pharma, Sanofi Aventis, Krka farma, Berlin Chemie Menarini Hrvatska, and Sandoz Hrvatska. D. Plavec has received research grants from GlaxoSmithKline, honoraria for advisory boards and/or lectures and/or clinical trials from GlaxoSmithKline, Menarini, Pliva, Boehringer Ingelheim, Belupo, AbbVie, MSD, and Chiesi. M. Labor, I. Dumbović Dubravčić, M. Fijačko and A. Včev declare that they have no competing interests.

Ethical standards All studies on humans described in the present manuscript were carried out with the approval of the responsible ethics committee (Institutional Review Board and Medical Faculty Ethics Committee) and in accordance with national law and the Helsinki Declaration of 1975 (in its current, revised form). Informed consent was obtained from all patients included in the study.

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