Study Protocol: COPD - Eosinophil-guided Reduction of Inhaled Corticosteroids (COPERNICOS)

A randomized, double-blinded, multicentre, four-arm intervention clinical trial on eosinophil-guided time-updated person-specific reduction of inhaled corticosteroid therapy and prophylactic low dose Azithromycin therapy in patients with severe or very severe chronic obstructive pulmonary disease (COPD)

Information regarding principal investigator and sites can be found in Clinical Trial Information System.

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Primary Committee

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Scientific Project Sponsor

Chronic Obstructive Pulmonary Disease Trial Network: COP:TRIN – a network of independent COPD researchers in Denmark: www.coptrin.dk

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1. Background

Chronic Obstructive Pulmonary Disease (COPD) is characterized by increased mucus production, destruction of alveoli, and constriction of the conductive airways. This causes respiratory distress, impaired lung function, and a tendency towards frequent lower respiratory tract infections. COPD is a chronic and potentially life-threatening disease affecting approximately 380 million people worldwide (1). Acute Exacerbation of COPD (AECOPD) is the leading cause of death in COPD patients and survivors often suffer from mental disabilities such as anxiety and depression with substantial negative impact on self-rated health (2, 3).

The Global Initiative for Obstructive Lung Disease (GOLD) recommends inhaled corticosteroids (ICS) for patients with COPD with frequent and/or severe exacerbations and blood eosinophils > 0.3×10^9 cells/L, and in those with ≥ $0{,}1 \times 10^9$ cells blood-eosinophils and recurrent exacerbations while on bronchodilators. The recommendations are derived from post-hoc-analyses from RCT's but yet no RCT has prospectively validated the recommendation (4, 5). Treatment with ICS is often prescribed in combination with inhaled Long-Acting Muscarinic Antagonist (LAMA) and Long-Acting $Beta_2$-Agonist (LABA). The effect of the treatment varies substantially among the patients studied, and in some cases, no improvement in the patients' condition is seen. Moreover, complications are seen, resulting from the use of ICS. Several studies have shown that treatment with ICS is associated with an increased risk of side effects such as pneumonia (6, 7), and in observational studies osteoporosis and diabetes (8, 9). Over-treatment with ICS is costly for both patients and society, associated with adverse events, and difficult to eradicate: approximately 70% of COPD patients continue to use ICS even when there is no obvious indication, and the effect is discrete or absent (10). The personal consequences of these complications for the patient have not been systematically addressed but are presumably of great importance to their quality of life.

Several clinical studies have shown that blood eosinophil count is a useful to predict treatment response to ICS in patients with COPD (11, 12). Airway inflammation plays a key role in AECOPD, which is mostly driven by neutrophilic inflammation, yet eosinophilic inflammation is the main driver in a subset of patients (13, 14). In COPD, ICS reduces the risk for AECOPD predominantly in the sub-population of with evidence of eosinophilic inflammation (15, 16). A personalized, eosinophil-guided, time-updated approach reduced systemic corticosteroid therapy during AECOPD (17). Yet, a personalized, eosinophil-guided approach to direct ICS in COPD patients with frequent AECOPDs has never been tested in a randomized trial. We wish to study this method with ICS in stable COPD to reduce ICS overtreatment and thus ICS-related adverse events.

AECOPDs can be prevented by prophylactic oral Azithromycin treatment. A small study (n=92) of showed that 12 months of Azithromycin treatment reduced the annual AECOPD among COPD patients classified as GOLD risk classes C or D, termed E in the lates GOLD version (21). A larger study (n=1142) confirmed these results; however, only 50% were in GOLD classes C or D and it is largely unknown how Azithromycin reduces exacerbation rates (22).

Long-term ICS treatment affects bacterial load in stable COPD, and lower eosinophil counts are associated with increased airway bacterial load (18, 19). Azithromycin exerts multiple effects on the structure and composition of the lower airway microbiota and has anti-inflammatory effects(20). No one has looked at the impact on the respiratory microbiota in patients receiving both treatments.

Thus, in the present four-arm facultative designed, randomized controlled, multicentre, parallel group study we have two aims: both to investigate whether an individualized and eosinophil-guided approach reduces ICS over-treatment and side effects, and whether oral low-dose prophylactic

Azithromycin 250 mg three times weekly reduces number of moderate-severe AECOPD and improves time alive and out of hospital.

2. Objectives

2.1 Clinical objectives

ICS

- Whether a strategy of eosinophil-guided[1] administration of ICS in COPD patients with GOLD class E and/or FEV_1 < 30% can lead to non-inferior[2] treatment responses to triple therapy (ICS/LABA/LAMA).
- Whether side effects[3] due to ICS exposure can be reduced with the eosinophil-guided administration of ICS.

Azithromycin

- Whether the use of prophylactic low dose Azithromycin[4] in COPD patients with GOLD risk E and/or FEV_1 < 30% can reduce moderate and severe exacerbations.

[1] Blood eosinophils are measured every 3rd month during the study. If blood-eosinophils < 0.3 x 10^9 cells/L, ICS treatment will be paused for the next 3 months. If blood-eosinophils are ≥ 0,3 x 10^9 cells/L, ICS treatment will be continued/resumed during the next 3 months.
[2] Unchanged number of COPD exacerbations or death from all cause within 365 days

[3] New diagnosis of diabetes mellitus, change in HbA1c and antibiotic requiring infections
[4] 250 mg Azithromycin three times weekly

2.2 Microbiota objectives

ICS

- Whether a strategy of eosinophil-guided administration of ICS in COPD patients with GOLD class E and/or FEV_1 < 30% leads to a change in (i) the respiratory microbiota abundance and diversity and (ii) the immunological profile in comparison to continuous triple therapy (ICS/LABA/LAMA).

Azithromycin

- Whether prophylactic low dose Azithromycin in COPD patients with GOLD class E and/or FEV_1 < 30% leads to changes in (i) the respiratory microbiota abundance and diversity and (ii) the immunological profile.

3. Hypotheses

ICS primary

- In COPD patients with GOLD class E and/or FEV_1 < 30%, an eosinophil-guided administration of ICS treatment regimen is associated with no apparent loss of efficiency[5] .

ICS secondary

- In COPD patients with GOLD class E and/or FEV_1 < 30%, eosinophil-guided administration of ICS will reduce the use of inhaled corticosteroids by > 25%[6].

[5] Measured by "admissions with COPD exacerbation or death from all causes within 365 days
[6] Measured by budesonide equivalent doses

- In COPD patients with GOLD class E and/or FEV_1 < 30%, eosinophil-guided administration of ICS will reduce the number of ICS related side effects.
- The respiratory microbiota have a composition of less "pathogenic" bacterial species in COPD patients with GOLD class E and/or FEV_1 < 30%, treated with the eosinophil-guided administration of ICS regimen compared to those treated with continuous triple therapy (ICS/LABA/LAMA) regimen.
- The complexity of the respiratory microbiota is lower among COPD patients with GOLD class E and/or FEV_1 < 30%, treated with continuous triple therapy (ICS/LABA/LAMA) regimen than among those treated with the eosinophil-guided administration of ICS regimen.
- The immunological profile of the upper airways changes in COPD patients with GOLD class E and/or FEV_1 < 30% treated with the eosinophil-guided ICS regimen compared to those treated with continuous triple therapy (ICS/LABA/LAMA) regimen.

Azithromycin primary

- In COPD patients with GOLD class E and/or FEV_1 < 30%, the use of low dose Azithromycin can result in a reduction in admissions with COPD exacerbation or death from all causes within 365 days" as compared to placebo.

Azithromycin secondary

- The difference in composition of the respiratory microbiota is substantial when COPD patients with GOLD class E and/or FEV_1 < 30% are treated with prophylactic low dose Azithromycin
- The immunological profile of the upper airways changes when COPD patients with GOLD class E and/or FEV_1 < 30% are treated with prophylactic low dose Azithromycin.

4. Methods

4.1 Study design

COPERNICOS is a 4-arm facultative designed, randomized controlled, multicentre, parallel group, double blinded, non-inferiority intervention study in participants with COPD (GOLD class E and/or FEV_1 < 30%).

Participants will be randomly allocated to one of the following four treatment groups:

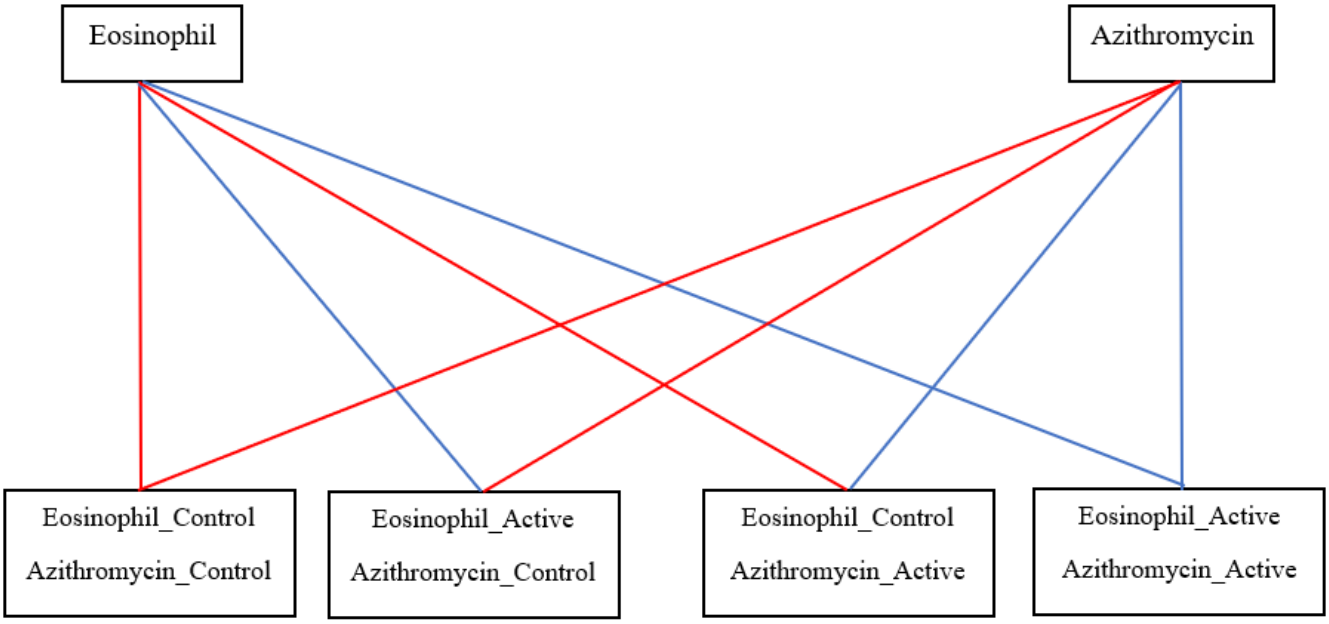

Figure 1: The four treatment groups **(Control – Active)**

1. **Eosinophil "Control"/Azithromycin "Control" group:**
   - Azithromycin: participants are given placebo
   - ICS: The participants are given the usual LAMA/LABA/ICS product in the usual dose.

2. **Eosinophil "Active"/Azithromycin "Control" group:**

- Azithromycin: placebo
- ICS: All participants will receive LABA/LAMA medication. The ICS medication will be switched on/off according to the most recent blood eosinophil count (at inclusion + every 3 months):

    i. If blood eosinophil ≥ 0.3 x 10^9 cells/L, ICS is continued in usual dose next 3 months.
    ii. If blood eosinophil < 0.3 x 10^9 cells/L, ICS is discontinued.

3. Eosinophil "Control"/Azithromycin "Active" group:

- Azithromycin: 250 mg Azithromycin three times weekly.
- ICS: The participants are given the usual LAMA/LABA/ICS product in the usual dose throughout the entire project period

4. Eosinophil "Active"/Azithromycin "Active" group:

- Azithromycin: 250 mg Azithromycin three times weekly.
- ICS: All participants will receive LABA/LAMA medication. The ICS medication will be switched on/off according to the most recent blood eosinophil count (at inclusion + every 3 months):

    i. If blood eosinophil ≥ 0.3 x 10^9 cells/L, ICS is continued in usual dose next 3 months.
    ii. If blood eosinophil < 0.3 x 10^9 cells/L, ICS is discontinued.

A microbiological and immunological study within the framework of the randomized study aims to investigate the respiratory microbiota and immunological profile in participants from the four randomized groups. A total of 40 participants from each group will participate in the sub-study and

will undergo tracheal aspirate collection and nasal swabs at baseline and 12 months follow-up. Collection of tracheal aspirates is a routine procedure at participating centres and serious complications are very rare. Nasal swab is not associated with any serious complications. Participants' data and laboratory specimens will be assigned a coded identification number to maintain participant confidentiality.

4.2 Recruitment

Participants for the trial will be recruited through advertisements and announcements on social media, in local newspapers, daily newspapers, and via the Danish Lung Association and its member magazine. Participants can contact trial staff by e-mail or telephone to receive the written participant information. Furthermore, patients at the outpatient clinic at each centre will be consecutively screened for eligibility for the trial. If a patient matches the criteria for the study, the patient will be invited to a screening meeting. The oral information session is given by a medical doctor who is duly qualified, including having knowledge of the trial to be able to provide information about it and answer any questions the subject may have. This meeting will take place in an undisturbed office.

Participants will be thoroughly screened for eligibility, and informed of the right to an assessor and companion at the first contact during an information meeting with trial staff. A 24-hour reflection period will be given to every participant, and informed consent will be obtained upon the signed consent declaration, provided that the participant will participate in the study. Only after receiving oral and written participant information, can informed consent be obtained. It is the primary investigator's responsibility at each centre to ensure that study personnel are trained, duly authorized, and competent to obtain informed consent. The consent to collect blood samples,

tracheal aspirates and nasal swabs to research biobank and future-research biobank is a separate part of the informed consent.
At recruitment health files regarding inclusion and exclusion criteria will be accessed for screening purposes before informed consent is obtained according to the Danish Health Care Act §46 (1). The following contact to possible participants will be at the next planned visit to the outpatient clinic.

During the project health files will be accessed by investigators for the following information:

- Medication
- Hospital admissions

If informed consent is obtained, the study sponsor, investigator, monitor, and the Danish Medicines Agency (Lægemiddelstyrelsen) will have access to health files, including electronic files, as part of the trial surveillance, quality control, etc.
Participants are allocated randomly via REDCap to one of the four arms; prior to randomization the inclusion and exclusion criteria will have been entered.
The azithromycin/placebo tablets are dispensed either as 500 mg tablets with a score line for splitting (patients are instructed to halve each tablet using the provided pill cutter, ***or*** as 250 mg capsules that should be swallowed whole without splitting. Patients will therefore be given very detailed instructions on the provided tablets or capsules, and on how to take the medication. The reason for having the medication in two different formulations is that the pharmacy producing the medication, for production reasons, has not been able to supply all the medication in the same formulation. Participants compliance to Azithromycin/placebo are monitored by both medicine diaries and leftover medicine returned at the 12-month examination. Blood eosinophil count will be monitored every 3 months from inclusion and participants will receive a phone call from a

nurse every 3 months to ensure that participants have taken ICS according to the physician's prescription and plan the next 3 months treatment according to protocol.

4.3 Inclusion criteria

- COPD (verified by a specialist in respiratory medicine + spirometry).
- GOLD class E anytime within the last 2 years (corresponding to 2 ≥ AECOPD and/or ≥1 AECOPD leading to hospitalization during a 12 month period within the last 2 years) and/or FEV_1<30%.
- Treatment for last 4 weeks including LAMA, LABA and ICS.
- Informed consent.

4.4 Exclusion criteria

- Known asthma.
- Male < 40 years.
- Female <40 years, if non-menopausal (had menstruation within the last 12 months) conditioned by a negative urine HCG test
- Severe mental illness which considerably complicates co-operation.
- Language problems that considerably complicate co-operation.
- Current treatment with systemic corticosteroids corresponding to > 5 mg prednisolone per day.
- Systemic antibiotic treatment (if to participate in microbiota sub-study) or systemic corticosteroid treatment within 14 days (also prophylactic Azithromycin).
- Contra-indication to treat with Azithromycin (as listed by the producer).
- Non-bacterial* exacerbation per investigator judgement in the last 3 months.

* Non-bacterial exacerbation per investigator judgement may be guided by
Exacerbation with blood eosinophil > 0.3 x 10^9 cells Exacerbation with CRP < 100 mg/L Exacerbation with chest x-ray without sign of pneumonia

4.5 Examinations

The following clinical tests will be performed during the project cf. figure 2.

	Enrollment	Inclusion (Baseline)	After 3 months	After 6 months	After 9 months	After 12 months
Informed consent	X					
Eligibility screening	X					
Blood sample		X	X	X	X	X
Tracheal aspirate and nasal swab		X				X
Lung function measurement (Spirometry)		X				X
Weight		X				X
Height		X				
COPD Assessment Test (CAT)		X				X
MRC-dyspnoea scale		X	X	X	X	X

Figure 2: An overview of examinations that each participant will undergo. The blood test can be performed either at the participant's home or at the outpatient clinic, with intervals of 3, 6, and 9 months.

Blood tests: Electrolyte parameters (sodium, potassium, albumin, creatinine, hemoglobin), liver parameters (conjugated bilirubin, ALT, alkalic phosphatase, INR, LDH), infection parameters (CRP, white blood cell differential count, thrombocytes), D-dimer and HbA1c.

Patients with COPD enrolled in the COPERNICOS clinical trial exhibit physical frailty due to the severity of their illness. Therefore, attendance at one of the trial sites in general is both demanding and stressful for the participants. To reduce the stress and to ease the access for the COPD patients to participate in the trial, the blood tests after 3, 6 9 and 12 months can be performed in the participants' home. Otherwise, they will be performed at one of the local trial sites. It's important to highlight that the full 12-month follow-up, including spirometry, will be conducted in patients' homes if they are unable to visit the trial sites. If the blood test is performed at the participants' home, it will be performed by trial staff either a doctor, a nurse, or a medical student. All staff will be trained in accordance with the current ICH-GCP guidelines. However, the investigator has the ultimate responsibility for all trial related medical decisions. The blood tests will be analyzed by the already approved laboratories for the trial. We aim to ensure that participants, needing the blood test to be taken at home more than one time, will be visited by the same trial staff to contribute to continuity for the patients. The blood tests will only be performed by doctors, nurses or medical student, who will follow all applicable and relevant guidelines and our standard operating procedures (SOP) on handling and storage of blood tests set by the Capital Region of Denmark ("Region Hovedstaden").

Questionnaires: Standardized questionnaires are used. CAT and MRC dyspnea scale are short and simple tests that provide an understanding of the severity and impact of COPD on the participant's daily life.

4.6 Data Collection

Data processing, statistical analysis and publication of the data material will be performed by a PhD student (coordinating investigator) together with health professionals (primary and secondary investigators) from the participating pulmonary medical outpatient clinics. The randomized trial started with the inclusion of participants 05JUL2021, and the last participant is expected to be

included by 31AUG2025. Data collection ceases 31AUG2026, and the project is scheduled to run until 31AUG2026.

A nurse will be responsible for collecting baseline data, performing lung function measurement, weight and height at baseline and the 12-month follow-up as well as performing blood sampling and questionnaires at three-, six- and nine-month follow-ups, according to Fig. 2. Data will be obtained via the electronic health record. Medical decisions are made by doctors only. Proper handling, storage, and delivery of medication and the primary daily project management is handled by the coordinating investigator. Recruitment, medical examinations, and distribution and accounting of medication will be assisted by primary investigators at each centre.

The data collected will be treated confidentially and only by personnel associated with the project. This includes demographic data, health status, current illnesses, medications, medicinal side effects, hospital admissions, and results from various examinations during the trial. Data will be reported in Electronic Case Report Forms (eCRF) specific for each participant. The data is encrypted, stored in online servers and protected by the Data Protection Authority through various security precautions. The physical copies of the CRF are kept in locked archives on the involved departments for 5 years.

Data in eCRF will be handled by the investigator at each centre and in accordance with the Law for Data Protection and the Danish Law for Privacy Regulation.

4.7 Replacement of Azithromycin/Placebo IMP

In the trial, the investigational medicinal product (IMP) consisting of Azithromycin/Placebo will be distributed to the participants. Some participants will receive Azithromycin/Placebo IMP with an

expiry date before the end of the 12-month study period. The data for all distributed Azithromycin/Placebo IMP, including expiry dates, are closely monitored.

Replacement will be coordinated as the expiry date of the Azithromycin/Placebo IMP approaches. In such instances, a designated member of the study team (comprising the investigator, sub-investigator, study nurse, or medical student, all operating under the investigator's supervision and responsibility) will schedule appointments with each participant whose Azithromycin/Placebo IMP is nearing expiration. These appointments will be arranged at a time when the participant is available at home for the replacement of Azithromycin/Placebo IMP. The Azithromycin/Placebo IMP will be transported by the vendor or a courier, both holding all applicable licenses and authorizations for carrying out transportation of medicine, to the participant's home. The medicine will be placed in a package that explicitly states not to open it without prior contact with a member of the study team. The medicine in the package will be delivered to the participant's home. After delivery of the new IMP to the participant's home a notice will be send to the research coordination center at Gentofte Hospital. The vendor or the courier will take the expiring IMP back to the research coordination center at Gentofte Hospital. Thereafter a member of the study team will visit the participant in the participant's home or, if deemed sufficient and safe, perform a phone call. The member of the study team will hereby give a complete instruction for the patient on how to open the new IMP package and ensure correct use of it. Furthermore, the study member will answer all questions that the participants might have. If any medical related issues or problems might appear at any time, the study member will contact the investigator. However, the new Azithromycin/Placebo IMP is almost identical to the expiring one both in look and in content. The Azithromycin/Placebo IMP does not require any specific temperature or storage measures; therefore, it will not be temperature surveilled during the transportation.

All participants in the trial will at time of inclusion get dispensed Azithromycin/Placebo IMP at the site. However, only in cases where Azithromycin/Placebo IMP is set to expiry, the above-mentioned procedure for replacement of Azithromycin/Placebo IMP will be carried out.

The replacement of the Azithromycin/Placebo IMP will be conducted directly at the participant's residence. A designated member of the study team will collect the soon-to-expire Azithromycin/Placebo IMP and document the exchange. Thereafter, the member of the study team will hand over Azithromycin/Placebo IMP with longer expiry date to the participating patient. Detailed list of the newly handed out Azithromycin/Placebo IMP will be made in accordance with the current applicable requirements including but not limited to expiry date, batch number, number of capsules, name and signature of the member of the study team handing over the new Azithromycin/Placebo IMP, study ID etc. After the replacement of the Azithromycin/Placebo IMP, the replaced Azithromycin/Placebo IMP with soon expiry date will be transported back to the research coordination center at Gentofte Hospital. The sponsor has the overall responsibility of the Azithromycin/Placebo IMP but due to the regulations in section 23(2) of the GDP Executive Order only the investigator can carry out IMP deliveries, and in this case IMP replacement. The replacement will be performed in accordance with all current data regulations.

4.8 Research Biobank

The purpose of establishing a research biobank is to investigate the frequency of ICS-induced side effects and the changes in respiratory microbiota in different treatment groups. This biobank will clarify the hypotheses and provide biological materials for future research (Providing that future

projects can obtain a separate approval from the Danish National Committee on Health Research Ethics).

The research biobank will store blood samples, tracheal aspirate samples, and nasal swabs. Tracheal aspirate and nasal swab samples are taken from 40 participants from each treatment arm, in total 160 participants. Approximately 1-5 ml of tracheal aspirate will be collected from each participant. Blood samples are taken from each participant at inclusion and at follow-up every 3 months during the 12-month study period. Approximately 210 ml of blood will be collected during the entire study period corresponding to a maximum of 36 ml per blood draw. All samples are stored in a freezer at -80 degrees Celsius. The freezers are kept in a locked room at each of the participating pulmonary medicine departments.

All samples are pseudonymized and kept for 15 years in accordance with present legislation and data protection laws. Establishment of this research biobank ends **31AUG**2027. Following the end of this study and the research biobank, all surplus biological material will be transferred to a future-research biobank. These samples will also be pseudonymized and kept for 15 years. Permission to store biological material from participants in the research biobank and future-research biobank is a part of the informed consent to participation in the project.

5. Statistical Considerations (Power Calculation)

Participants will be randomized using stratified block randomization (REDCap) to ensure equal distribution of participants at the site, age (less than 70 years vs 70 years or more) and number of hospital-requiring exacerbations within the past 12 months (0-1 vs 2 or more).

The trial will have a target of 444 participants, , included in the project within 48 months, and 111 participants will be allocated to each of the four groups to be examined.

Two sample size calculations have been conducted, one for ICS and one for azithromycin, as there are two interventions being tested (factorial design):

A non-inferiority (1-sided) test for ICS.
Type 1 error limit (α) 2.5%, Power (1-β) of 80%. (α = 0.025, β = 0.2)
Control group exacerbation/death: 0.3 (30%)
Expected maximum worsening >11% absolute (up to 0.41)
Sample size 444 (222 x 2)

A 1-sided superiority test for azithromycin.
Type 1 error limit (α) 2.5%, Power (1-β) of 80%. (α = 0.025, β = 0.2)
Control group exacerbation/death: 0.3 (30%)
Expected effect: >10% absolute (up to 0.2)
Sample size 444 (222 x 2)

These two interventions will be published separately in two primary publications, but there will be adjustments in the main analysis to account for whether they received the other intervention. These two analysis teams (separate) will be blinded to an effect estimate of the other intervention.

40 participants from each group will participate in the microbiota sub-study.

Scheduled analyses of data (Interim analysis) will be assessed when half of the participants are recruited. These assessments will be made by the independent Data and Safety Monitoring Board (DSMB) on the two primary endpoints, and for the secondary endpoint “Death or “uncontrolled AECOPD tendency” within 365 days”. Data on primary and secondary power targets will be used for this purpose. DSMB will review the protocol, monitor the guideline, evaluate the trial concerning the recruitment of participants, participants' risk and, based on interim analyses, make recommendations to the investigators on whether to continue or cease the study. DSMB may at any time require an extraordinary interim analysis

5.1 Primary Endpoints

ICS and Azithromycin

The primary outcome targets admissions and death within 365 days of randomization. This is assessed in the following analysis:

- Number of hospitalization-requiring COPD exacerbations and/or death within 365 days (continuous data: analysis with chi squared test)

5.2 Secondary Endpoints

ICS and Azithromycin

- "Days alive and out of hospital within 365 days after recruitment" (continuous data: analysis with T-test)Death or "uncontrolled AECOPD tendency"[7] within 365 days.
- Number of moderate/severe exacerbation within 365 days
- Cumulative dose of inhaled corticosteroids within 365 days
- Cumulative dose of systemic corticosteroids within 365 days
- Change in lung function (ΔFEV1) from baseline to 365 days

5.3 Exploratory

ICS

- Change in blood eosinophils from baseline to 365 days (eosinophilic trajectories)

[7] ≥3 exacerbations within 12 months

- New diagnosis of diabetes mellitus within 365 days
- Change in HbA1c from baseline to 365 days
- Antibiotic-requiring infections within 365 days

ICS and Azithromycin

- Difference in respiratory microbiota abundance and diversity from baseline to 12 months between treatment arms
- Difference in immunological profile including cytokines and chemokines in the upper airways from baseline to 12 months between treatment arms
- Change in COPD-related quality of life (Based on COPD Assessment Test - CAT) from baseline to 365 days
- Number who progress to MRC -dyspnea score from < 3 to ≥3 anytime during follow-up (assessed every 3 months).
- Number of admission requiring non-invasive ventilation (NIV) treatment or admissions to intensive care within 365 days
- Mortality within 365 days

5.3 Data analysis

The four treatment groups will be compared in terms of endpoints at inclusion and follow-up visits every 3 months from trial inclusion with standard statistical tests such as t-test (Dichotomous outcomes), chi-squared test, Fisher's exact test, and time-to-event analyses.

Recurrence of AECOPD, the period between index AECOPD and the next AECOPD, and number of admission-requiring NIV treatment within 365 days are analyzed using Fisher's exact test or Chi-

squared test. Time to AECOPD within 365 days and number of hospitalization-requiring exacerbations within 365 days will be analyzed with t-test and Cox proportional-hazards regression model (unadjusted and adjusted). Mortality rate within 365 days will be analyzed with Chi-squared test or Fisher's exact test (unadjusted) and with Cox proportional-hazards regression model (adjusted).

New onset of diabetes during the study period or occurrence of antibiotic-requiring infections within 365 days will be analyzed using Fisher's exact test or Chi-squared test. Changes in lung function, health-related quality-of-life (CAT), and level of dyspnea (MRC) will be analyzed using analysis of variance (ANOVA). The cumulative ICS dose will be analyzed as mean total cumulative dose from recruitment to end of the study period using t-test, Wilcoxon signed-rank test or Mann-Whitney U test.

6. Risks and Side Effects

Possible side effects from the use of ICS (Beclometasondiproprionat) according to pro.medicin.dk are:

Common (1-10%): Pharyngitis, Coughing, Hoarseness, Headache, Candidiasis.

Uncommon (0.1-1%): Blurry vision, Dizziness, Tremor.

Rare (0.01-0.1%): Allergic reactions, Angioedema, Bronchospasm.

Very rare (<0.01%): Adrenal insufficiency. Eosinophilic pneumonia. Erythema, purpura. Decrease in bone mineral density. Glaucoma, cataract. Growth retardation.

Unknown prevalence: Disturbed behavior, Aggressiveness, Anxiety, Depression, Hallucinations, Hyperactivity, Hypercorticism.

Possible side effects from the use of Azithromycin according to pro.medicin.dk are:

Very common (>10%): Abdominal pain, diarrhea, flatulence, nausea.

Common (1-10%): Tiredness or weakness. Decreased appetite. Vomiting, taste perversion. Decreased lymphocyte count. Decreased serum bicarbonate. Arthralgia. Headaches, Paresthesia, Dizziness. Skin itching, rash. Visual disturbances.

Uncommon (0.1-1%): Pain. Hepatitis, Oral candidiasis. Dyspnea, Pneumonia, Edema. Eosinophilia, Leukopenia, Neutropenia. Elevated serum bicarbonate, Hyperchloremia, Hyperglycemia, Hyperkaliemia, Hypernatremia, Hypokalemia, Hyponatremia. Arthritis, Back Pain. Nervousness, Somnolence. Facial oedema, Dermatitis, Photosensitivity. Candidiasis, Infections. Metrorrhagia, Kidney Pain, Testicular Disease, Vaginitis. Hearing loss, Tinnitus.

Rare (0.01-0.1%): Cholestasis, Liver Impact. Agitation. Acute generalized exanthemata, pustulosis. Allergic reactions, Angioedema, Hypersensitivity.

Unknown prevalence: Fulminant hepatitis, Hepatotoxicity, Hepatic insufficiency, Pancreatitis, Pseudomembranous colitis. Arrhythmias, Extended QT interval, Hypotension, Torsades de pointes tachycardia. Hemolytic anaemia, Thrombocytopenia. Aggravated myasthenia gravis. Aggressiveness, Anxiety, Delirium, Hallucinations, Hyperactivity, Hypesthesia, Seizures, Syncope. DRESS - drug reaction with eosinophilia and systemic symptoms, Erythema multiforme, Stevens-Johnson syndrome, toxic epidermal necrolysis. Anaphylactic reaction. Acute renal failure, Interstitial nephritis.

Sample collection

When drawing a blood sample, there is a small risk of infection, slight discoloration at the puncture site, and transient pain/discomfort. Tracheal aspirate collection is associated with a small risk of minimal bleeding, infection, temporary breathing difficulties, and a low blood oxygen level during the examination. There are no known risks associated with nasosorption sample collection.

Documentation and reporting of adverse events

In the present study, the IMPs are not under stricter reporting requirements as per DKMA list available at webpage (https://laegemiddelstyrelsen.dk/en/sideeffects/side-effects-of-medicines/medicines-with-stricter-reporting-requirements/). Furthermore, inhaled corticosteroids (ICS) and azithromycin have extensive market presence, in the specified patient population, both with well-established safety profiles. In addition, the morbidity and mortality within this clinical trial's patient group is very high. Therefore, a risk-based safety reporting will be conducted, meaning only SUSARs will be expedited reported from the investigator to sponsor

All severe adverse events (SAE) will be documented in the electronic case report forms (eCRFs) as soon as the investigators are made aware of the events. Every 3 months at study follow-up visits, the study participants will be asked about SAEs, and the electronic patient files will be assessed for events since last visit. Participants are also advised to contact the investigators during the trial in case they experience an adverse event. Investigators will then immediately assess whether a

causal association to the study drugs is suspected. If the SAE – in the opinion of the investigator - exhibits potential causality with the study drugs, the event will be considered a serious adverse reaction (SAR). This will also be documented in the eCRFs. The investigators will then be prompted to assess if the SAR is expected. If the event is unexpected, as outlined above in the Summary of Product Characteristics for ICS and azithromycin, the investigators will be prompted to report immediately to the sponsor, as this will be considered a SUSAR. SUSARs must reported to sponsor within 24 hours after the study site have been aware of the event, by email or telephone. An immediate report ensures a duly and timely report to the EudraVigilance system if it is deemed to be a SUSAR. In addition to this, the investigator will be able to report any SAE immediately at their own discretion.

The sponsor will ensure prompt registration and reporting through the EudraVigilance system of all information regarding eligible SUSARs that are fatal or life-threatening, no later than 7 days after the sponsor becomes aware. Within 8 days of filing the report, the sponsor must update the EudraVigilance system including all pertinent details regarding the sponsor's and investigator's follow-up on the report. Test managers at other participating centers must be promptly informed of these SUSARs.

SAEs and SARs that do not qualify as SUSARs will not be reported immediately from investigators to sponsor, however these events will all constitute either a primary or secondary outcome according the protocol, and therefore will be registered by investigators in the eCRF. As described previously sponsor will be notified at every 3 months corresponding to study follow-up visits, where the participants will be inquired about events. At any time, the sponsor will be able to assess all registered SAE/SARs via the eCRF.

Events will be considered exclusively within the trial period, encompassing participant enrollment from screening to the last visit day. An annual safety report (ASR) of all SARs and SUSARs occurring during the trial period along with a description of the rules of registration and reporting of adverse events as given in this protocol will be submitted to the Clinical Trials Information System (CTIS). All SAEs, SARs, SUSARs will be reported in the final report to the CTIS at the conclusion of the trial.

7. Exclusion from or interruption of the trial

Participants may at any time terminate the study if the physician responsible for the study deems it necessary with medical justification (allergy to the medicine, safety risk, or other adverse circumstances). The investigator can furthermore at all hours unblind the Azithromycin/placebo treatment by contacting Glostrup Apotek. Termination and unblinding should preferably be done in agreement with the coordinating investigator of the project. The participant in question will be informed immediately of health concerns, project termination, and future treatment plan. Additionally, participants may, at any time, withdraw their informed consent. This will not have any consequence on further treatment.

8. Economy

The initiative for the trial was taken by the steering committee of COP:TRIN (Chronic Obstructive Pulmonary Disease: Trial Network) and the departments of pulmonary medicine at Gentofte Hospital, Herlev Hospital, Hvidovre Hospital, Bispebjerg Hospital, North Zealand Hospital, and Aalborg Hospital. The project budget is approximately DKK 7.433.600. This amount will cover salary to researchers, supervisors, nurses and auxiliary staff, the cost of data collection, equipment, medication, laboratory analyses, diagnostic tests, and potential hospitalizations.

Funding has been sought from various foundations, including Region H Research Fund. The Scientific Ethics Committee and the trial participants will be informed of the sponsors, their financial contribution and its part in the project, and information on grant recipient (Whether the support is paid directly to the investigators or their department/institute).

The Novo Nordisk Foundation has supported the initiative with 4.6 million DKK by a grant to Jens-Ulrik Stæhr Jensen.

The investigators have no economic interests in the research project in question and are not financially linked to private companies, foundations etc. with economic interests in the study.

8.1 Remuneration

Participants will not receive remuneration for project participation, but the cost of Azithromycin/placebo treatment during the trial period will be covered by the sponsor.

9. Publications of Trial Results

The trial is a part of a PhD thesis. The results from the trial will be published regardless of whether they are positive, negative, or inconclusive. The trial will be registered and published at clinicaltrials.gov, clinicaltrialsregister.eu as well as published in an international peer-reviewed scientific journal and in The Journal of the Danish Medical Association. At least one publication in a high impact scientific journal is expected. If publication in a scientific journal is not possible, the results will be published as an online report. In addition, a summary of the results will be submitted

to the CTIS and the Clinical Trials database (www.clinicaltrials.gov) as soon as possible and no later than one year after the trial has ended.

10. Scientific Perspective

AECOPD are often accompanied by markedly reduced lung function and increased likelihood of progression of COPD and mortality. In many cases, the illness progression following an exacerbation is irreversible. Therefore, the everyday life of COPD patients is often characterized by fear and anxiety of these acute exacerbations. In addition to the risk of exacerbations, the risk of side effects from the medical treatment of COPDoften creates additional concerns for COPD patients. Corticosteroids in the dosages given both for maintenance and for AECOPD carry high risks for the patient: dysregulation of glucose hemostasis (17), high risk of pneumonia and other infections (23, 24), and emergence or worsening of osteoporosis and the consequent fractures (8). Such side effects can lead to a vicious cycle of anxiety, physical deconditioning, muscle wasting, loss of social life, increased risk of further AECOPD, more corticosteroids, and again more side effects. We believe that this trial has the potential to impact the current COPD guidelines seeing that it can provide relevant information for the development of a treatment plan that has the potential to reduce the usage of ICS by 80%[8], minimize ICS-induced complications and increase the patients' quality of life.

As for Azithromycin, little is known about the mechanism of its effect. Only one large, randomized trial has been performed patients with severe COPD (50% had an exacerbation in the previous year) (22). We aim to determine how prophylactic long-term Azithromycin treatment alters airway inflammation and the respiratory microbiota, and whether Azithromycin can reduce exacerbation rates in COPD patients with GOLD class E and/or FEV_1 < 30%.

[8] 20% of COPD patients in class C/D have blood eosinophil counts ≥ 0,3 x 10^9 cells/L.

We recognize the importance of understanding individual differences in clinical response, biological information, and microbiota changes in response to combined medication. We hope to provide an in-depth understanding of key biological and physiological mechanisms that lead to AECOPD with this study. With a better understanding of the interplay between microbiota changes and therapeutic influences on this by ICS and Azithromycin, the treatment of COPD symptoms can be continuously reevaluated and adapted to each patient.

11. Research Ethical Statement

The research project will be carried out in accordance with the Helsinki Declaration, the Danish Data Protection Act, the Danish Health Act, and the General Data Protection Regulation (EU) 2016/679 (GDPR).

The study is approved through the CTIS and will be conducted in compliance the Clinical Trials Regulation (EU) No 536/2014 (CTR) and with the principles of good clinical practice, as this statement a requirement under the CTR.

The study will be registered in the US Clinical Trials database (www.clinicaltrials.gov), which is based on guidelines defined by The Food and Drug Administration.

Since study participants will not be exposed to irresponsible risks and can greatly benefit from the study's results and participation in the study, we believe that the study is scientifically and ethically sound.

12. Informed Consent

Participation in the trial is voluntary, and participants will not receive any form of financial compensation for participation. . Participants are protected under the Personal Data Processing Act, and the study is reported to the Regional Science Ethics Committee (VEK), the Danish Medicines Agency (Lægemiddelstyrelsen), and the Danish Data Protection Agency (Videnscenter for Dataanmeldelser).

13. Compensation and Reimbursement Schemes

The trial is covered by the patient compensation scheme. Participants can apply for compensation in accordance with Statutory Order No. 1113 of 7 November 2011 on the right of appeal and compensation in the healthcare system if participants experience unexpected damage during the trial or at inclusion.

14. Authorship Criteria

This study investigates two interventions: Eosinophil-guided administration of inhaled corticosteroids treatment regimen and prophylactic treatment with low dose Azithromycin

respectively. Both interventions are planned to result in one main publication each. In addition to these, the microbiota sub-study is also planned to result in a main publication.

In addition to the main publications a number of secondary publications are planned, but are not further dealt with in this section.

Authorships to one of the main publications are granted to a person who have made a substantive contribution. A substantive contribution includes one or more of the following:

- design or conceptualization of the study[9], or
- major role in the acquisition of data[10], or
- analysis or interpretation of the data[11], or
- drafting or revising the manuscript[12].

All authors approve the final version to be published.

[9] Protocol drafting and major protocol revising along application for national ethics committee and national health authority approval.
[10] Inclusion of 10 patients at a centre is considered a major role in the acquisition of data and entitles one authorship for every 10 patients included at the same centre. It is the responsibility of the primary investigators at each centre, that authorship(s) at the centre are granted to the contributing investigator(s). Also, coordinating across centres (coordinating investigator) is considered a major role in the acquisition of data and entitles one authorship.
[11] Data analysis and interpretation are conducted by DMSB, sponsor, coordinating investigator and the writing group.
[12] The writing group is constituted after data acquisition has been completed, but will at a minimum consist of the sponsor, coordinating investigator and primary investigators of sites which have recruited a minimum of 10 patients.

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