Influence of COVID-19 on immune responses to pneumococcal Polysaccharide and influenza vaccinations in older persons

Acronym: CoVac

(COVID-19 invloed op afweerreactie na pneumokokkenvaccinatie in ouderen)

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LIST OF ABBREVIATIONS AND RELEVANT DEFINITIONS

AE	Adverse Event
AR	Adverse Reaction
BRP	Basis Registratie Personen
CI	Confidence Interval
CoVac	COVID influence on Vaccination
CRF	Case Report Form
EU	European Union
EudraCT	European drug regulatory affairs Clinical Trials
GCP	Good Clinical Practice
GDPR	General data protection regulation
GM	Geometric Means
GMC	Geometric Mean Concentration
GMT	Geometric Mean Titter
HA	Hemagglutinin
HI	Hemagglutinin Inhibition
IC	Informed Consent
IIV	Immunology of Infectious diseases and Vaccines
ILI	Influenza-Like Illness
IMP	Investigational Medicinal Product
METC	Medical research ethics committee (MREC)
MIA	Multiplex Immunoassay
PIF	Subject information leaflet
PPV23	23-valent pneumococcal polysaccharide vaccine
(S)AE	(Serious) Adverse Event
SES	Social Economic Status
SF36	Short-form 36
SOP	Standard operation procedure
SPC	Summary of Product Characteristics
Sponsor	The sponsor is the party that commissions the organization or performance of the research, for example a pharmaceutical company, academic hospital, scientific organization or investigator. A party that provides funding for a study but does not commission it is not regarded as the sponsor, but referred to as a subsidizing party.
SUSAR	Suspected Unexpected Serious Adverse Reaction
WMO	Medical Research Involving Human Subjects Act
QIV	Quadrivalent inactivated Influenza Vaccine

SUMMARY

Rationale: SARS-CoV-2 infections have a profound impact on the immune system, which may hamper subsequent immune reactions against novel challenges. Currently it is unknown how the observed immune suppression will affect responses against primary or secondary infections, but also vaccinations. As vaccination is the prime prevention measure to protect elderly against other (respiratory) infections, there is an urgent need to investigate whether previous SARS-CoV-2 infection influences these vaccination responses. We hypothesize that previous SARS-CoV-2 infection will lead to lower vaccination response in older adults.

To study this hypothesis, we will investigate the immune/antibody response to pneumococcal vaccination in older adults with and without previous proven SARS-CoV-2 infection. As of autumn 2020 all inhabitants of the Netherlands 73-79 of age will be offered a 23-valent polysaccharide pneumococcal vaccine (PPV23). We will invite persons with proven SARS-CoV-2 infection and an aged sex matched control group of individuals to participate in a study where blood will be collected before and after (pneumococcal) vaccination. In individuals who receive the influenza virus vaccination at the same time, also the antibody response against influenza will be measured.

The aim of the study is to identify whether (severe) COVID-19 disease will have long-lasting effects on subsequent vaccination responses in older adults.

The main objective of this study will be to get a better insight in the influence of COVID-19 on subsequent vaccine-induced immune responses and gain knowledge on the immune hypo responsiveness after a SARS-CoV-2 infection in older adults, with the ultimate goal to formulate evidence-based strategies to improve immunity to vaccination in the ageing population.

Study design: Longitudinal observational study

Study population: Human volunteers 73-79 years of age, birth cohort 1941-1947

Main study parameters/endpoints:

The primary objective of the study is to compare anti-pneumococcal antibody responses against pneumococcal strains in blood of older individuals who have had laboratory confirmed COVID-19 with those measured in their healthy peers stratified by age and sex. IgG, IgM and IgA antibodies against all 23 pneumococcal serotypes included in the vaccine will be measured pre and four weeks post-vaccination.

A secondary objectives is to assess the influenza-specific antibody response in participants that concomitantly received the influenza vaccination. Assess potentially cross-reactive antibodies against other coronaviruses.

Exploratory endpoints

Exploratory analyses will involve linking COVID-related disease characteristics (identified by questionnaires), and quality of life (using SF36 questionnaires) data as well and SARS-CoV-2-specific antibody concentrations at the pre-vaccination time point to the height of pneumococcal and if available influenza vaccination response.

Nature and extent of the burden and risks associated with participation, benefit and group relatedness: The burden associated with participation involves self collection of blood samples by finger stick (300 ul per timepoint). In addition, the subject will be asked to fill in a questionnaire. The potential risks are considered minimal. The results of the study may contribute to a better control of respiratory diseases in older persons.

Planning:

- First Subject, First Visit (FPFV): October 2020
- Last Subject, First Visit (LPFV): November 2020
- Last Subject, Last Visit (LPLV): December 2020

1. INTRODUCTION AND RATIONALE

Increased life expectancy is a global phenomenon. Predictions indicate that by the year 2050, the global population above 60 years of age will have more than doubled. Although the increased life expectancy is caused by improved health in general, the rising prevalence of age-related frailty and multimorbidity indicates that much is to be gained from improved understanding of how to maintain good health up to old age. A prerequisite for healthy ageing is a robust immune system that protects the body against incoming pathogens. Ageing-related changes of the immune system, comprehensively referred to as immunosenescence, (1,2) contribute to the increased susceptibility of elderly persons to infectious diseases and the low efficacy of vaccination. Moreover, dysregulated immune responses may give rise to chronic immune-mediated inflammatory diseases that are most prevalent among the elderly. Both immunosenescence as well as increased comorbidities also underly the relatively high susceptibility of elderly for COVID-19. On top of that SARS-CoV-2 infections have a profound impact on the immune system, including decreased numbers of lymphocytes and exhaustion of adaptive immune cells.(3-6) This may hamper subsequent immune responses against novel challenges. Currently it is unknown how long the observed immune suppression lasts and how this will affect responses against subsequent primary or secondary infections, but also vaccinations. As vaccination is the prime prevention measure to protect elderly against other (respiratory) infections, there is an urgent need to investigate whether previous COVID-19 influences vaccination responses.

In older adults, influenza virus infections and community-acquired pneumonia (CAP) and invasive pneumococcal disease (IPD) form a global problem causing millions of deaths worldwide (7). In persons suffering from pneumonia due to viral infections, such as an influenza virus infection, coinfections by bacteria such as *S. pneumoniae* increase the severity and mortality rates. (8) Infants and older adults, especially those above 75 years of age and immunocompromised elderly, are at increased risk of severe infections and death. CAP is associated with a six-fold increased mortality and 16% lower quality-of-life in the post-discharge year among patients surviving hospitalization for CAP, compared to non-diseased persons (8,9). Moreover, CAP occurs especially in individuals with chronic diseases such as COPD, diabetes mellitus and chronic heart disease. These not only increases the risk for acquiring pneumococcal disease, but can also adversely affect the severity and outcome of that disease. Both COPD and diabetes have been shown to be significant predictors of hospitalization in patients with CAP. Furthermore, the risk of respiratory and cardiac complications both of which are associated with increased mortality is greater in individuals with chronic lung and/or heart disease. Due to the chronic nature of these conditions,

affected individuals are at risk of CAP and IPD the whole year round but particular in winter time during influenza season (10-12). This highlights the need for timely pneumococcal vaccination of such patients.

In 2020, vaccination with the 23-valent pneumococcal vaccine (PPV23) will be implemented in the Dutch National Immunization Program for older adults.(13) In the fall of 2020 older adults between 73-79 years of age (birth cohort 1941-1947) will be invited for a PPV23 and a seasonal influenza vaccination. This non conjugated polysaccharide vaccine should elicit protective antibodies against 23 of the most common Pneumococcal serotypes. The considerable number of older adults that have had COVID-19 since March 2020, emphasizes the relevance of the question whether COVID-19 adversely affects vaccination responses, but also provides the unique opportunity to investigate the effect of a recent SARS-CoV-2 infection on pneumococcal and influenza vaccination (14) responses in this vulnerable age group.

Although in general individuals aged between 73-79 are more vulnerable for infections heterogeneity exist between individuals within this age group and having had a SARS-CoV-2 infection or not may add to this heterogeneity. Therefore we will assess both SARS-CoV-2 related vulnerability (experienced disease severity) as well as general vulnerability (comorbidities, general frailty score based on quality of life, physical health etc.). The questionnaires on Quality of Life (SF36), existence of comorbidities, independent living abilities, physical condition and life style factors allow us to quantify the variation in frailty and how this associates with COVID19 severity and subsequent responses to vaccination. The results from this study could allow identification of individuals with a lower response to vaccination based on SARS-CoV-2 exposure/infection and will aid future studies to improve vaccination responses in older adults.

In this study, the humoral immune responses induced by a pneumococcal polysaccharide and a seasonal influenza vaccine (in those receiving the flu vaccine concomitantly) will be measured in older men and women (73-79 years) with previous proven PCR confirmed SARS-Co-V2 infection and compared to those in healthy COVID-19 negative aged-matched healthy peers. Potential participants of the healthy control group, will be screened for SARS-CoV-2 specific antibodies pre vaccination as well details on having had COVID-related symptoms and known direct contact with COVID-patients. The pneumococcal vaccination will be considered as a primary vaccination since there have not been an adult pneumococcal vaccination program in The Netherlands before and persons with a previous pneumococcal vaccination will be excluded from the study. The influenza vaccination will in most cases be a

booster vaccination as most individuals will have received a seasonal flu vaccine in previous years.

In addition, data on experienced COVID-19 disease and health and comorbidities will be collected to classify individuals with lower resilience in general and stratify the groups based on these parameters.

In conclusion, pneumococcal specific antibody responses will be compared between heathy and previous COVID-19, >= 28 days after positive SARS-CoV-2 PCR, in older adults aged 73-79 years to evaluate the effect of the infection on vaccine induced immune responsiveness.

2. OBJECTIVES

2.1 Primary objective

Assess whether COVID-19 in 73-79 year old male and female persons is related to low antibody responses to PPV23 by measuring IgG, IgM and IgA antibody concentrations to all 23 vaccine pneumococcal polysaccharide serotypes pre- and post- vaccination. Subsequent analyses will be performed comparing vaccine responses stratified by age, sex and co-morbidities.

2.2 Secondary objectives

- Assess whether COVID-19 in 73-79 years old male and female persons is related to low antibody responses to Influenza vaccine strains by measuring HI titers pre and post vaccination by comparing vaccine responses in ex-COVID-19 patients with age and sex matched controls. Subsequent analyses will be performed comparing vaccine responses to frailty stratifying by age and sex
- Assess potentially cross-reactive antibodies against other coronaviruses
- Identify parameters of the immune system, specific morbidities and/or other specific health related parameters that correlate with low vaccine responsiveness

2.1 Exploratory objectives

- Determine the effect of the level of COVID-19-specific antibodies (as potential indication of severity of infection) to pneumococcal and influenza vaccine antibody responses

- Determine the impact of severity of experienced COVID-19-related disease and clinical baseline status (quality of Life) as a predictive marker for vaccine response

3. STUDY DESIGN

All persons of 73-79 years of age that have had PCR confirmed COVID-19 and are still alive will be identified from Osiris (n=5449 disease cases, of which 4198 are still alive, dd September 17th 2020) and a control group frequency matched for age but without COVID-19 from the BRP from the same regions as the cases will be invited for participation in the current study. Ex COVID-19 patients with a positive SARS-CoV-2 PCR >= 28 days before study entry will be identified and will receive a study invitation letter explaining the goal of the study and a reply card.

Pre vaccination blood samples will be collected for baseline antibody analysis using fingerprick collection. The participants will receive an envelope with a fingerprick set (2x) for blood self sampling including instructions. After blood collection the vial with the blood sample will be shipped to the RIVM in the provided return envelope specifically for biological samples by regular mail within 24 h of blood draw. This will enable fast and easy collection of samples from 600-800 older adults in a short timeframe.

Participants will be vaccinated with the PPV23 vaccine and concomitantly a yearly influenza vaccine if they choose so. Pre vaccination and at 4 weeks postvaccination participants will be asked to collect blood samples using self sampling by fingerprick. Both blood samples will be used for (functional) antibody analysis.

Group	Source	Expected number of invitations	Expected number of participants
COVID-19 positives	OSIRIS	4200	300
COVID-19 negatives	BRP	~7500	300

4. STUDY POPULATION

4.1 Population (base)

Older adults born between 1941-1947, (73-79 years of age) that are eligible for PPV23 vaccination will invited for participation. Use of immunosuppressive agents other than systemic corticosteroids 2 weeks before inclusion and/or diagnosis of primary or secondary immune deficiencies are no exclusion criteria in order to have a representative group of elderly.

4.2 Inclusion criteria

In order to be eligible to participate in this study, a subject must meet all of the following criteria:

- born between 1941-1947 (73-79 years of age)
- COVID-19 group: Having had a proven (SARS-CoV-2 PCR test positive) COVID-19 infection at least 1 month before study entry
- Control group: Have not had a positive SARS-CoV-2 PCR test
- Willing to receive the PPV23 vaccine
- Be capacitated
- Have signed Informed Consent.

4.3 Exclusion criteria

A potential subject who meets any of the following criteria will be excluded from participation in this study:

- Having had a previous pneumococcal vaccination (PCV or the 23-valent pneumococcal polysaccharide vaccine (PPV23))

4.4 Temporary exclusion criteria

- If a subject has an elevated body temperature less than 48 hours before a blood collection the blood collection should be postponed until the criteria are met, or the blood collection is cancelled

- Receipt of any vaccine(s) within 1 month of vaccination and within 2 weeks of blood collection.

4.5 Withdrawal criteria during study

4.6 Sample size calculation

The primary objective of the study is to compare pneumococcal (and influenza vaccine-specific) antibody responses in older adults that have had COVID-19 with controls.

The sample size calculation is based on a 1.5-fold difference in IgG GMC (GMC ratio = 1.5) between COVID-19 patients and non-COVID-19
"Direct evidence of the protective effect of anticapsular antibodies comes from studies where passively administered antibodies have provided protection from otitis media or invasive pneumococcal disease.137, 138 Furthermore, vaccines that induce antibodies to pneumococcal capsular polysaccharides are protective against invasive pneumococcal infections (see Chapter 47)." Uit Plotkin 7th edition 2017.
Besides this the WHO has determined, on the basis of three clinical effectivity trials on pneumococcal conjugate vaccines in children, that the protective levels of pneumococcal specific IgG antibodies is 0.35 ug/ml. Adults and elderly have baseline antibody levels higher than the protective levels in children. There are however no published data of randomized controlled effectivity trials in adults. Therefore no correlate of protection can be determined for adults.

The height of the antibody concentrations mostly determines the duration of protection. A lower antibody response after immunization will result in a quicker decline to below the levels of protection.

The clinical relevance of a 1.5-fold difference in IgG GMC's is not determined yet. In publications of comparable trials normally the only the number of included patients is mentioned and not the sample size calculation. In another trial on the same age group (not looking at COVID infections) we choose at a 2-fold difference, but for the current trial we expect the difference to be smaller, therefore we choose 1.5-fold difference.

The sample size calculation is based on pneumococcal serum IgG antibody concentrations 1 month post pneumococcal vaccination. The sample size needed to detect a 1.5-fold difference in IgG GMC (GMC ratio = 1.5) between COVID-19 patients and non-COVID-19 subjects is 288 subjects per group, with 90% power and a significance

level of 5%. The standard deviation (on the natural logarithm scale) was assumed to be 1.5 (based on CAPiTA trial performed in elderly) (16).

When including more non-COVID-19 subjects, e.g. with a ratio of 1.5, 240 COVID-19 patients and 360 non-COVID-19 subjects would be needed to detect a GMC ratio of 1.5, with 90% power and a significance level of 5%.

The number of lab-confirmed COVID-19 cases between 73 and 79 years of age in the Netherlands is around 5449, of which 4198 survived (September 17th 2020). When assuming a response rate for the study of 20% and a PPV23 vaccination uptake of 50%, we could include 300 COVID-19 cases. This would be enough to detect a 1.5-fold difference in antibody levels with 90% power. To reach these 300 participants in the COVID+ group we will invite all known COVID-19 cases in this age group in the Netherlands and ~2.5 times more non-COVID-19 subjects.

The standard deviation for hemagglutinin inhibition (HI) titers for influenza-specific antibodies is smaller (≤ 1.3 based on the datasets of the different influenza vaccine strains published by Rümke et al (17) and is therefore covered by this sample size when a similar effect is expected with a 90% power and a significance level of 5%.

5. TREATMENT OF SUBJECTS

5.1 Investigational product/treatment

Not applicable.

5.2 Use of co-intervention

Not applicable.

5.3 Escape medication

Not applicable.

6. INVESTIGATIONAL PRODUCT

Here we describe the vaccines implemented in the National Immunization Program. However, the vaccines will be administered by the GPs, as part of the routine program for this age group. Therefore, this intervention is not part of our study.

6.1 Name and description of investigational product(s)

Not applicable.

(the vaccine response studied is that after vaccination by the GPs with Pneumovax 23, PPV23, is a 23 valent pneumococcal polysaccharide vaccine (Merck Sharp & Dohme). Each dose of 0,5 ml contains 25ug of each of the following 23 serotypes: 1, 2, 3, 4, 5, 6B, 7F, 8, 9N, 9V, 10A, 11A, 12F, 14, 15B, 17F, 18C, 19F, 19A, 20, 22F, 23F, 33F).The other vaccine response studied is that of the 2020-2021 QIV seasonal unadjuvated quadrivalent inactivated influenza vaccine containing 15ug Hemagglutinin (HA) of each influenza vaccine strain, as recommended by the WHO for the northern hemisphere, that will be used for the national immunization program (NIP) in the Netherlands. All subjects will be vaccinated with the same vaccine, which will be either Vaxigrip Tetra (Sanofi Pasteur, France) or Influvac Tetra (Mylan, the Netherlands).

6.2 Summary of findings from non-clinical studies

PPV23, summary of findings from non-clinical studies are not mentioned.

QIV, summary of findings from non-clinical studies are not mentioned.

6.3 Summary of findings from clinical studies

Not applicable.

6.4 Summary of known and potential risks and benefits

Not applicable.

6.5 Description and justification of route of administration and dosage

Not applicable.

6.6 Dosages, dosage modifications and method of administration

Not applicable.

6.7 Preparation and labelling of Investigational Medicinal Product

Not applicable.

6.8 Drug accountability

Not applicable.

7. NON-INVESTIGATIONAL PRODUCT

7.1 Name and description of non-investigational product(s)

Not applicable.

7.2 Dosages, dosage modifications and method of administration

Not applicable.

7.3 Preparation and labelling of Non Investigational Medicinal Product

Not applicable.

7.4 Drug accountability

Not applicable.

8. METHODS

8.1 Study parameters/endpoints

8.1.1 Main study parameter/endpoint

The primary study parameter is pneumococcal serotype- specific serum IgG antibody concentrations (GMCs) pre-vaccination and four weeks post PPV23 vaccination measured by bead-based multiplex immune assay.

8.1.2 Secondary study parameters/endpoints (if applicable)

- Influenza vaccine strain-specific serum IgG antibody titers (GMTs) will be measured pre- vaccination and four weeks post-influenza vaccination by Hemagglutinin Inhibition (HI) assay
- pneumococcal serotype-specific IgM and IgA antibody titers in serum pre- and post-vaccination
- health status assessment will be done by questionnaires.

8.1.3 Exploratory endpoints:

Other study parameters that will be investigated:
- cross-reactive antibodies against other previous coronaviruses infections
- COVID-19 details, including, onset, symptoms and severity

8.2 Randomization, blinding and treatment allocation

Not Applicable

8.3 Study procedures

Potential participants that received subject information and show interest in the study will receive an instruction letter, a self-sampling set and an informed consent form (pre-signed by investigator). The informed consent form (ICF) is signed by the subject (described in section 11.2).

The participants are requested to fill out the questionnaire. In addition, the participants will be asked to donate finger prick blood sample (maximum of 0.5 ml)

twice by the provided self-sampling set according to the detailed instruction, and are requested to return the sample(s) in the stamped, addressed safety-envelope provided.

Upon arrival at RIVM; blood samples will be processed for serum separation and subsequently aliquoted and stored at -80 °C.

Serum samples will be tested for the presence of (functional) antibodies by quantitative multiplex serology immune-assays. Selected positive serum samples will be tested for functionality of the antibodies

The self-sampling and questionnaire is repeated 1 month after vaccination.

8.4 Withdrawal of individual subjects

Subjects can leave the study at any time for any reason if they wish to do so without any consequences. The investigator can decide to withdraw a subject from the study for urgent medical reasons.

8.5 Replacement of individual subjects after withdrawal

Subjects who withdraw or are withdrawn from the study will be replaced. Not applicable

8.6 Follow-up of subjects withdrawn from treatment

Not applicable

8.1 Premature termination of the study

QIV and PPV23 are licensed products. The products are routinely used in several countries in the same age groups and considered safe. It is therefore unlikely that serious side effects will occur that can lead to premature termination of the study.

Nevertheless, the sponsor may end the study prematurely if this is beneficial to the health or welfare of the subjects, if continuation no longer serves a scientific purpose,

In case the study is terminated prematurely, subjects will be notified about this. All study materials should be returned to the sponsor as soon as possible.

9. SAFETY REPORTING

9.1 Temporary halt for reasons of subject safety

In accordance to section 10, subsection 4, of the WMO, the sponsor will suspend the study if there is sufficient ground that continuation of the study will jeopardize subject health or safety. The sponsor will notify the accredited Medical research ethics committee (METC) without delay of a temporary halt including the reason for such an action. The study will be suspended pending a further positive decision by the accredited METC. The investigator will take care that all subjects are kept informed.

9.2 AEs, SAEs and SUSARs

9.2.1 Adverse events (AEs)

Adverse events (AE) are defined as any undesirable experience occurring to a subject during the study, whether or not considered related to the investigational product or trial procedure. Only adverse events reported spontaneously by the subject will be recorded.

The trial staff will only record any adverse event spontaneously reported by the subject occurring within 28 days after vaccination and more than a week after blood sampling.

Any adverse event recorded as described above after routine PPV23 and QIV vaccination will be reported according to the standard procedure of the Netherlands pharmacovigilance Centre 'Lareb'(https://www.lareb.nl/Meldbijwerking/Meldformulier).

9.2.2 Serious adverse events (SAEs)

A serious adverse event is any untoward medical occurrence or effect that:

- results in death;
- is life threatening (at the time of the event);
- requires hospitalization or prolongation of existing inpatients' hospitalization;
- results in persistent or significant disability or incapacity;
- is a congenital anomaly or birth defect; or

- any other important medical event that did not result in any of the outcomes listed above due to medical or surgical intervention but could have been based upon appropriate judgement by the investigator.

A scheduled hospital admission will not be considered as a serious adverse event.

Subjects will be asked to report to the trial staff any serious adverse event occurring within 28 days after vaccination.

All SAE's occurring more than 28 days after vaccination and more than a week after blood sampling will not be reported.

The sponsor will report the SAEs through the web portal *ToetsingOnline* to the accredited METC that approved the protocol, within 7 days of first knowledge for SAEs that result in death or are life threatening followed by a period of maximum of 8 days to complete the initial preliminary report. All other SAEs will be reported within a period of maximum 15 days after the sponsor has first knowledge of the serious adverse events.

9.2.3 Suspected unexpected serious adverse reactions (SUSARs)

Adverse reactions (AR) are all unfavorable and unintended responses to an investigational product related to any dose administered.

Unexpected adverse reactions are SUSARs if the following three conditions are met:

1. the event must be serious (see chapter 9.2.2);
2. there must be a certain degree of probability that the event is a harmful and an undesirable reaction to the medicinal product under investigation, regardless of the administered dose;
3. the adverse reaction must be unexpected, that is to say, the nature and severity of the adverse reaction are not in agreement with the product information as recorded in:
    - Summary of Product Characteristics (SPC) for an authorised medicinal product;
    - Investigator's Brochure for an unauthorised medicinal product.

The sponsor will report expedited the following SUSARs through the web portal *ToetsingOnline* to the METC

- SUSARs that have arisen in the clinical trial that was assessed by the METC;
- SUSARs that have arisen in other clinical trials of the same sponsor and with the same medicinal product, and that could have consequences for the safety of the subjects involved in the clinical trial that was assessed by the METC.

The remaining SUSARs are recorded in an overview list (line-listing) that will be submitted once every half year to the METC. This line-listing provides an overview of all SUSARs from the study medicine, accompanied by a brief report highlighting the main points of concern.

The expedited reporting of SUSARs through ToetsingOnline is sufficient as notification to the competent authority.

The expedited reporting will occur not later than 15 days after the sponsor has first knowledge of the adverse reactions. For fatal or life threatening cases the term will be maximal 7 days for a preliminary report with another 8 days for completion of the report.

9.3 Annual safety report

Not applicable

9.4 Follow-up of adverse events

All AEs will be followed until they have abated, or until a stable situation has been reached. Depending on the event, follow up may require additional tests or medical procedures as indicated, and/or referral to the general physician or a medical specialist.

9.5 Data Safety Monitoring Board (DSMB) / Safety Committee

Not applicable

10. STATISTICAL ANALYSIS

10.1 Primary study parameter(s)

Geometric mean concentrations (GMCs) and 95% confidence intervals (CIs) will be calculated for the pneumococcal serotype specific IgG antibodies in COVID and control groups. Differences in GMCs within and between groups will be analyzed by ANOVA and t-tests.

10.2 Secondary study parameter(s)

- Geometric mean titers (GMTs) and 95% confidence intervals will be calculated for influenza vaccine strain-specific antibodies. Differences in GMTs within and between groups pre-and post-vaccination will be analyzed by ANOVA and by t-tests.
- GMTs or GMCs and 95% CIs will be calculated for antibodies. Differences in geometric means (GMs) in antibodies within and between groups at each time point will be analyzed by ANOVA and t-test.
    - Determine pre-and post-vaccination 1) the frequency of individuals with a HI titer of 40 or higher at T0 and T4 (seropositivity rates), 2) the frequency of subjects with > 4 fold increase in HI titers pre- versus post- versus pre-vaccination (seroprotection rates) 3) and frequency of subjects with an antibody titer ≥40 post vaccination and a 4-fold increase in HI titers and (seroconversion rates).
    - Subjects with a response of <40 at four weeks after influenza vaccination are defined as hypo-responders; subjects with antibody titer ≥40 post vaccination and a 4-fold increase in HI titers will be defined as responders; intermediate responses will be defined as weak responders.
    - Vaccination response rates will be determined and compared between groups. The percentage of hypo-responders and responders will be determined per vaccine and compared between groups by t-tests.
- Demographic and other baseline data such as age (category), gender, co-morbidity, health status, vaccination status and medication usage will be presented per group and overall. Appropriate descriptive statistics will be presented. Differences between the groups will be tested by non-parametric Mann-Whitney test or t-tests and if necessary adjusted by means of regression analysis or stratification.

10.3 Other study parameters

- COVID and ILI data such as symptoms, duration, will be presented per group and overall. Appropriate descriptive statistics will be presented. Differences between the groups will be tested by non-parametric Mann-Whitney test or t-tests and if necessary adjusted by means of regression analysis or stratification

10.4 Interim analysis

Not applicable

11. ETHICAL CONSIDERATIONS

11.1 Regulation statements

This clinical study will be conducted conform the current rules for Good Clinical Practice (GCP), as described by the Committee for Proprietary Medical Products (CPMP) of the European Union and the International Committee on Harmonisation (ICH) in "Note for Guidance on Good Clinical Practice, document CPMP/ICH/135/95", effective since January 17th 1997,Clinical Trial Directive 2001/20/EC, the principles of the Declaration of Helsinki (The World Medical Declaration of Helsinki adopted by the 18th WMA General Assembly, Helsinki, Finland, June 1964 and last amended by the 649th WMA General Assembly, Fortaleza, Brazil, October 2013) and in accordance with the Medical Research Involving Human Subjects Act (WMO)

The EU General Data Protection Regulation (GDPR) of the European Parliament and of the Council (2018) will be followed.

11.2 Recruitment and consent

Older adults, age 73 to 79 years, with a positive PCR test result for SARS-CoV-2 will be identified in the OSIRIS database. Subjects for the control group will be identified in the BRP (Basis Registratie Personen). Recruitment will be done by inviting the subjects by mail and simultaneously send the subject information. Participants can show their interest to participate in the study by returning the reply card. Subsequently, participants that are willing to participate will receive two self-sampling sets with detailed instructions, a questionnaire on paper and an informed consent form (pre-signed by investigator). The ICF, pre-signed by the investigator, will have to be countersigned at home by the subject

and together with the first blood sample and completed questionnaire will be returned to the RIVM in the stamped, addressed safety-envelope. The time the subjects have to respond to the invitation, is depending on the date of vaccination by the GPs. After return of the reply card, it will at least take 2 days before the subjects receive the self-sample envelope and can sign the informed consent.

11.3 Objection by minors or incapacitated subjects (if applicable)

No incapacitated subjects will be recruited for this study, if during the study a subject becomes or is assessed incapable, this subject will be withdrawn from the study by the investigator.

11.4 Benefits and risks assessment, group relatedness

Risks assessment

Blood collection by fingerpick is a standard procedure which is generally accepted. The sensation of a fingerprick can be discomforting for some participants. The risk of blood collection is considered minimal.

Group relatedness and benefits

There are no personal benefits for the participants of the study. The results of the study may contribute to a better vaccination strategy to control respiratory diseases in older adults on a population level in the future.

11.5 Compensation for injury

By law, the RIVM is exempted from the compulsory subjects' insurance as laid down in article 7, paragraph 7 of the WMO. Subjects can claim damage resulting from the clinical trial directly at the RIVM. The subjects are informed of this arrangement in the Subject Information Form. The coverage for damage to research subjects through injury or death caused by the study is listed below:

€ 5.1.2b

5.1.2b

5.1.2b

5.1.2b

€ 5.1.2b

5.1.2b

for all damage disclosed by scientific research for the Sponsor as 'verrichter' in the meaning of said Act in each year of insurance coverage. The coverage applies to the damage that becomes apparent during the study or within 4years after the end of the study.

11.6 Incentives (if applicable)

No incentives are given to the participants. Hopefully, participants are willing to participate in the study to provide data that may help protect older adults better against infectious diseases.

12. ADMINISTRATIVE ASPECTS, MONITORING AND PUBLICATION

12.1 Handling and storage of data and documents

The Good Clinical Data Management Practices (GCDMP) guideline, a document that provides guidance on the accepted practices in Clinical Data Management (CDM) that are consistent with regulatory practices will be followed. A Data Management Plan (DMP) will be designed to ensure optimal collection, processing, storage, and dissemination of data. The Guidelines on FAIR (findable, accessible, interoperable and reusable) Data Management in Horizon 2020 as well as the General Data Protection Regulation (GDPR) the Declaration of Helsinki (and the clinical trial regulation/GCP if appropriate) will be followed. Direct identifiable information, like name and contact information, of study subjects will only be accessible by selected, authorized members of the project team, by monitors and auditors, by members of the METC and the Dutch competent authorities. To secure sensitive, private information from study subjects, all subjects participating in the study will receive a unique subject number. All study documents, data (both retrospective and newly generated) and materials will be recorded/collected using this subject number. The direct identifiable information will be stored separate from all study data, which is used for scientific purposes, including human samples like blood and saliva. The identification key will be stored at the participating sites. The investigator of the trial site or a designated representative will be responsible for the key to the code.

Pseudonymization will be applied to minimize the risk of tracing data back to individual subjects and prevents the leaking of private, sensitive information. Data processing of pseudonymized data will be performed in compliance with the GDPR. Data will be accessible to authorized, trained persons only.

Clinical Data Management is a crucial part of a clinical trial and includes, electronic Case Record Forms (eCRF), database design and validation, query handling, medical history and medication coding, database locking, data extraction and assessment of data quality and completeness and integrity at regular intervals during the conduct of the trial.

A remote data capture system will be used for data collection. The electronic CRFs are created and completed in a validated system that complies with the Code of Federal Regulations (CFR), 21 CFR Part 11, that ensures accurate, reliable data by using a secure computer-generated, date and time-stamped audit trail of operator entries modifications and/or deletion of data or electronic records. Procedures are in place to guarantee data integrity and confidentiality.

The questionnaires will be, as much as possible, completed by the subject directly in the Data Management System. Data from the data management system will be transferred to a central database over the internet using secured data communication protocols (with specific consent of the data subject). Pseudonymized data, and collected samples, of individual subjects are only accessible in EU countries. All data will be stored automatically and regular back-ups will make sure that data will not be lost. Databases and web servers of data management systems will be hosted in data centers that meet the highest possible security requirements. Procedures will be implemented to guarantee that study subjects can exercise their rights as required by the GDPR and GCP.

Specific categories will be created to indicate the level of security/safety involved, and for each respective category appropriate guidelines will be designed. The data categories will be as follows: 1) anonymous, non-sensitive data will be marked as “public data”; 2) data regarding cohort information and statistics, as well as low-risk aggregated data, which have been adequately anonymized, will be marked as “low-risk”. Access to this data will be possible for study team members and the research community; 3) data which is high risk, sensitive, or other info which can be led back to an individual will be marked as “classified”. The latter category contains all individual and pseudonymized data of study subjects as well as all genetic data and human samples. Access to this data will only be possible with specific authorization, which can be acquired only after accepting the Data Access Agreements and notification to the lead investigators. In addition, specific authorization can

only be obtained by members study team.

After project closure, all data (taking into account the requirements for data minimization) is stored on a secured network for at least 15 years.

12.2 Handling and storage of samples

All collected samples will be coded as described in section 8 and stored at the RIVM for a minimum of 15 years after the end of the study. The RIVM has an infrastructure for collecting, storing and managing biomaterial and associated clinical data in a standardized manner, sample processing SOPs and standardized sample storage conditions.

Samples will be used for the study purposes and objectives of the clinical study. The types of analyses that will be done on the samples during the project are described in the protocol. Samples that are left over after all analysis are done will only be used to answer other research questions of the study. Samples that are left over after study termination will only be stored and used if the subject had given consent to use his/her materials for further research as described in the PIF. The coordinating investigator and the principal investigator of the study decide on the use of the samples for further research, after all research questions have been answered. They can also ask for advice of the Clinical Expertise Centre and the privacy coordinator of the RIVM.

12.3 Monitoring and Quality Assurance

A detailed risk based monitoring plan will be written by the sponsor detailing the monitoring activities and frequencies. Monitoring will assess informed consent procedure and protocol adherence. Informed consent forms of subjects will be reviewed for completeness as described in the monitoring plan. During the study, 1-2 monitoring visits per year will be performed, the number of monitoring visits per year depends on the number of study visits in a year. During these monitoring visits the following items will be verified: adherence to protocol, presence of personally signed and dated Informed Consent forms, accuracy and completeness of study data recorded on the CRFs, consistency between source data and data recorded in the CRF/database, reporting of (serious) adverse events incl. investigators response, presence of an up-to-date study site file, sample labelling, storage and transport. Source data verification will be performed 100% for first 10 subjects, if acceptable, followed by 10% of all subjects

(actual numbers will be determined in the risk-based monitoring plan). In case of increase or decrease in findings the percentage can be adjusted.

For the purpose of compliance with Good Clinical Practice it may be necessary to conduct a site audit performed by authorized representatives of the sponsor and/or a Regulatory Authority and/or the Ethics Committee. This may occur at any time from start to after conclusion of the study

12.4 Amendments

A 'substantial amendment' is defined as an amendment to the terms of the METC application, or to the protocol or any other supporting documentation, that is likely to affect to a significant degree:

- the safety or physical or mental integrity of the subjects of the trial;
- the scientific value of the trial;
- the conduct or management of the trial; or
- the quality or safety of any intervention used in the trial.

All substantial amendments will be notified to the METC and to the competent authority.

Non-substantial amendments will not be notified to the accredited METC and the competent authority, but will be recorded and filed by the investigator.

12.5 Annual progress report

The investigator will submit a summary of the progress of the trial to the accredited METC once a year. Information will be provided on the date of inclusion of the first subject, numbers of subjects included and numbers of subjects that have completed the trial, serious adverse events/ serious adverse reactions, other problems, and amendments.

12.6 Temporary halt and (prematurely) end of study report

The investigator will notify the accredited METC and the competent authority of the end of the clinical study within a period of 90 days. The end of the clinical study is defined as the last subject's last visit.

The investigator will notify the METC immediately of a temporary halt of the study, including the reason of such an action.

In case the study is ended prematurely, the investigator will notify the accredited METC and the competent authority within 15 days, including the reasons for the premature termination.

Within one year after the end of the clinical study, the investigator will submit a study report with the results of the primary endpoints of the study and a study outline to the accredited METC and the Competent Authority.

12.7 Public disclosure and publication policy

The study results will be reported in progress reports and submitted for publication in peer-reviewed, and possibly open access, journals.

Prior to the start of the study, the study will be registered in a primary registry (NTR).

13. STRUCTURED RISK ANALYSIS

13.1 Potential issues of concern

Not applicable

13.2 Synthesis

All subjects will receive an offer for the seasonal flu and PPV23 vaccination through the usual channels because of their age (73-79 years).

The seasonal QIV and the pneumococcal PPV23 vaccine are registered products to be used within the indication as mentioned within the SPC, therefore no potential issues of concern are expected in addition to those that have already been described in the current SPC.

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