SAP for Cod Liver Oil for COVID-19 Prevention Study

Skriv inn tekst her

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Abbreviations

Abbreviation	Full text
BMI	Body mass index
CLOC	Cod Liver Oil for COVID-19 Prevention Study
ITT	Intention to treat
Koronastudien	The Norwegian Corona Cohort
MSIS	Norwegian Surveillance System for Communicable Diseases
PCR	Polymerase chain reaction
SAP	Statistical Analysis Plan

1. Administrative Information

1.1 Title and trial registration

A randomized, parallel-group treatment, participant, investigator, care provider and outcomes assessor masked, two-arm study to assess the effectiveness of cod liver oil compared to placebo in the prevention of COVID-19 and airway infections in healthy adults

Short: Cod Liver Oil for COVID-19 Prevention (CLOC) Study

1.2 SAP version

SAP version 1.0 - 23-November-2021

1.3 Protocol version

Cod Liver Oil for C-19 Prevention Study, protocol version 1.02, November 10, 2021

1.4 SAP revisions

Version No. – Date	Justification	
0.1 – 3-Jun-2021	Initial draft	Pre-analysis
0.9 – 12-Aug-2021	Second draft	Pre-analysis
0.99 – 24-Aug-2021	Draft for approval	Pre-analysis
1.0 – 24-Nov-2021	SAP approved	Pre-analysis
1.1 – 10. Des-2021	Amendment 1	Pre-analysis

Amendment 1 (December 10, 2021)

Added the time period 'from one week after the start of cod liver/placebo taking to the end of the study period' to primary endpoint 1-4 in accordance to the protocol.

1.5 Roles and responsibility

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1.6 Signatures of:

Senior statistician responsible: Trond Haider, Cand. real.

Signature: Trond Haider

Date signed: 13. Dec. 2021

Chief investigator/clinical lead: Arne Søraas, MD, PhD

Signature: _____

Date signed: 13. Dec 2021

Responsible researcher: Sonja Hjellegjerde Brunvoll, PhD

Signature: _____

Date signed: 13. Dec 2021

Data Scientist: Anders Benteson Nygaard, PhD

Signature: _____

Date signed: 13. dec. 2021

2. Introduction

2.1 Background and rationale

Preliminary evidence from literature and an ongoing study (the Norwegian Corona Cohort, Koronastudien) in our lab suggests that cod liver oil may prevent COVID-19 and complications of COVID-19. In this Cod Liver Oil for COVID-19 Prevention (CLOC) study, we will investigate whether this is actually the case by randomizing volunteers to take cod liver oil or placebo (corn oil) during the winter months of 2020-2021.

2.2 Objectives

Based on the encouraging results from Koronastudien[1] as well as existing evidence from the literature, we aim to investigate whether cod liver oil can prevent COVID-19, serious COVID-19, or other airway infections.

Adverse events will also be presented.

We also aim to explore and confirm already known health effects and possible new health effects in particular rare adverse events associated with cod liver oil use. This will be done during a follow-up period of up to two years after the end of the cod liver oil period.

3. Study Methods

3.1 Trial design

The CLOC study aim to include 80,000 participants that will be randomized in a 1:1 ratio to consume 5 ml cod liver oil or placebo (corn oil) with lemon taste per day for 6 months.

The study was organized as an ancillary study of Koronastudien, a cohort study that aims to identify risk factors associated with the community- and workplace acquisition of COVID-19 virus and which also aims to identify risk factors for the progression of the

[1] In Koronastudien, the Norwegian Corona Cohort, 140.000 participants have answered a questionnaire about their use of dietary supplements, demographics, airway symptoms, and risk factors for Covid-19. In univariate analyses, it was found that participants using vitamin D and or cod liver oil were associated with a reduced risk of Covid-19 (OR 0.8, p<0.001) and of serious (hospitalized) Covid-19 with hospitalization (OR 0.3, p=0.07).

disease and to understand the virus and the disease itself. Koronastudien has collected extensive electronic questionnaire data using the University of Oslo web-based solution "Nettskjema". Participants of the CLOC study will be a subgroup of participants in Koronastudien, and relevant data from these participants will be shared among the studies.

The hypotheses are:

Intake of cod liver oil can prevent COVID-19 disease
Intake of cod liver oil can prevent serious COVID-19
Intake of cod liver oil can prevent other airway infections

These hypotheses will be tested in a triple-blinded randomized placebo-controlled study where 80.000 participants will be assigned to cod liver oil or placebo in a 1:1 ratio.

The safety of cod liver oil will be explored.

Finally, it is a separate aim of the study to identify the mechanisms of action of any health effects seen. These will be explored under another SAP.

3.2 Randomization

Willing and eligible participants will be randomly assigned to one of two treatment groups: 5 ml of cod liver oil x1 per day or placebo together with the first meal each day. Participants will be assigned to the cod liver oil or placebo group in a 1:1 ratio.

Concealment of allocation: Randomization will be conducted at the Department of Research Support, Oslo University Hospital, by personnel not involved in the study. The list of participants with address and treatment assignment will be provided to the packaging company, which will send cod liver oil or placebo for the whole study period to each participant based on that list. The responsible personnel at the packaging company will not be involved in the study, and no personnel involved in the study will have access to this list.

The number of participants to be randomized: In the case of successful recruitment of an excess number of participants, participants belonging to high-risk groups (> 70 years old and dark-skinned) will be prioritized for randomization. The remaining non-randomized participants will be included as an extra control group that will not receive any treatment but will otherwise be followed as the other participants and analysed for the exploratory endpoints.

Previous use of vitamin D

Caucasians using cod liver oil or an equivalent supplement (containing vitamin D) less frequently than 5 days per week can be included in the study. In addition, dark-skinned individuals may be included regardless of previous use of vitamin D (and cod liver oil). We consider the risk of overdosing of vitamin D to be very low in this group and while the group will contribute less to the total power of the study than participants not using such supplements their intake will increase during the study period and they will contribute to the study. Based on data from Koronastudien we expect less than 20-30% such participants.

3.3 Sample size

The fallback method (https://www.fda.gov/media/102657/download) will be used to correct for testing of multiple primary endpoints.

The total alpha (0.05) will be divided among the endpoints, and a fixed sequence for the testing will be maintained. As the testing sequence progresses, a successful test preserves its assigned alpha as "saved" (unused) alpha that is passed along to the next

test in the sequence. This passed-along alpha is added to the assigned alpha of the next endpoint, and the summed alpha is used for testing that endpoint. Thus, as sequential tests are successful, the alpha accumulates for the endpoints later in the sequence; these endpoints are then tested with progressively larger alphas.

Endpoints in testing order with power calculation:

1. Serious COVID-19 (MSIS-confirmed COVID-19 with dyspnoea, hospitalization, or death). (MSIS: Mandatory Norwegian Surveillance System for Communicable Diseases). Assigned α=0.018.

   Based on the observations in Koronastudien of a 40% reduction in serious COVID-19 in the group reporting use of cod liver oil, and an expected incidence of 0.25%, a power of 70%, 67,000 participants will have to be included for this endpoint.

2. COVID-19 positive PCR (MSIS). Assigned α=0.03.

   Based on the observations in Koronastudien of a 20% reduction in COVID-19 incidence in the group reporting use of cod liver oil, and an expected incidence of 1%, a power of 70%, 65,000 participants will have to be included for this endpoint.

3. A negative SARS-CoV-2 test (MSIS). Assigned α=0.001.
   COVID tests (SARS-CoV2-2), real time (RT)-PCR, are mostly obtained because of a suspicion of COVID-19. The number of negative COVID tests from one week after starting taking cod liver oil/placebo and to the end of the intervention period. This endpoint has been added as an alternative to the Positive PCR or bacterial culture endpoint (now a secondary endpoint, as it is not available at this time).
4. Self-reported airway infection. Assigned α=0.001.

   Based on the expected frequency of airway infections in Koronastudien of >30% and as a threshold of clinically interesting reduction of all airway infections of 10%, 23,000 participants will have to be included for this endpoint. The study has a power for this endpoint >95%. 16,000 participants in the study will give an 80% power for this endpoint.

The power of the study is dramatically influenced by the incidence of COVID-19, and this will be considered when the statistical analysis plan is finalized before unblinding to maximize the scientific output of the study. Thus, the statistical analyses including p-values and testing-order above can be changed if the COVID-19 situation dictates such changes. Any change will be done before unblinding. The time of unblinding can be changed based on the prevalence of COVID-19 to increase the scientific output from the study.

3.4 Framework

The background for the study is that data suggest that cod liver oil may prevent COVID-19 and complications of COVID-19. Hence, this is a superiority study, placebo-controlled, blinded and randomised, designed to show that cod liver oil has a positive effect on COVID-19 and complications of COVID-19, compared to corn oil, the placebo.

3.5 Statistical interim analyses and stopping guidance

No interim analyses were planned in this study. The study's duration has been specified to last during the winter months of 2020–2021, with a duration from 1–6+ months for the participants.

3.6 Timing of final analysis

All primary variables will be analysed after ending the intervention part of the present study. Analyses of the four primary variables will be conducted immediately pursuant to the approval of this SAP. Further analysis of the secondary variables will be specified in another SAP.

3.7 Timing of outcome assessments

Outcome assessment

Time points at which the outcomes are measured including visit "windows"

4. Statistical Principles

4.1 Confidence intervals and *P* values

An overall significance level of 0.05 is used for the composite primary endpoints and are tested in the specified order as seen in section 3.3, above. To control the overall significance level pertaining to the primary hypotheses, the fallback method for alpha spending is used for the statistical analyses of relative risk comparing the two randomisation groups, Cod liver oil and Corn oil. If the first analysis results in significant results, i.e., $p \leq 0.018$, according to section 3.3, the second null-hypothesis can be tested at a significance level of 0.018+0.030=0.048. If the first null hypothesis is not rejected, the second is tested at the stated significance level of 0.03. Each following null hypothesis to be tested has a significance level of its stated level plus the sum of the levels of previous tests that result in a significant result. Supplementary analyses, such as regression analyses, will not be part of the fallback method, as these are expected to be highly correlated to the primary analyses. Confidence interval and p-values will be provided for all variables tested statistically.

4.2 Adherence and protocol deviations

The Intention-to-treat (ITT) population will be used for statistical testing of the primary variables. Hence, anyone who received bottles of cod liver/corn oil will participate in the primary variables' analyses. As a number of bottle shipments were for several reasons returned to sender, such as wrong address, address in another country or did not collect the bottles at the post office despite several urgings, the ITT population is defined as those who had the choice to start taking cod liver/corn oil. Primary variable statistics will also be presented for all who were sent cod liver/corn oils. Participants with missing data on primary variables will be considered as having a negative outcome. Supplementary analyses will be conducted on participants who have reported on the primary variables and on taking oil at given fractions, to be specified when analysing the data, of "the last fortnight".

5. Trial Population

5.1 Screening data

Data from the first reported questionnaire will be considered as baseline wherever such values are considered necessary.

5.2 Eligibility

All participants invited to this study are considered eligible.

5.3 Recruitment

Information to be included in the CONSORT flow diagram.

5.4 Withdrawal/follow-up

The level of reported oil consumption per time unit and overall will be presented. The number of participants with last reported data will be presented for each data collection time-point.

5.5 Baseline patient characteristics

The following baseline variables[2] will be presented for each treatment group and overall:

- Age group
- Gender
- Weight
- Height
- BMI
- Occupational status
- Education level
- Household income
- Household count
- Children living at home
- Smoking habits
- Chronic disease (Yes/No)
- Parents ethnic origin
- Skin type
- Sun exposure (July to October 2020)
- Dietary intake:
    - Vitamin D supplement
    - Omega-3 supplement
    - Cod liver oil
    - Fatty fish intake

Follow-up variables:

- Compliance
- Airways infections
- SARS-CoV-2-tested (negative and positive)
- Dyspnoea (among COVID positive participants)
- Side effects (counts (%)), self-reported free-text fields

Lab

- Vitamin D (baseline and change from baseline)
- Omega-3 index (baseline and change from baseline)
- Baseline serology

MSIS data:

- Positive and negative test results

[2] The specific SAS variable names in the received data set are presented in Appendix 1.

For continuous baseline variables N, mean, standard deviation, median, minima and maxima will be presented. For categorical variables, N and Per cent will be presented for each oil group.

6. Analysis

6.1 Outcome definitions

Primary variables:

- First primary: Serious COVID-19 (MSIS-confirmed COVID-19 with dyspnoea, hospitalisation, or death)
- Second primary: COVID-19 defined by positive PCR obtained from MSIS
- Third primary: A negative SARS-CoV-2 test (MSIS).
- Fourth primary: Self-reported airways infection

The first incidence of report of any of the above primary variables will be used in the analyses. The specified order will be used for the fallback analyses. The values of all variables will be Yes/No to the condition together with time from one week after start of oil consumption to first reporting of the condition, i.e. Yes (except for third primary variable, where total number of negative tests is reported). Those not reporting a Yes answer, the last reported time will be used as censoring time. The date from one week after oil-consumption starts to the date of reporting of a Yes will be converted to number of days from the first to the second date.

The primary analysis set is the defined ITT sample with missing data on outcome counting as the negative outcome.

Compliance

For sensitivity analyses on compliance, we will define compliant participants as those having taken CLO for at least 2 months. We will also analyse participants based on volume CLO taken (more than 250 ml).

6.2 Analysis methods

The first, second and fourth primary variables will be analysed for relative risk (risk ratio) of acquiring a condition using cod liver oil versus corn oil using the Wald test[3]. Time to first occurrence of the primary variables will be plotted using the Kaplan-Meier approach. The third primary variable will be compared for the two treatment groups tested using study duration-adjusted counts. If the use of student's t-test cannot be used due to underlying assumptions not holding up, a median test will be used. If appropriate, the Cox proportional hazard regression will be used to assess the effect of the outcome of the above specified baseline variables on time to occurrence of the primary variables. Otherwise, logistic regression on the occurrence of outcome will be undertaken with the same explanatory variables. The explanatory variables to be included in the main regression analyses are:

- Age at CLOC screening
- Gender
- BMI

[3] © SAS Institute Inc. https://documentation.sas.com/doc/no/pgmsascdc/v_016/statug/statug_freq_details70.htm, 18-Aug-2021

- Occupational status (employed/not employed?)
- Children at home (Y/N)
- Chronic disease (Y/N ≥1 chronic diseases)
- Dietary intake (Y/N)
    - Cod liver oil
    - Vitamin D
    - Omega 3
    - Fish consumers
- Present smoker (Y/N)

Other variables will be included in ad hoc/exploratory analyses.

The sample size in this study is so large that normal assumptions are likely to be valid. If this should not be appropriate for specific variables, appropriate non-parametric alternatives will be used. Should the assumptions to use Cox proportional hazard model not be satisfied, an alternative approach is logistic regression. The assumptions for methods used will be assessed. Should they be violated and not rectified by the sample size, alternative approaches will be used.

Sensitivity analyses will be conducted by including subsets of the study sample based on compliance. Other baseline variables may also be proposed as basis for ad hoc sensitivity analyses. Overdispersion will be considered for models where that can be an issue.

Further ad hoc subgroup analyses may be performed based on the findings of the primary analyses.

6.3 Missing data

The primary variables will be assumed to be negative, A "No" answer, if no positive answer is reported.

6.4 Harms

Adverse events will be presented for each treatment group, at each reporting time and accumulated over the whole study duration from start of oil consumption.

Data will be summarized in tables of means and medians for continuous variables and tables of counts for discrete variables. Sufficient detail on summarizing safety data, e.g., information on severity, expectedness, and causality; details of how adverse events are coded or categorized; how adverse event data will be analysed, i.e., grade 3/4 only, incidence case analysis, intervention emergent analysis

6.5 Statistical software

SAS© version 9.4 will be used to analyse the data.

6.6 References

1. Study protocol: Arne Søraas – Principal Investigator, *Protocol for the Cod Liver Oil for Covid-19 Prevention study.* Oslo University Hospital, Department of Microbiology, Section of Research, Oslo, Norway

Appendix 1: SAS variable names used in the received data set

The table below presents the SAS variable names as they appear in the original SAS data set received.

Variables	SAS variable names
Age group	
Gender	
Weight	
Height	
BMI	
Occupational status	
Education level	
Household income	
Household count	
Children living at home	
Smoking habits	
Chronic disease (Yes/No)	
Parents ethnic origin	
Skin type	
Sun exposure (July to October 2020)	
Dietary intake (Yes/No):	
Vitamin D supplement	
Omega-3 supplement	
Cod liver oil	
Fatty fish intake	
Compliance	
Airways infections	
SARS-CoV-2-tested	
Self-reported free-text side effects	
Laboratory measurements:	
Vitamin D	
Omega-3 index	
Baseline serology	

Primary variables:	
1. Serious COVID-19 (MSIS-confirmed COVID-19 with Dyspnoea, hospitalisation, or death)	*CLOC_primary_1_severe_c19*
2. COVID-19 defined by positive PCR obtained from MSIS	*CLOC_primary_2_msis_status*
3. A negative MSIS SARS-CoV-2 test	*CLOC_primary_3_negative_tests*
4. Self-reported airways infection	*CLOC_primary_4_selfrep_airway*

Description of the calculation of the four primary variables

- **First primary:** Serious COVID
  - *CLOC_primary_1_severe_c19*
    - Created by merging *deriv_sym_dyspnea_post_cov_yn_ever* and *deriv_hosp_post_cov_yn_ever* (see description below).
    - 1 = ever answered yes for dysp or hosp
    - 0 = only answered no for dysp or hosp
    - 999 = has filled out forms, has not answered for dysp or hosp question
    - NA = has not filled out forms / not tested positive (second primary)
  - Only participants fulfilling the criteria for the second primary, will be included in this analysis. In addition, participants who have tested positive within one week after start of oil consumption are excluded from the analysis (*exclude_MSIS_transtart7d*).
  - Dyspnoea criteria
    - Variable: *deriv_sym_dyspnea_post_cov_yn_ever*
      - Answered dyspnoea in forms (R1-R6, E2, Q1) submitted after positive cov test (MSIS)
      - 1 = ever answered yes in any form
      - 0 = only answered no in any form
      - 999 = has filled out form, has not answered dypnoea question
      - NA = has not filled out form
  - Hospitalized criteria
    - Variable: *deriv_hosp_post_cov_yn_ever*
      - Answered hospitalized because of COVID in forms (B1, B2, R1-R6, E2) submitted after positive cov test (MSIS)
      - 1 = ever answered yes in any form
      - 0 = only answered no in any form
      - 999 = has filled out form, has not answered hospitalized question
      - NA = has not filled out form
  - Death criteria
    - Participant registered as dead/deceased in the National Population Register after a positive cov test (MSIS), but only if the death was related to COVID-19 based on hospital records or information from relatives.

- **Second primary:** New MSIS positive

- *CLOC_primary_2_msis_status*
  - Created from *MSIS_CLOC_test_status*
  - 1 = tested positive for the first time during **CLOC participation period**
  - 2 = only tested negative during **CLOC participation period** (never positive)
  - 3 = untested during **CLOC participation period** (never positive)
- Participants who have tested positive within one week after start of oil consumption are excluded from the analysis (*exclude_MSIS_transtart7d*).
- MSIS criteria
  - Variable: *MSIS_CLOC_test_status*
    - 1 = tested positive for the first time during CLOC
    - 2 = only tested neg during CLOC (never positive)
  - *Variables obtained through MSIS (translated): "Test date" and "Test result"*
  - Variable to be analyzed as second primary endpoint identifying only tests registered during the **CLOC participation period** (defined as period from start of cod liver oil/placebo consumption (*deriv_Date_CLO_start_corr*) to June 2, 2021).

- **Third primary:** Negative test
  - *CLOC_primary_3_negative_tests*
    - Generated from *MSIS_CLOC_TotalTests_7d_neg*
    - Sum of total negative tests registered during the period from one week after start of cod liver oil/placebo consumption (*deriv_Date_CLO_start_corr*) to June 2, 2021.
    - 0 if no tests registered
  - Negative test criteria
    - Variable: *MSIS_CLOC_TotalTests_7d_neg*
      - Sum of total negative tests registered during the period from one week after start of cod liver oil/placebo consumption (*deriv_Date_CLO_start_corr*) to June 2, 2021.
  - Supplementary variables
    - *MSIS_CLOC_FirstNegative*
      - Date of first negative test registered during the **CLOC participation period**.
    - *MSIS_CLOC_LastNegative*
      - Date of last negative test registered during the **CLOC participation period**.

- **Forth primary:** Self-reported airway infections
  - *CLOC_primary_4_selfrep_airway*
    - Generated from *deriv_respinf_date_yn*
      - 1 = ever answered yes and provided valid date in any form
      - 0 = only answered no, or provided non-valid date in any form
      - 999 = has filled out form, has not answered airway infections question
      - NA = has not filled out form
    - Any self-reported airway infections during the period from one week after start of cod liver oil/placebo consumption (*deriv_Date_CLO_start_corr*) to June 2, 2021.
  - Self-reported airway infections criteria
    - Variable: *deriv_respinf_date_yn*

- Answered question *has had respiratory infection since last cloc report* in any CLOC questionnaire (R1-R6)
    - *deriv_respinf_report_yn_ever*
- Date of self-reported airway infections in the period from one week after start of cod liver oil/placebo consumption (*deriv_Date_CLO_start_corr*) to June 2, 2021.
    - *deriv_respinf_date_sums*
-

- Supplementary variable
    - *deriv_respinf_count report_sum*
        - Number of respiratory infections reported
    - *R**x**_Date_respiratoryinfection**x**_sym_start_report*
        - Date of each infection reported in each report form