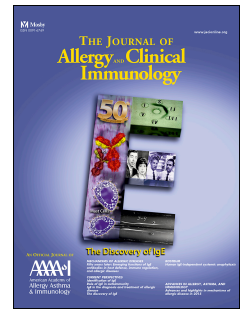


Accepted Manuscript

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PII: S0091-6749(17)30675-9

DOI: [10.1016/j.jaci.2017.01.046](https://doi.org/10.1016/j.jaci.2017.01.046)

Reference: YMAI 12771

To appear in: *Journal of Allergy and Clinical Immunology*

Received Date: 20 September 2016

Revised Date: 3 December 2016

Accepted Date: 23 January 2017

Please cite this article as: Leitao Filho FS, Won Ra S, Mattman A, Schellenberg RS, Fishbane N, Criner GJ, Woodruff PG, Lazarus SC, Albert R, Connett JE, Han MK, Martinez FJ, Leung JM, Paul Man S, Aaron SD, Reed RM, Sin DD, Serum Immunoglobulin G and Risk of Exacerbations and Hospitalizations in Chronic Obstructive Pulmonary Disease, *Journal of Allergy and Clinical Immunology* (2017), doi: 10.1016/j.jaci.2017.01.046.

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Serum Immunoglobulin G and Risk of Exacerbations and Hospitalizations in Chronic Obstructive Pulmonary Disease

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Keywords: COPD; Immunoglobulin G; IgG Deficiency; Exacerbation; Hospitalization.

Capsule Summary

Hypogammaglobulinemia (total IgG levels < 7.0 g/L) is present in 1 in 4 patients with moderate to severe COPD and is associated with 50% to 100% increase in the risk of exacerbations and hospitalizations.

Abbreviations used

COPD: Chronic Obstructive Pulmonary Disease

Ig: Immunoglobulins

IgG: Immunoglobulin G

MACRO: Macrolide Azithromycin for Prevention of Exacerbations of COPD trial

STATCOPE: Simvastatin for the Prevention of Exacerbations in Moderate-to-Severe COPD trial

FEV₁: forced expiratory volume in the first second

FVC: forced vital capacity

BMI: body mass index

CI: confidence intervals

HR: hazard ratios

ICS: inhaled corticosteroids

LABA: long-acting beta2-agonist

LAMA: long-acting muscarinic antagonist

To the Editor:

Reduced circulating total IgG levels have been associated with recurrent respiratory tract infections.^(1, 2) Respiratory infections are the major triggers of exacerbations in Chronic Obstructive Pulmonary Disease (COPD).⁽³⁾ To date, the association between total IgG levels and COPD exacerbations or hospitalizations is not well understood because previous studies have been limited by small sample sizes and a scarcity of robust data on hospitalizations.⁽⁴⁻⁶⁾

To investigate the relationship between circulating total IgG levels and the risk of COPD exacerbations and hospitalizations, we used samples from two large COPD trials: 1) Macrolide Azithromycin for Prevention of Exacerbations of COPD (MACRO)⁽⁷⁾ – Derivation Cohort; and 2) Simvastatin for the Prevention of Exacerbations in Moderate-to-Severe COPD (STATCOPE)⁽⁸⁾ – Validation Cohort. This present study received approval from the University of British Columbia Research Ethics Committee.

IgG deficiency was defined when serum total IgG levels were below the lower limit of normal for adults (< 7.0 g/L).⁽⁹⁾ For analytic purposes, we divided participants into quartiles of total IgG levels. Additional details related to IgG measurements are presented in the Methods section in this article's Online Repository at www.jacionline.org.

Total IgG levels were quantified in 976 MACRO participants (468 in the azithromycin arm; 508 in the placebo arm) and in 653 STATCOPE participants (322 in the simvastatin arm; 331 in the placebo arm), totaling 1,629 samples. There were no significant differences in the total IgG levels or in the prevalence of IgG deficiency between the two cohorts. The details of participant characteristics for each cohort (Table E1) and according to quartiles of total IgG

(MACRO, Table E2; STATCOPE, Table E3) are presented in this article's Online Repository at www.jacionline.org.

For both cohorts, the frequency of COPD exacerbations and hospitalizations were significantly higher in the lowest quartile (p for trend <0.05 for both outcomes; see Figure E1 in this article's Online Repository at www.jacionline.org). The rate of COPD exacerbations significantly increased from the highest to the lowest quartile in both cohorts: MACRO (1.15 ± 1.55 vs. 2.17 ± 3.51 exacerbations per person-year; p for trend <0.001 ; Figure 1A), STATCOPE (0.94 ± 1.30 vs. 1.98 ± 2.11 exacerbations per person-year; p for trend <0.001 ; Figure 1B).

In MACRO, the median time to first COPD exacerbation was progressively longer from the lowest quartile (158 days, 95% CI, 121 to 204 days) to the highest quartile (304 days, 95% CI, 239 to 375 days; log-rank test for trend: $p<0.001$; Figure 1C). In STATCOPE, the median time to first COPD exacerbation was 140 days (95% CI, 104 to 205 days) in the lowest quartile, increasing to 411 days (95% CI, 300 to 525 days) in the highest quartile (log-rank test for trend: $p<0.001$; Figure 1D). Similarly, the participants in the lowest quartile in both cohorts also experienced the greatest risk of hospitalizations (log-rank test for trend: $p<0.001$; Figures 1E and 1F, respectively). The interaction term between total IgG levels and study drug (azithromycin in MACRO; simvastatin in STATCOPE) was not statistically significant in both cohorts ($p=0.88$, and $p=0.25$, respectively). No significant differences were observed in the follow-up period across total IgG quartiles in both cohorts.

Table 1 shows the unadjusted and adjusted hazard ratios (HR) for COPD exacerbations and hospitalizations for both cohorts. In MACRO, the unadjusted HRs for both outcomes increased significantly from the highest to the lowest quartile, and these results remained

significant after adjustments for several covariates (see Methods in this article's Online Repository at www.jacionline.org). In STATCOPE, the adjusted HRs for these outcomes were not statistically significant, however we observed a trend for higher adjusted HRs from the highest to the lowest quartile.

In both cohorts, we also observed higher serum IgG levels among participants who did not have any exacerbations during follow-up versus those who had 1 or 2 exacerbations, or those with 3 or more exacerbations, whereas participants with 3 or more exacerbations had the lowest levels (see Figure E2 in this article's Online Repository at www.jacionline.org).

In the present study, we found that total IgG deficiency was common, affecting ~1 in 4 participants with COPD who were at a high risk for exacerbations. Most importantly, we showed that in both cohorts, reduced total IgG levels, in a dose-dependent manner, were significantly and independently related to COPD exacerbations and hospitalizations. Since patients with selective IgG deficiency are at increased risk of respiratory infections^(1, 2) and since respiratory infections are the leading cause of COPD exacerbations and hospitalizations⁽³⁾, these data raise the possibility that selective IgG deficiency may be a common (modifiable) contributor of exacerbations in COPD. However, in the present study, we did not determine whether IgG replacement therapy could mitigate the risk of exacerbations in those with reduced serum IgG levels. Thus, before we can fully accept this notion, a large randomized controlled trial would be needed to evaluate the risk-benefit of IgG replacement therapy in COPD.

Our study had some limitations. Firstly, as both MACRO and STATCOPE cohorts specifically recruited patients at increased risk for exacerbations, the association between total IgG levels and exacerbations in milder COPD patients is uncertain. Secondly, we only analyzed

blood samples collected at baseline and thus we were unable to determine whether IgG levels are modified during exacerbations or with systemic corticosteroids. Thirdly, we did not evaluate whether patients with reduced total IgG levels had deficient antibody responses to vaccination, which provides a better evaluation of the adaptive immune response. Fourthly, we did not phenotype the exacerbations; thus, the etiology of the exacerbations was not ascertained (though we presumed most were infectious in origin).

In sum, our data demonstrate that total IgG deficiency is relatively common among COPD patients, and that reduced total IgG levels are associated with increased risk of exacerbations and hospitalizations, with the greatest risk observed among participants with total IgG levels below 7.0 g/L. Future clinical trials are warranted in determining whether IgG replacement in those with reduced total IgG levels decreases the risk of COPD exacerbations and hospitalizations.

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This work was supported by the BC Lung Association, Canadian Respiratory Research Network (CRRN) and the Flight Attendants Medical Research Institute (FAMRI). The MACRO and STATCOPE trials were funded by the US National Heart Blood and Lung Institute (NHLBI).

We thank the COPD Clinical Research Network for conducting both the MACRO and STATCOPE trials and for providing us access to all available samples in both cohorts. Dr. Leitao Filho FS gratefully acknowledges postdoctoral support from CNPq-Brazil and the Canadian Institutes of Health Research (CIHR) Integrated and Mentored Pulmonary and Cardiovascular Training program (IMPACT). SWR acknowledges support from the Ulsan University Hospital (Biomedical Research Center Promotion Fund, 10-03).

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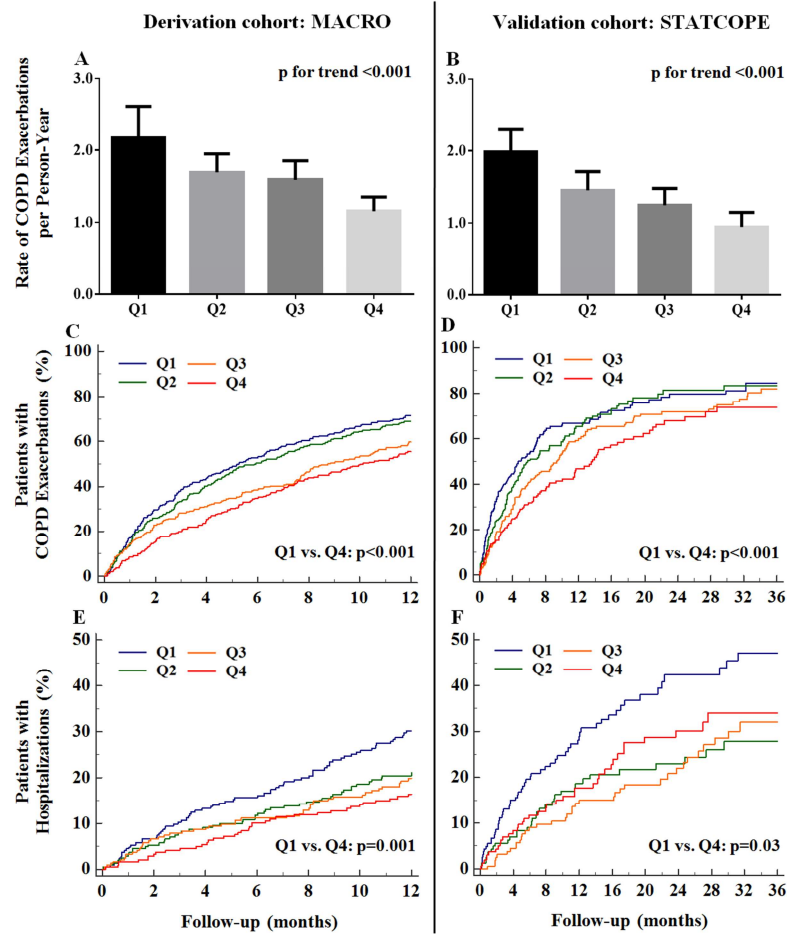
Table 1. Unadjusted and adjusted Hazard Ratios for COPD exacerbations and hospitalizations according to quartiles of serum total IgG levels in the derivation (MACRO) and validation (STATCOPE) cohorts

Derivation Cohort – MACRO					
Outcomes	Quartile 1	Quartile 2	Quartile 3	Quartile 4	
COPD Exacerbation					p for trend
Unadjusted	1.65 (1.32 – 2.08)	1.54 (1.22 – 1.93)	1.17 (0.92 – 1.48)	Reference	<0.001
Adjusted*	1.39 (1.09 – 1.78)	1.38 (1.09 – 1.76)	1.10 (0.86 – 1.40)	Reference	<0.01
Hospitalization					p for trend
Unadjusted	1.94 (1.31 – 2.87)	1.30 (0.86 – 1.98)	1.22 (0.80 – 1.87)	Reference	0.001
Adjusted*	1.92 (1.26 – 2.93)	1.39 (0.90 – 2.15)	1.30 (0.84 – 2.01)	Reference	<0.01
Validation Cohort – STATCOPE					
Outcomes	Quartile 1	Quartile 2	Quartile 3	Quartile 4	
COPD Exacerbation					p for trend
Unadjusted	1.69 (1.28 – 2.22)	1.52 (1.15 – 2.01)	1.24 (0.94 – 1.63)	Reference	<0.001
Adjusted*	1.27 (0.94 – 1.73)	1.30 (0.97 – 1.75)	1.08 (0.80 – 1.44)	Reference	0.06
Hospitalization					p for trend
Unadjusted	1.59 (1.05 – 2.39)	0.86 (0.55 – 1.37)	0.80 (0.51 – 1.26)	Reference	0.01
Adjusted*	1.57 (0.99 – 2.48)	0.88 (0.54 – 1.43)	0.85 (0.53 – 1.36)	Reference	0.04

*Adjusted for study drug (azithromycin vs. placebo in the derivation cohort; simvastatin vs. placebo in the validation cohort), age, gender, ethnicity (Caucasian vs. Non-Caucasian), FEV₁ (% of predicted values), smoking status (current vs. former smokers), oxygen dependence, inhaled steroid use, and treatment with systemic steroids in previous year as additional covariates in a Cox Proportional Hazards model. Quartile 1 – lowest total IgG levels; Quartile 4 – highest total IgG levels.

Figure Legend

Figure 1. Lower serum total IgG levels were associated with worse outcomes among COPD participants in both cohorts (Q1 – lowest total IgG levels; Q4 – highest total IgG levels). **A (MACRO); B (STATCOPE):** Rate of COPD exacerbations per person-year according to quartiles of serum total IgG. Error bars = 95% confidence interval. **C and E (MACRO); D and F (STATCOPE):** Participants with COPD exacerbations and hospitalizations during the study period according to quartiles of serum total IgG (Log-rank test for trend: $p < 0.001$ for all quadrants). Log-rank test used for comparisons between Q1 and Q4.



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Chronic Obstructive Pulmonary Disease**

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Methods

Derivation Cohort – MACRO trial

The MACRO trial investigated the efficacy of azithromycin, a macrolide antibiotic with immunomodulatory and antibacterial effects, for the prevention of COPD exacerbations. Eligible participants included COPD patients aged ≥ 40 years who were former or current smokers (≥ 10 pack-years) with a post-bronchodilator forced expiratory volume in the first second (FEV₁) $< 80\%$ of the predicted value. Subjects also needed to meet at least one of the following inclusion criteria: use of supplemental oxygen, receipt of systemic steroids or antibiotics for a respiratory problem, or a history of emergency department (ED) visit or hospitalization for a COPD exacerbation within the previous year of the trial enrollment.

The primary outcome of interest was the time to first exacerbation of COPD, defined as a complex of respiratory symptoms (increased or new onset) consisting of two or more of the following: cough, sputum, wheezing, dyspnea, or chest tightness with a duration of at least three days and requiring treatment with antibiotics or systemic steroids or both in combination.⁽¹⁾ Severity of exacerbations was graded using the following scale: 1) mild - home management, with or without contacting a health care provider; 2) moderate - requiring a visit to ED; 3) severe - requiring hospitalization; and 4) very severe - resulting in intensive care unit (ICU) admission.

Validation Cohort – STATCOPE trial

The STATCOPE trial was designed to investigate whether simvastatin could reduce COPD exacerbations. The inclusion criteria and outcomes of interest were the similar to those for the MACRO trial, except that the primary outcome was the effect of simvastatin on the exacerbation rate.

IgG Measurements

Blood samples of participants from both cohorts were collected at their baseline evaluation when they were free of exacerbations for at least 4 weeks prior to the blood draw and stored in temperature-monitored -80°C freezers. To measure IgG levels, these samples were thawed at room temperature for 1 hour, with IgG subclass levels measured in serum by immunonephelometry using a Siemens BNII nephelometer (BNII; Erlangen, Germany) and Binding Site IgG subclass (IgG1-4) reagents (Birmingham, UK). We acquired serum total IgG levels by summing the individual four IgG subclass measurements. We processed all samples in the clinical chemistry laboratory at St. Paul's Hospital, Vancouver, British Columbia, Canada.

The normal range for total IgG levels in adults used in this analysis were derived from a healthy population and included mean levels ± 2 standard deviations (7.0 - 16.0 g/L).^(2, 3)

Clinical and Exacerbation Data

Demographic variables (age, gender, body mass index (BMI) and ethnicity), active smoking, medication use, lung function measurements (forced vital capacity (FVC) and FEV_1) and pneumococcal vaccination status were recorded at baseline. The number and severity of exacerbations during follow-up, time to first COPD exacerbation, and time to first hospitalization (due to severe or very severe COPD exacerbations) were assessed prospectively throughout the duration of both trials.

Statistical Analysis

We used the Mann–Whitney U-test to examine whether total IgG levels were similar in both the derivation and validation cohorts. For each cohort, we divided participants into quartiles of total IgG levels for analysis. The Jonckheere-Terpstra and Cochrane-Armitage

tests were employed for continuous variables and categorical variables, respectively.⁽⁴⁾ We calculated the rates of COPD exacerbations in person-years by dividing the number of acute exacerbations by the duration of follow-up in years for each participant (i.e., person-year of follow-up). To compare these rates across total IgG quartiles in both cohorts, we applied the Kruskal–Wallis test.

We obtained a Kaplan-Meier estimate of survival function for the time to first COPD exacerbation or hospitalization according to total IgG quartiles, and compared the curves using log-rank test. We applied bootstrap methods to generate confidence intervals (CI) for the estimates of the median times-to-event. We performed multivariate Cox proportional-hazards regression models to obtain the adjusted hazard ratios (HR) for these outcomes across the quartiles of total IgG (using the highest quartile as the reference). The multivariate models included all variables with biological plausibility or that showed an association with the outcome of interest (exacerbations or hospitalizations) to the level of $p < 0.2$ in the univariate analysis. The HRs related to total IgG levels were adjusted for the following variables: study drug (azithromycin or simvastatin), age, sex, ethnicity (Caucasian vs. Non-Caucasian), smoking status, FEV₁ (% of predicted value), long-term oxygen therapy, inhaled steroid use, and history of treatment with systemic steroids in the year prior to enrollment. To investigate any possible differential effect of total IgG levels by study drug assignment, we included an interaction term in our multivariate models using IgG as a continuous variable. We tested for trend across quartiles of total IgG in the Cox regression models by using orthogonal contrasts for IgG quartiles as an ordinal factor.

We also classified the participants from both cohorts into three groups according to their exacerbation status (no exacerbation vs. one or two exacerbations vs. three or more

exacerbations during study period) and compared their total IgG levels using the Kruskal-Wallis test.

We performed all statistical analyses using SPSS version 21.0 (IBM Corp., Armonk, NY, USA) and R version 3.2.2 (available from <http://www.R-project.org>). The level of statistical significance was set at $p < 0.05$ for all tests.

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Table E1. Baseline characteristics of participants in the derivation (MACRO) and validation (STATCOPE) cohorts

Variable	MACRO N=976	STATCOPE N=653	P*
Total IgG, g/L	9.2 ± 3.2	9.3 ± 3.0	0.64
Total IgG deficiency (< 7.0 g/L)	239 (24.5)	158 (24.2)	0.91
Age, years	65.5 ± 8.6	62.8 ± 8.4	<0.001
Female Sex	392 (40.2)	272 (41.7)	0.55
Caucasian	806 (82.6)	509 (77.9)	0.02
BMI, kg/m ²	27.7 ± 6.2	27.1 ± 6.8	0.062
Current Smokers	207 (21.2)	188 (28.8)	<0.001
FVC, % predicted	70.2 ± 18.1	71.1 ± 19.1	0.29
FEV ₁ , % predicted	39.8 ± 15.7	41.5 ± 17.8	0.049
Long-term oxygen therapy	587 (60.1)	318 (48.7)	<0.001
Steroid use in previous year	822 (84.2)	550 (84.2)	0.99
ICS use – any formulation	764 (78.3)	484 (74.1)	0.06
LABA use – any formulation	732 (75.0)	507 (77.6)	0.22
ICS + LABA use	524 (53.7)	366 (56.0)	0.35
LAMA use	621 (63.6)	416 (63.7)	0.97

* Student's t-test, Mann-Whitney U-test (for comparisons of total IgG levels), or Chi-square test, as appropriate. Values expressed as means ± SD or number (%). Definition of abbreviations: BMI - body mass index, FVC - forced vital capacity, FEV₁ - forced expiratory volume in one second, ICS - inhaled corticosteroids; LABA - long-acting beta2-agonist; LAMA - long-acting muscarinic antagonist; IgG - immunoglobulin G.

151 **Table E2. Baseline characteristics of participants according to quartiles of serum total**
 152 **IgG in the derivation cohort (MACRO)**

Variable	Quartile 1 (3.4 - 7.0) N = 244	Quartile 2 (7.0 - 8.7) N = 244	Quartile 3 (8.7 - 10.7) N = 244	Quartile 4 (10.7 - 37.2) N = 244	P for trend
Allocation to azithromycin	106 (43.4)	122 (50.0)	110 (45.1)	130 (53.3)	0.09
Age, years	66.6 ± 7.9	65.1 ± 8.4	65.9 ± 8.7	64.1 ± 9.2	<0.01
Female Sex	117 (48.0)	115 (47.1)	84 (34.4)	76 (31.1)	<0.001
Caucasian	224 (91.8)	216 (88.5)	207 (84.8)	159 (65.2)	<0.001
BMI, kg/m ²	26.8 ± 5.9	27.3 ± 6.1	28.6 ± 5.9	28.4 ± 6.9	<0.01
Current Smokers	42 (17.3)	54 (22.1)	48 (19.7)	63 (25.8)	0.048
FVC, % predicted	69.4 ± 18.9	70.1 ± 17.2	70.2 ± 19.5	70.9 ± 17.0	0.35
FEV ₁ , % predicted	37.5 ± 15.4	39.8 ± 15.0	39.6 ± 15.3	42.1 ± 16.6	<0.01
Long-term oxygen therapy	166 (68.0)	140 (57.4)	142 (58.4)	139 (57.0)	0.02
Steroid use in previous year	225 (92.2)	207 (84.8)	195 (79.9)	195 (79.9)	<0.001
ICS use – any formulation	193 (79.1)	193 (79.1)	198 (81.1)	180 (73.8)	0.24
LABA use – any formulation	180 (73.8)	183 (75.0)	190 (77.9)	179 (73.4)	0.89
ICS + LABA use	124 (50.8)	139 (57.0)	142 (58.2)	119 (48.8)	0.73
LAMA use	161 (66.0)	151 (61.9)	166 (68.0)	143 (58.6)	0.25
Pneumococcal Vaccination*	167 (68.4)	165 (67.6)	161 (66.0)	156 (66.5)	0.26

153 Values expressed as means ± SD or number (%). The range of total IgG levels (in g/L) is displayed for each
 154 quartile. Definition of abbreviations: BMI - body mass index, FVC - forced vital capacity, FEV₁ - forced
 155 expiratory volume in one second, ICS - inhaled corticosteroids; LABA - long-acting beta2-agonist, and LAMA
 156 - long-acting muscarinic antagonist. *Administered at the baseline evaluation.

Table E3. Baseline characteristics of participants according to quartiles of serum total IgG in the validation cohort (STATCOPE)

Variable	Quartile 1 (3.5 - 7.0) N = 163	Quartile 2 (7.0 - 8.8) N = 163	Quartile 3 (8.8 - 11.0) N = 164	Quartile 4 (11.0 - 22.9) N = 163	P for trend
Allocation to simvastatin	72 (44.2)	81 (49.7)	85 (51.8)	84 (51.5)	0.17
Age, years	64.2 ± 7.8	65.0 ± 8.3	61.5 ± 8.5	60.6 ± 8.3	<0.001
Female Sex	87 (53.4)	67 (41.1)	62 (37.8)	56 (34.4)	<0.001
Caucasian	148 (90.8)	142 (87.1)	132 (80.5)	87 (53.4)	<0.001
BMI, kg/m ²	25.6 ± 5.8	25.9 ± 6.3	28.3 ± 7.6	28.7 ± 6.9	<0.001
Current Smokers	38 (23.3)	43 (26.4)	50 (30.5)	57 (35.0)	0.01
FVC, % predicted	65.6 ± 19.0	71.8 ± 19.1	72.9 ± 18.3	74.3 ± 19.0	<0.001
FEV ₁ , % predicted	35.5 ± 17.2	40.6 ± 16.3	43.4 ± 17.8	46.3 ± 18.0	<0.001
Long-term oxygen therapy	93 (57.1)	83 (50.9)	68 (41.5)	74 (45.4)	0.01
Steroid use in previous year	139 (85.3)	141 (86.5)	141 (86.0)	129 (79.1)	0.14
ICS use – any formulation	127 (77.9)	124 (76.1)	123 (75.0)	110 (67.5)	0.03
LABA use – any formulation	133 (81.6)	128 (78.5)	133 (81.1)	113 (69.3)	0.02
ICS + LABA use	99 (60.7)	86 (52.8)	97 (59.1)	84 (51.5)	0.22
LAMA use	114 (69.9)	106 (65.0)	104 (63.4)	92 (56.4)	0.01
Pneumococcal Vaccination*	111 (68.1)	96 (58.9)	88 (53.7)	90 (59.0)	0.01

Values expressed as means ± SD or number (%). The range of total IgG levels (in g/L) is displayed for each quartile. Definition of abbreviations: BMI - body mass index, FVC - forced vital capacity, FEV₁ - forced expiratory volume in one second, ICS - inhaled corticosteroids; LABA - long-acting beta2-agonist, and LAMA - long-acting muscarinic antagonist. * Administered at the baseline evaluation.

Figure Legends

Figure E1. Frequency of participants with COPD exacerbations, recurrent exacerbations (two or more exacerbations), and hospitalizations during the study period across quartiles of serum total IgG levels in the derivation (MACRO) and validation (STATCOPE) cohorts (Q1 – lowest quartile of total IgG levels; Q4 – highest quartile of total IgG levels).

Figure E2. Serum total IgG levels according to the number of exacerbations during study period in the derivation (MACRO) and validation (STATCOPE) cohorts. MACRO: 9.7 ± 3.2 g/L for non-exacerbators vs. 9.0 ± 3.0 g/L for 1-2 exacerbations/yr vs. 8.6 ± 3.4 g/L for 3 or more exacerbations/yr; p for trend <0.001 . STATCOPE: 9.9 ± 3.1 g/L for those with no exacerbations; 9.3 ± 3.1 g/L for those with 1 or 2 exacerbations/yr; and 8.6 ± 2.7 g/L for those with 3 or more exacerbations/yr (p for trend <0.001). * $P<0.05$ for comparisons between groups (Kruskal–Wallis test with adjustments for multiple comparisons). Error bars represent 95% confidence interval for the mean.

Derivation cohort: MACRO N = 976 participants	Validation cohort: STATCOPE N = 653 participants
614 participants (62.9%) experienced at least one COPD exacerbation: • Q1: 172/244 (70.5%) • Q2: 166/244 (68.0%) • Q3: 146/244 (59.8%) • Q4: 130/244 (53.3%)	434 participants (66.5%) experienced at least one COPD exacerbation: • Q1: 119/163 (73.0%) • Q2: 113/163 (69.3%) • Q3: 113/164 (68.9%) • Q4: 89/163 (54.6%)
337 participants (34.5%) experienced two or more COPD exacerbations: • Q1: 105/244 (43.0%) • Q2: 86/244 (35.2%) • Q3: 81/244 (33.2%) • Q4: 65/244 (26.6%)	308 participants (47.2%) experienced two or more exacerbations: • Q1: 88/163 (53.9%) • Q2: 82/163 (50.6%) • Q3: 83/164 (50.3%) • Q4: 55/163 (33.7%)
208 participants (21.3%) required hospitalizations: • Q1: 71/244 (29.1%) • Q2: 50/244 (20.5%) • Q3: 48/244 (19.7%) • Q4: 39/244 (16.0%)	167 participants (25.6%) required hospitalizations: • Q1: 58/163 (35.8%) • Q2: 35/163 (21.6%) • Q3: 36/164 (21.5%) • Q4: 38/163 (23.3%)

