

Vaccine responsiveness to SARS-CoV2 in aged persons

Monitoring immunogenicity of SARS-CoV-2
vaccination in Dutch middle-aged and older
individuals
(participating in the Doetinchem Cohort Study)

incl Amendment 1, 2, 3 and 4

Acronym: VIDO

Vaccinatie tegen SARS-CoV-2 en Immuniteit in Doetinchem Onderzoek

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Vaccine responsiveness to SARS-CoV2 in aged persons

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Vaccine responsiveness to SARS-CoV2 in aged persons

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Vaccine responsiveness to SARS-CoV2 in aged persons

TABLE OF CONTENTS

1. INTRODUCTION AND RATIONALE.....	11
2. OBJECTIVES.....	14
2.1 Primary objective	14
2.2 Secondary objectives.....	14
2.3 Exploratory objectives.....	15
3. STUDY DESIGN	15
4. STUDY POPULATION.....	18
4.1 Population (base).....	18
4.2 Inclusion criteria	18
4.3 Exclusion criteria.....	18
4.4 Temporary exclusion criteria.....	18
4.5 Withdrawal criteria during study.....	19
4.6 Sample size calculation	19
5. TREATMENT OF SUBJECTS	20
6. INVESTIGATIONAL PRODUCT	21
6.1 Name and description of investigational product(s)	21
6.2 Summary of findings from non-clinical studies.....	21
6.3 Summary of findings from clinical studies.....	21
6.4 Summary of known and potential risks and benefits.....	21
6.5 Description and justification of route of administration and dosage.....	21
6.6 Dosages, dosage modifications and method of administration.....	21
6.7 Preparation and labelling of Investigational Medicinal Product.....	21
6.8 Drug accountability	21
7. NON-INVESTIGATIONAL PRODUCT	21
8. METHODS	22
8.1 Study parameters/endpoints.....	22
8.1.1 Main study parameter/endpoint	22
8.1.2 Secondary study parameters/endpoints	22
8.1.3 Exploratory endpoints:	22
8.2 Randomization, blinding and treatment allocation	23
8.3 Study procedures.....	23
8.4 Other study procedures	24
8.4.1 Antibody analysis in serum and plasma	24
8.4.2 Plasma.....	24
8.4.3 Absolute numbers of cells in whole blood.....	24
8.4.4 Questionnaires.....	24
8.5 Withdrawal from the study	25
8.6 Specific criteria for withdrawal	25
8.7 Replacement of individual subjects after withdrawal	25
8.8 Follow-up of subjects withdrawn from treatment.....	26
8.9 Premature termination of the study.....	27
9. SAFETY REPORTING.....	28
9.1 Temporary halt for reasons of subject safety.....	28
9.2 AEs, SAEs and SUSARs	28
9.2.1 Adverse events (AEs).....	28
9.2.2 Serious adverse events (SAEs).....	28
9.2.3 Suspected unexpected serious adverse reactions (SUSARs).....	29

Vaccine responsiveness to SARS-CoV2 in aged persons

9.3	Annual safety report.....	30
9.4	Follow-up of adverse events	30
10.	STATISTICAL ANALYSIS	31
10.1	Primary study parameter(s)	31
10.2	Secondary study parameter(s).....	31
10.2.1	Absolute numbers of Cells.....	31
10.2.2	Plasma Biomarkers.....	31
10.2.3	Health questionnaire.....	31
10.3	Associations of baseline frailty characteristics with baseline immunological parameters and further with vaccine responsiveness.....	32
11.	ETHICAL CONSIDERATIONS	33
11.1	Regulation statement	33
11.2	Recruitment and consent.....	33
11.3	Objection by minors or incapacitated subjects (if applicable)	35
11.4	Benefits and risks assessment, group relatedness.....	35
11.5	Incidental findings	35
11.6	Compensation for injury	35
11.7	Incentives.....	36
12.	ADMINISTRATIVE ASPECTS, MONITORING AND PUBLICATION.....	37
12.1	Handling and storage of data and documents	37
12.1	Handling and storage of samples	38
12.2	Monitoring and Quality Assurance	39
12.3	Amendments.....	39
12.4	Annual progress report	40
12.5	Temporary halt and (prematurely) end of study report	40
12.6	Public disclosure and publication policy.....	40
13.	STRUCTURED RISK ANALYSIS.....	41
13.1	Potential issues of concern	41
13.2	Synthesis	41
14.	REFERENCES	41
15.	Amendment 1	42
15.1	Goal of amendment	42
16.	Amendment 2	43
17.	Amendment 3	44
17.1	Goal of amendment	44
17.2	Justification for amendment.....	44
18.	Amendment 4	45
18.1	Goal of amendment	45
18.2	Justification for amendment.....	45

Vaccine responsiveness to SARS-CoV2 in aged persons

LIST OF ABBREVIATIONS AND RELEVANT DEFINITIONS

AE	Adverse Event
AR	Adverse Reaction
BMI	Body Mass Index
CA	Competent Authority
CDM	Clinical Data Management
CPMP	Committee for Proprietary Medical Products
CRF	Case Report Form
CRP	C-reactive protein
DCS	Doetinchem Cohort Study
DMP	Data Management Plan
EU	European Union
FPFV	First Subject First Visit
GCP	Good Clinical Practice
GCDMP	Good Clinical Data Management Practices guideline,
GDPR	General Data Protection Regulation; in Dutch: Algemene Verordening Gegevensbescherming (AVG)
GP	General practitioner
HBV	Hepatitis B virus
HCV	Hepatitis C virus
HIV	Human immunodeficiency virus
IC	Informed Consent
ICF	Informed Consent Form
ICH	International Committee on Harmonisation
IIV	Immunology of Infectious diseases and Vaccines
LPFV	Last Participant First Visit
LPLV	Last Participant Last Visit
METC	Medical research ethics committee (MREC); in Dutch: medisch-ethische toetsingscommissie (METC)
MIA	Multiplex Immunoassay
PBMC	Peripheral Blood Mononuclear Cells
PIF	Participants Information Leaflet
(S)AE	(Serious) Adverse Event
SARS-CoV-2	Severe acute respiratory syndrome coronavirus-2

Vaccine responsiveness to SARS-CoV2 in aged persons

Sponsor	The sponsor is the party that commissions the organisation or performance of the research, for example a pharmaceutical company, academic hospital, scientific organisation 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 subsidising party.
SUSAR	Suspected Unexpected Serious Adverse Reaction
WMO	Medical Research Involving Human Subjects Act; in Dutch: Wet Medisch-wetenschappelijk Onderzoek met Mensen

Vaccine responsiveness to SARS-CoV2 in aged persons

SUMMARY

Rationale: Vaccination against SARS-CoV-2 is the most effective way to end the current pandemic, although systemic immune responses induced by the various vaccines may vary considerably in amplitude, longevity and quality per person. In addition, with ageing there is a decline in the functioning of the immune system, making older people more vulnerable to infections and prone to lower responses to vaccination. Since there is great heterogeneity in the rate at which immune function declines, it is important to identify elderly at risk for potential low vaccination responses and thus at higher risk for infection. We will assess the induction of antibody responses to vaccination in older and middle-aged men and women of 52-90 years of age. In addition we will investigate whether age, co-morbidities, frailty or underlying lingering inflammation in these individuals might underly lower responses to the SARS-CoV-2 vaccines.

To study this, we will capitalize on the infrastructure and data provided by the ongoing longitudinal Doetinchem Cohort Study (DCS) (NL63779.041.17), which includes available data on lifestyle, metabolic factors, co-morbidities and frailty of all participants. In the DCS, persons originally 20-59 years of age at study start, have been followed for over 30 years (N>3700). Various frailty parameters have been assessed in these participants. Based on these, a frailty score (index) has been determined by using the most recent information on 36 frailty-related "health deficits", i.e. specific (co)morbidities, tests of physical (dys)functioning and cognition. Because of these data the DCS provides a unique opportunity to get insight into factors affecting vaccine immunogenicity in older and middle-aged persons. Recent data from a sub-cohort of the DCS aged 60-80 years revealed that lingering inflammation is associated with frailty. Therefore, frailty may be linked to reduced immune function and vaccination responses.

The main objective The primary objective is to assess antibody responses against the SARS-CoV-2 vaccines in blood of individuals of 52-90 years of age at several timepoints post vaccination, with respect to age, co-morbidities and frailty, taking sex differences into account. The secondary aim is to address which specific pre-vaccination frailty characteristics and metabolic and immunological biomarkers are related to low or high vaccination responses using state of the art statistical analyses. Altogether, being able to identify frailty or unfavorable 'biological ageing' may be useful in conceiving individualized approaches for improved vaccination strategies or alternative interventions to better protect older persons against SARS-CoV-2 disease.

Study design: Longitudinal observational study

Study population: Human volunteers 52-90 years of age already participating in the long term DCS cohort study and receiving SARS-CoV-2 vaccination and volunteers above 50-

Vaccine responsiveness to SARS-CoV2 in aged persons

ears of age living in a nursing home who received SARS-CoV-2 vaccination less than 7 months ago.

Intervention (if applicable): Not applicable.

Main study parameters/endpoints: The **primary endpoints** are vaccine-specific (Spike-protein-specific) antibody levels as well as functional neutralizing antibodies in serum at 1 month till 12 months following the second SARS-CoV-2 vaccination. Vaccine responses will be related to age, sex, co-morbidities and frailty characteristics.

Secondary endpoints; Vaccine-specific (Spike-protein-specific) antibody levels in serum at 1 month after the first SARS-CoV-2 vaccination. In addition, the possible interference of infection with SARS-CoV-2 in vaccine responsiveness will be determined by measuring serum antibodies to SARS-CoV-2 virus core proteins. For participants receiving a booster; Vaccine-specific (Spike-protein-specific) antibody levels in serum pre booster and at 1 month till 12 months following the booster SARS-CoV-2 vaccination.

Exploratory endpoints Cellular and serological biomarkers before vaccination will be identified and related to antibody responses to vaccination. Potentially, differences between vaccines might be assessed depending on the vaccines to be used in this age group of study participants in Doetinchem. In addition, antibody responses to SARS-CoV-2 vaccination in a frail sub cohort of older persons living in nursing homes will be determined from 6 months onwards after the last vaccination and related to those of age-matched persons in the DCS.

Nature and extent of the burden and risks associated with participation, benefit and group relatedness: The burden associated with participation involves collection of blood samples by finger pricks performed by the participant at home. In addition, the subject will be asked to fill in brief questionnaires. The potential risks of the finger prick are considered minimal. The results of the study may contribute to a better control of SARS-CoV-2 disease in older and middle aged persons.

Planning:

- First Participant, First Visit (FPFV): Feb-April 2021
- Last Participant, First Visit (LPFV): July-November 2021
- Last Participant, Last Visit (LPLV): Maart 2024

Vaccine responsiveness to SARS-CoV2 in aged persons

1. INTRODUCTION AND RATIONALE

Vaccination against SARS-CoV-2 is the most effective way to end the current pandemic. In the Netherlands vaccination will at first be targeted at health care workers and specific risk groups. Subsequently, older persons above 60 years of age and, in time, the entire adult population will be offered vaccination. Various vaccines will be used to the population, depending on national availability. Some Immune data is provided by the manufacturers before registration, but immune responses induced by the various vaccines may vary considerably between individuals, especially in older persons. High levels of neutralizing antibodies against SARS-CoV-2 Spike protein, inhibiting ACE2-receptor binding, are pivotal in preventing SARS-CoV-2 infection. Knowledge on systemic antibody responses, that will vary considerably in amplitude, longevity and quality per person, is crucial to estimate the protection they provide to the vaccinated individual against disease and possible effect on viral transmission. Monitoring the immune responses induced by the vaccines, especially in the older most vulnerable, population of the Netherlands, is needed. More generally, this will contribute to the understanding of vaccine-induced correlates of protection against disease and transmission and will assist public health policy in future vaccine strategies.

Ageing-related changes of the immune system, comprehensively referred to as immunosenescence, 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. Consequently, the population of adults over 50 years of age shows a huge heterogeneity in health status that even further increase during ageing. This diversity is caused by a complex interplay of physiological changes in metabolism, organ function, the immune system, and differences in exposure to risk factors over the life course. Little is known on the combined effects of these changes on the ability of the body to control infectious diseases and to vaccine responsiveness in older adults. (1-5).

Especially in older adults, community-acquired infection by Sars-Cov-2 by the current pandemic form a global problem causing millions of deaths worldwide While many younger people experience no or mild symptoms on infection, older adults are highly susceptible to life-threatening respiratory and systemic conditions (6-7). The emergence of SARS-CoV-2 and its inordinately rapid spread is posing severe challenges to all people and health care systems worldwide. Although vaccine effectivity have been shown by the Pharmaceutical companies, the very urgent question remains what the longevity of the vaccine response will be in older persons and especially in the most vulnerable persons. Data on vaccine immunogenicity in older persons who are at the highest risk of developing severe disease are of utmost importance. As indicated above protective responses induced by vaccination

Vaccine responsiveness to SARS-CoV2 in aged persons

tend to be much lower in older adults than in the young, but there is an enormous inter-individual variation in older people. The high vaccine effectivity of the new vaccines based on mRNA suggest that these might induce good adaptive immune responses at least shortly post vaccination. Therefore, identification of immune responses to the SARS-CoV-2 vaccines and determination of their kinetic patterns during time are critical to address.

We recently constructed and validated a frailty index in older adults by documenting the accumulation of 36 co-morbidities of all participants in the unique longitudinal Doetinchem cohort study (DCS) currently comprising persons of 52-90 years of age (N≈3700) (8-10). This cohort study is still running (NL63779.041.17) and offers us the opportunity to study immunogenicity to the SARS-CoV-2 vaccines in middle-aged and older persons of the Dutch population. The parameters already present of chronic diseases, overweight, measures of physical activity, cognitive functioning and life style habits, resulted in a validated frailty index which is indicated in appendix A. We have shown recently that frailty in persons 60-85 years of age was associated with increased low grade inflammation indicated by elevated CRP trajectories and altered myeloid cell numbers (10). By using such detailed information we can further relate the vaccine responsiveness to various risk-groups in the population. So persons can be identified who not only will be more vulnerable for infection but also for vaccine responsiveness that even may last less longer than in the most healthy older persons at an individual level. By studying frailty and inflammatory parameters in combination with SARS-CoV-2 vaccination responses, we will be able to relate the inflammatory state of the immune system to vaccine responsiveness. Such a predictive immune profile is highly needed to improve vaccination strategies or alternative interventions for the more vulnerable group of older adults

In this study, several antibody responses induced by the current new SARS-CoV-2 vaccines will be measured in middle-aged and older men and women (52-90 years) and compared with respect to their health status. Immunoassays to detect various SARS-Cov-2 specific antibodies have been developed and validated by our department at the RIVM (ref). Evaluation of vaccine efficacy is not an objective of the study, but we will monitor the subjects for SARS-CoV-2 like illness and complaints during the study follow up. Moreover, the possible interference of infection with SARS-CoV-2 with vaccine responsiveness will be determined by measuring virus-specific serum antibodies to SARS-CoV-2 core protein of the virus and questionnaires on positive PCR tests as well.

In addition, when logistics of the vaccination strategy gives us the opportunity, data on biological baseline parameters will be collected to identify individuals with potential lower

Vaccine responsiveness to SARS-CoV2 in aged persons

resilience and predictors for vaccine responsiveness. These parameters will include parameters considered to be associated with a low grade inflammation and immunosenescence i.e.. C-reactive protein (CRP), and numbers of monocytes, neutrophils and lymphocytes.

In conclusion, vaccine responses in individuals aged 52-90 years in the DCS and relation to co-morbidities or frailty will be evaluated using state of the art statistical analysis. In addition, antibody responses to SARS-CoV-2 vaccination in a sub cohort of older persons living in nursing homes that represents the most frail elderly, will be determined from 6 months onwards after the last vaccination and related to those of age-matched frail persons in the DCS.

Early recognition of unfavorable 'biological ageing' may be useful in conceiving approaches for further vaccination strategies or alternative interventions for risk groups to be able to prevent possible SARS-CoV-2 infection in middle aged and older persons.

In case the government decides to recommend booster vaccination in this age group, amendment 3 will go into place. In those participants who take this booster vaccination vaccine-specific antibody levels pre and post vaccination will be followed after informed consent. This follow-up can be repeated in case of booster vaccination with any other (booster) vaccine against SARS-CoV-2, using this similar basic structure of 4 fingerpricks (pre-vaccination, 28 days, 6 months and 12 months after vaccination). In this case other sampling moments will expire or are pushed forward.

The 3th vaccination for specific immune compromised target groups is not seen as a booster vaccination but is part of the primary series.

Vaccine responsiveness to SARS-CoV2 in aged persons

2. OBJECTIVES

The overall aim is to address the magnitude, quality and persistence of the antibody responses to SARS-CoV-2-vaccinations in the Dutch population of above 50 years of age. Furthermore, inter-individual differences in the presence of co-morbidities, frailty characteristics and parameters of lingering inflammation will be related to vaccine responsiveness using state of the art statistical analyses. This may lead to individualized approaches to future vaccination strategies or alternative supportive interventions that might better protect vulnerable older persons to SARS-CoV-2 infection.

2.1 Primary objective

- Assess the SARS-CoV-2 vaccine systemic antibody responses in 52-90 years old male and female persons in the DC at a month after the last vaccination.
- Determine the kinetics and longevity of SARS-CoV-2 antibody responses based on the data at all timepoints related to frailty in 52-90 years old male and female persons in the DC.
- Assess the relation of age, frailty and specific co-morbidities in 52-90 years old male and female persons with antibody responses to SARS-CoV-2 and identifying subgroups of individuals more at risk for lower vaccine responsiveness

2.2 Secondary objectives

- Assess the SARS-CoV-2 vaccine systemic antibody responses in 52-90 years old male and female persons in the DC at a month after the first vaccination and comparison of these data with those at a month after the last vaccination.
- Assess the possible interference of infection with SARS-CoV-2 with vaccine responsiveness, by measuring virus-specific serum IgG antibodies to SARS-CoV-2 Core protein and using data on the results of PCR testing and from questionnaires
- For participants taking a booster dose; vaccine-specific (Spike-protein-specific) antibody levels pre booster and 28 days (-7 till +14 days) till 12 months (+/- 4 weeks) following the booster SARS-CoV-2 vaccination.

Vaccine responsiveness to SARS-CoV2 in aged persons

In subgroups of participants functional antibodies will be measured at 28 days (-7 till +14 days) , 6 months (+/- 4 weeks) and 12 months (+/- 4 weeks) post booster vaccination.

2.3 Exploratory objectives

- Assess numbers of immune cells as well as the inflammatory status at baseline and integration of these data for their association with age and vaccine responsiveness.
- If different vaccines will used in this age group of persons, comparison of antibody responses between these vaccines will be monitored.
- In addition, antibody responses to SARS-CoV-2 vaccination in a sub cohort of frail elderly living in nursing home, male and female above 50 years of age, will be determined from 6 months onwards after the last vaccination and related to those of age-matched frail persons in the DCS.

3. STUDY DESIGN

All persons still participating in round 6 (2013-2017) of the DCS and not participating in other studies currently running (n=3200) will be invited for participation in the current study.

Persons 52-60 years of age will be vaccinated later this year and will receive a reminder.

After approving participation, pre vaccination (T0) finger prick EDTA blood will be sampled for assessing both the numbers of immune cells and biomarkers in whole blood, and for possible baseline SARS-COV2 antibodies as well. In persons who already received the first vaccination, T1 or incidentally T2 will be the first sampling point. Participants will be vaccinated with one of the SARS-CoV-2 vaccine either via the normal procedure by the GPs, or provisional vaccination sites. They will communicate the date of vaccination to the coordinating study investigators. Before vaccination (T0), at 1 month after the first vaccination (T1), at 1 month (T2) at 3 month (T3), at 6 months (T4) and at 1 year (T5) post vaccination, serum will be sampled by finger pricks. We will assess IgM, IgG and IgA antibody responses and functional antibody responses to vaccination and if this is impacted by frailty and specific co-morbidities. Depending on the results, an extra timepoint at 9 month post vaccination will be included. The blood collections by finger prick will be performed by the participant him/herself, a family member or a care taker. Study nurses at the RIVM or at the GGD will be stand by for questions and further assistance when needed.

Via the questionnaires, possible SARS-CoV-2 related illness and other health questions will be monitored (figure 1).

Vaccine responsiveness to SARS-CoV2 in aged persons

Optional part COVID-19 booster vaccination

In case of SARS-CoV-2 booster vaccination, participants will be asked if they want to participate in extra sampling timepoints. They will communicate the date of booster vaccination to the coordinating study investigators. Before vaccination, at 28 days (-7 till +14 days) after the booster vaccination, at 6 months (+/- 4 weeks) and at 12 months (+/- 4 weeks) post (last) booster vaccination, serum will be sampled by finger pricks at home by the participants themselves. The timing of the booster in relation to the original study timepoints will determine whether the original timepoints take place or can/will be used as pre booster vaccination timepoint. If booster vaccination will take place <28 days after a previous blood draw, this blood sample will be used as pre booster vaccination sample. Other sampling timepoint (e.g. 6 and 12 months post booster will be pushed forward).

Blood samples received out of window will still be used for the study analyses (Intention To Treat analyses).

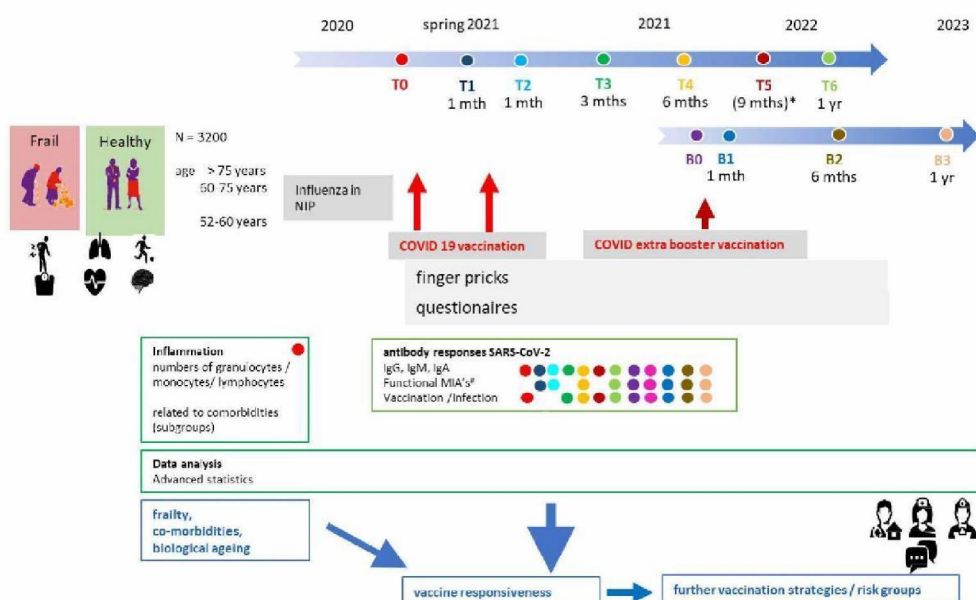


Figure 1. Study design. Persons of middle and older age, 52-90 years, participating in the Doetinchem cohort study, for assessing SARS-CoV-2 antibody responses post vaccination in relation the biological ageing. *= optional time point. In case of a booster vaccination participants can continue with B0-B3. Depending on the timing of the booster vaccination time points of the original study can be skipped. #= Functional MIA's; optional, will not be tested at all timepoints.

Vaccine responsiveness to SARS-CoV2 in aged persons

Older participants of the DCS are home dwelling. To also collect vaccine immune response related information on extremely frail elderly a group of older persons living in nursing homes will be added to the study. Approximately 450 persons, above 50 years of age, living in nursing homes and capable of signing an ICF, excluding incapacitated, will be invited for participation in the current study from T4 (6 months post-vaccination) onwards. The blood collections by finger prick will be performed by a RIVM hired study nurse at the nursing home locations. A short questionnaire will be included for information on age, gender, vaccination background, possible SARS-CoV-2 related illness background, comorbidities and important health questions. If needed, the nursing home practitioner will be contacted for additional or lacking information on vaccination background, possible SARS-CoV-2 related illness background and important health questions.

Vaccine responsiveness to SARS-CoV2 in aged persons

4. STUDY POPULATION**4.1 Population (base)**

Adult persons (52-90 years of age), having participated in round 6 (2013-2017) of the DCS and not participating in studies currently running will be invited for participation (n=3200).

Participants above 50 years of age living in a nursing home will be invited for participation (n=400-500).

4.2 Inclusion criteria

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

- Having participated in round 6 of the Doetinchem Cohort study or living in a nursing home
- Willing to receive the SARS-CoV-2 vaccine or received the vaccinations not longer than 1 month in advance for the DC participants or no longer than 7 months ago for nursing home residents
- Willing to sign the Informed Consent.

4.3 Exclusion criteria

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

- Participant of the DC already received their last SARS-CoV-2 vaccination more than 1 month before signing the ICF, so sampling at T2 and further is not possible.
- participants living in a nursing home already received both SARS-CoV-2 vaccinations more than 7 months before signing ICF.
- Incapacitated participants

4.4 Temporary exclusion criteria

Not applicable.

Vaccine responsiveness to SARS-CoV2 in aged persons

4.5 Withdrawal criteria during study

Receipt of any high-dose (≥ 20 mg of prednisone daily or equivalent) daily corticosteroids (local incl. inhaled steroids are acceptable) within 2 weeks of sampling, or, as medically prescribed, within two weeks after the vaccination.

4.6 Sample size calculation

This is an observational study for which all current participants in the DCS will be invited, at the occasion of their being offered vaccination against the virus as part of national health care. The study population may be considered to be fairly representative of the general Dutch population aged 50 years or above that is going to be vaccinated. In total approximately 3200 persons will be invited, which is expected to result in approximately 1500 participants being included in study, taking an estimated vaccination uptake of about 80% and a participation rate of about 60% into account.

The primary objective of the study is to assess the immunogenicity of SARS-CoV-2 vaccinations in middle aged and older persons, and relate this to age, sex and frailty characteristics including co-morbidities. Immunogenicity will be evaluated on the basis of measurements of antibody levels at 6 points in time, which can be considered as continuous variables. When antibodies remain high at 6 months post vaccination a, 7th time point at 9 months post vaccination will be included.

The ultimate sample size, which is beyond our control, should be sufficient to be able to develop a multivariable prediction model with antibody levels as dependent (continuous) variable, with the possibility of internal (split sample or cross-) validation. Data on antibody responses to SARS-CoV-2 are still scarce, but measurements of antibodies (virus-specific anti-S1 and/or RBD) in people who have been infected may provide an indication. For example, Jennifer M. Dan, et al. (Immunological memory to SARS-CoV-2 assessed for up to 8 months after infection. *Science* 10.1126/science.abf4063 (2021), measured anti-RBD IgG antibodies at various points in time after COVID-19 infection, in cases of different ages. Standard deviation (calculated from the supplementary data file) for the individuals above 50 years of age was 1.5 (units/volume) on the log-scale versus 1.4. in the whole group. With the expected sample size, this would provide virtually 100% power to detect a two-fold difference in mean values between two groups (for instance frail versus non-frail persons).

Moreover, with all continuous data being used as continuous parameters, the sample size should be sufficient to develop a multivariate model that "predicts" the antibody responses based on co-morbidities, frailty, frailty characteristics, numbers of immune cells at

Vaccine responsiveness to SARS-CoV2 in aged persons

baseline, and biomarkers in blood as predictors, allowing also internal validation of the model using cross-validation or a boot strapping method. This will lead to sufficient power to be able to find associations of vaccine responsiveness between frail and non-frail men and women, and even for subgroups based on age and specific frailty parameters or co-morbidities (13-14) Samson et al, 2019; Samson, Van den Berg et al, 2020).

For the third explorative objective, we will invite 400-500 nursing home residents who are aged above 50 years. However, the majority of these persons, 85%, is above 70 years of age and 70% is above 75 years age. Based on all our experience in population based studies and the minimal invasive blood sampling by just a finger prick, we expect that at least 30% of these invited nursing home residents will participate. This may lead to the participation of more than 100 most frail persons above 75 years of age

In the current VIDO participants living at home, about 150 persons are above 75 years of age who show a variety in frailty index from more healthy to more frail. By using both frailty data and antibody units as continuous parameters, this will lead to sufficient power to be able to find a possible difference in vaccine responsiveness at 6 months and 1 year post vaccination between older persons living at home and the most frail persons living in nursing homes.

For a possible booster vaccination, the number of participants depends on the amount of persons that take the booster vaccination and want to continue participating in the booster part of the study.

5. TREATMENT OF SUBJECTS

Not applicable.

Vaccine responsiveness to SARS-CoV2 in aged persons

6. INVESTIGATIONAL PRODUCT

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

6.1 Name and description of investigational product(s)

Not applicable.

The SARS-CoV-2 vaccines have been authorized and will be used at the time of study inclusion for the age-group in this study.

6.2 Summary of findings from non-clinical studies

Not applicable.

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

Not applicable.

Vaccine responsiveness to SARS-CoV2 in aged persons

8. METHODS**8.1 Study parameters/endpoints****8.1.1 Main study parameter/endpoint**

Circulating IgG, IgM and IgA antibodies concentrations (specific for SARS-CoV-2 spike protein), at all timepoints after the second or last vaccination (T2-T6). Antibody levels will be measured by bead-based multiplex immune assay in blood obtained by finger prick at the different time points. Neutralizing antibodies will be determined at 1 month (T2), 6 months (T4) and 1 year (T6) post vaccination.

8.1.2 Secondary study parameters/endpoints

1. Antibody levels specific for SARS-CoV-2 pre vaccination (T0) and at 1 month after the first vaccination (T1)
2. IgG antibody titers to other Coronaviruses at the study timepoints.
3. Self-reported experiences with possible SARS-CoV-2 infection from answers to a short questionnaire.
4. Integrated endpoint analysis of all antibody analysis with frailty data and data of specific co-morbidities (subgroups).
5. Circulating antibody concentrations (specific for SARS-CoV-2 spike protein), at all timepoints after the booster vaccination (B0-B3). Antibody levels will be measured by bead-based multiplex immune assay in blood obtained by finger prick at the different time points. Functional antibodies will be determined in subgroups at 1 month (-7 till +14 days) (B1), 6 months (+/- 4 weeks) (B2) and 12 months (+/- 4 weeks) (B3) post vaccination.

8.1.3 Exploratory endpoints:

1. Absolute numbers of immune cells: lymphocytes, granulocytes and monocytes, in whole EDTA blood pre vaccination T0 or otherwise T1.
2. Inflammatory profiles by measuring biomarkers associated with frailty, such as CRP creatinine and cystatin C in plasma pre vaccination.
3. Antibody responses to SARS-CoV-2 vaccination in a sub cohort of older persons living in nursing homes will be determined from 6 months onwards after the last vaccination and related to those of age-matched frail persons in the DCS.

Vaccine responsiveness to SARS-CoV2 in aged persons

8.2 Randomization, blinding and treatment allocation

Not applicable.

8.3 Study procedures

DCS participants:

Persons still participating in the DCS that received study information and show interest in the study and are willing to be vaccinated will receive an instruction letter, a self-sampling set, login code for access to the digital questionnaire and an informed consent form (pre-signed by investigator). The informed consent form (ICF) is signed by the subject (described in section 11.2).

All potential participants will be marked at the start of the study if they fall within the upper 20% of the frailty index of the DCS and also if they scored 1 at the cognition tests. If people participate that are marked they will be contacted by phone before receiving their first self-sampling set with IC.

The participants are requested to fill out the digital questionnaire (or questionnaire on paper, if they indicated they preferred that). In addition, the participants will be asked to donate finger prick blood sample (maximum of 0.8 ml) 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. Personnel at the RIVM or study nurses at the GGD will be stand by for further assistance. They will contact persons who have indicated that on the reply card.

The self-sampling and questionnaire is repeated 6-7 times during the study.

Nursing home participants:

Blood samples from participants living in a nursing home will be collected by RIVM hired study nurses at the nursing home.

To reduce the burden for conduct of the study by the nursing home staff, mobile study nurses will be hired to conduct the finger prick and provide support with completing the questionnaires. The mobile nurse will ship the blood samples to the RIVM and completed questionnaires. These mobile study nurses are contracted by the sponsor of the study and are qualified and trained for the tasks assigned to them. The study nurses work under supervision of the sponsor.

Sampling and questionnaire is repeated 2-3 times during the study.

In case of boostervaccination(s) the participants will receive an extra instruction letter and informed consent. When the participant decides to participate in the extra blood sampling time points they will sign the informed consent. Sampling and questionnaire is repeated a maximum of 4 times for each booster vaccination. All following steps are continued as before.

Vaccine responsiveness to SARS-CoV2 in aged persons

Laboratory:

Upon arrival at the laboratory, blood samples will be processed and subsequently plasma (T0) and serum (T1 and onwards) will be aliquoted and stored at -80 °C. At T0, whole blood cell analysis will be performed.

Serum samples will be tested for the presence of SARS-CoV-2 specific antibodies by quantitative multiplex serology (Luminex), against appropriate controls (SARS/MERS and human coronaviruses). Selected SARS-CoV-2 positive serum samples will be tested for antibody binding strength (avidity) and virus neutralization, providing a quantitative measure of immune protection.

8.4 Other study procedures

8.4.1 Antibody analysis in serum and plasma

Plasma and serum will be used for all systemic IgG, IgM and IgA antibody measurements by bead-based immunoassay specific for SARS-CoV spike and core proteins. At 1 month, 6 months and 1 year post vaccination neutralizing antibodies will be determined by SARS-CoV-specific neutralizing assay. Both assay have been developed and are currently running at our institute (Den Hartog et al, 2020, 13,14). Post booster vaccination SARS-CoV-specific (functional) antibodies will be determined at 1 month, 6 months and 12 months.

8.4.2 Plasma

Plasma at T0 will be stored in aliquots for frailty markers such as CRP, creatinine and cystatin C and as a potential back up for other serological assays.

8.4.3 Absolute numbers of cells in whole blood

Whole blood from EDTA tube will be used for the determination of numbers of lymphocytes, granulocytes monocytes and thrombocytes by an hemo-analyzer.

8.4.4 Questionnaires

A short questionnaire will be filled in focusing on experiences of infectious diseases, in particular flu-like diseases including possible SARS-CoV-2 infection.

Vaccine responsiveness to SARS-CoV2 in aged persons

8.5 Withdrawal from the study

Subjects can leave the study at any time for any reason if they wish to do so without any consequences. If a subject decides to withdraw from the study, the samples and data collected up to that point will be used in the analysis unless the subject specifically requests not to include or to remove his/her data from the analysis. In case the subject doesn't approve storage of the collected samples after the study is finished, samples will be destroyed after the study analysis is considered complete. The investigator can decide to withdraw a subject from the study for urgent medical reasons or safety concerns. In that case, the subject will be followed until the urgency of their condition will 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.

Participant without booster vaccination: If a participant decides not to take a SARS-CoV-2 booster vaccination nothing will change for the participant.

Participant with booster vaccination not willing to participate in booster study:

The participant cannot finish the study as originally intended. The study team will contact the participant and will ask if the participant is willing to take a sample before and/or 1 month after the booster vaccination instead of the T5 and T6 timepoint from the original protocol. The demand for the participant will not change compared to the original protocol. If participant doesn't want this the study will end for the participant.

Participant with booster vaccination not willing to participate in the follow up

The participant cannot finish the study as originally intended. The study team will contact the participant and will ask if the participant is willing to take a last blood sample 1 month after the 4th vaccination instead of the other time points earlier indicated. If participant doesn't want this the study will end for the participant.

8.6 Specific criteria for withdrawal

Not applicable.

8.7 Replacement of individual subjects after withdrawal

Not applicable. All subjects invited are allowed to participate.

Vaccine responsiveness to SARS-CoV2 in aged persons

8.8 Follow-up of subjects withdrawn from treatment

Not applicable.

Vaccine responsiveness to SARS-CoV2 in aged persons

8.9 Premature termination of the study

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, and if this is confirmed by the METC. The METC will be informed about the decision. Premature termination has no effect on the subjects.

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.

Vaccine responsiveness to SARS-CoV2 in aged persons

9. SAFETY REPORTING

SARS-COV-2 vaccines 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. These vaccines are given by the participants' own GP or the GGD as part of the routine immunization program for this age group, not as part of this study. We will therefore not collect and report any AE, SAE or SUSAR regarding vaccination. This is the responsibility of the GP.

We will only report on AEs related to the blood collection, in predefined time periods.

9.1 Temporary halt for reasons of subject safety

In accordance with section 10, subsection 4, of the WMO, the sponsor will suspend the study if there is sufficient ground to conclude that continuation of the study will jeopardize subjects' health or safety. The sponsor will notify the accredited METC without undue 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 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. All adverse events reported spontaneously by the subject or observed by the investigator or his staff will be recorded.

The study staff will only record any adverse event spontaneously reported by the subject related to and occurring within one week after blood sampling.

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;

Vaccine responsiveness to SARS-CoV2 in aged persons

- 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.

An elective hospital admission will not be considered as a serious adverse event.

Subjects will be asked to report to the study staff any serious adverse event related to blood collection occurring within a week after blood sampling.

The investigator will report all SAEs to the sponsor (RIVM) without undue delay but not later than 24 hours after obtaining knowledge of the events, except for the following SAEs.

All SAE's occurring 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.

If a subject reports an(S)AE in the month after vaccination, possibly related to vaccination, we will take the following steps; ask the subject if the (S)AE has been reported to Lareb (by the subject/GP/GGD); if not ask the subject to report to Lareb; if the subject is not willing/able to report to Lareb, ask the subject if we can report to Lareb.

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:

- the event must be serious (see chapter 9.2.2);
- 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;

Vaccine responsiveness to SARS-CoV2 in aged persons

- 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.

These vaccines are given by the GP or GGD as part of the routine immunization program of this age group, not as part of this study. The GP is responsible for the correct handling of any side effects regarding vaccination. We have no access to the medical records related to a SUSAR. If a SUSAR occurs and the study team is notified by the participant, it's family or a caretaker we will contact the METC if reporting through ToestingOnline is needed.

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.

Vaccine responsiveness to SARS-CoV2 in aged persons

10. STATISTICAL ANALYSIS

The data consist of results from laboratory analyses of the donated blood, the answers to the health questionnaire, and what is known about their health status from the DCS. Analyses will be performed using appropriate packages from the R software platform, and in SAS.

10.1 Primary study parameter(s)

Concentrations of antibodies against SARS-CoV-2 will be determined at all time-points.

Summary statistics for concentrations and titers of neutralizing antibodies will be expressed and stratified by age and sex. Longevity and kinetics of antibody concentrations over time per person will be analyzed

Data will be weighted for age and gender according to the Dutch population.

The main outcome in this study is SARS-CoV-2, vaccine responsiveness as measured by antibody levels. The primary aim will be to determine to what extent antibody levels depend on sex, age, frailty index and co-morbidities or frailty characteristics. For analysis, antibody levels will be considered as continuous "response" variables. Also, the frailty index and in additional analyses individual frailty characteristics will be used as a continuous variable. Multiple covariates and their interactions will be explored by linear regression models. If needed the Benjamini-Hochberg method will be used to take into account multiple testing. Further analysis is described below in 10.3.

Analysis as described above will also be used for the booster time points.

10.2 Secondary study parameter(s)

10.2.1 Absolute numbers of Cells

Analyses of white blood cell, granulocytes and monocytes will result in absolute numbers of various subsets of immune cells (plus confidence intervals). For further analysis, absolute numbers will be transformed in order to obtain a more or less normal distribution, for instance using a log-transformation.

10.2.2 Plasma Biomarkers

Biomarkers like CRP in plasma will be presented as concentrations. If appropriate, values will be transformed for further analysis. Groups (as defined in Section 3) will be compared using t tests when (transformed) values are approximately normal in distribution.

10.2.3 Health questionnaire

The results of the health questionnaire will be presented as descriptive statistics.

10.3 Associations of baseline frailty characteristics with baseline immunological parameters and further with vaccine responsiveness

By linking the data of the participants to their files in the DCS, a detailed description will be given of the study population based on demographic descriptors (age, sex, social-economic status), exposure to risk factors such as smoking status, BMI, and various aspects of health status, such as the presence or absence of diabetes. Categorical variables will be summarized by means of frequency counts and percentages, whereas for continuous variables mean, standard deviation, minimum, maximum, or median, where appropriate, will be presented.

In addition to the main analysis relating antibody levels to the frailty index, a number of association studies will be performed to investigate in greater depth which characteristics and aspects of frailty influence antibody responses and how frailty is related to the various leukocyte subset numbers. Also associations will be tested between antibody levels and biomarkers. This will be done in men and women separately, using robust non-parametric methods, which depend on fewer assumptions regarding the underlying data, such as normality of distribution and linearity, and accounting for multiple testing. For the aim to be able to identify predictors of reduced antibody responses, we aim to gain more insight into the underlying biology of the immune response to SARS-CoV-2 vaccination, for which we employ a more explorative approach.

In order to develop a comprehensive prediction model that is able to identify individuals at increased risk of an insufficient immune response to vaccination multivariate analyses will be performed. For these, random forest algorithms will be used, the various potential predictors including frailty characteristics and measured biomarkers are entered as variables and the best predictors in a multivariate model are identified. (Samson et al, 2020, 13,14)

No interim analysis is planned for this study.

11. ETHICAL CONSIDERATIONS

11.1 Regulation statement

The study will be conducted according to the principles of the World Medical Association Declaration of Helsinki and its amendments effective since 1964 and in accordance with the Medical Research Involving Human Subjects Act (WMO). The guideline ICH Good Clinical Practice (GCP) will be followed, however it is foreseen that some items cannot be adhered to, mainly because of logistical reasons. The ICF, pre-signed by the investigator, will be countersigned at home by the subject and self-sampling by fingerpick and completion of the questionnaire will be taken place at home.

11.2 Recruitment and consent

DSC participants:

All persons still participating in the DCS who have participated in round 6 of the DCS (n=3200) will be invited for participation in the current study.

Recruitment will be done by a personal letter inviting the subjects to participate. It will be made clear that the participants are invited because they take part in the DCS. Home addresses will be obtained from their DCS files.

The subjects will receive the personal invitation letter including a Patient Information Form (which includes the Informed Consent Form (ICF)) and a reply card, by mail before the first finger prick will take place. A telephone number and an email address will be provided for questions. If the subject decides to participate, he/she will be asked to respond via the website or email preferably or otherwise sending in a reply card.

In advance the study subjects will be categorized based on their frailty index which is already known from DCS. When the frailty index of a person indicating to participate is within the 20% highest value of the group and also if a person scores 1 at the cognition tests, we will contact the participant by telephone before receiving their first self-sampling set. By this we will explain the content of the package they will receive, the procedure to sign the informed consent form and the procedure of the finger prick blood sampling. We will further emphasize that they can phone us or the study nurses of the GGD location in Doetinchem for further help with signing the IC form and the finger prick sampling after receiving package. Shortly after the phone call of the study team the finger prick sampling set will be received.

Participants will be asked to fill in the questionnaire via the website but will be given the opportunity to indicate whether they want to receive a questionnaire on paper instead of a digital questionnaire. Subjects can also indicate on the replay card if they would like to be

Vaccine responsiveness to SARS-CoV2 in aged persons

called at the moment they receive their fingerstick package so information can be provided on how to sign the ICF and/or how to execute the self-sampling set.

Subsequently, the potential participants will receive an instruction letter, a self-sampling set with detailed instructions, a login code for access to the digital questionnaire (or a questionnaire on paper) and an informed consent form (pre-signed by investigator). The ICF, pre-signed by the investigator, will be countersigned at home by the subject and returned to the investigator together with the blood sample and the potential hard-copy completed questionnaire in the stamped, addressed safety-envelope. Subjects that indicated on the reply card to want assistance will be called when they receive their first self-sampling set with ICF.

During the study a health care professional at the RIVM will be stand by for questions and further explanation regarding the finger prick procedures.

The participant will be asked to inform us of the vaccination dates and the vaccine name via the website, email or paper (reply form), and in the questionnaires. In case of uncertainty about the vaccination details given by the participant, information can be extracted from the "COVID-vaccinatie informatie en monitoringsysteem (CIMS)" of the RIVM. The participant will be asked to give consent for this.

Subsequently the participants will receive a self-sampling set at respectively T1-T5 with the detailed instructions.

Nursing home participants:

The nursing home geriatrician will provide potential subjects who might be eligible for participation. With an invitation letter, reply card and patient information & informed consent form. Also a telephone number and an email address will be provided for questions. If a nursing home resident indicates he/she will participate the reply card will be given to the nursing home geriatrician who will send all reply cards to the RIVM appointments for home visits at the residence will be made to perform the study related procedures. After the resident provides consent a mobile nurse will perform the blood draw and collect the questionnaire. After the visit the mobile nurse will provide the collected items to the sponsor.

For the booster timepoints participants will receive an information letter and IC form. In case they are willing to participate they can send in a signed IC. They will inform the team of the date of booster vaccination. Study procedures will continue as before with fingerprick sets and questionnaires.

Vaccine responsiveness to SARS-CoV2 in aged persons

11.3 Objection by minors or incapacitated subjects (if applicable)

All subject are adults capable of giving informed consent.

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

11.4 Benefits and risks assessment, group relatedness

Benefits

There is no personal benefit for the subjects.

Risks assessment

All subjects will receive an offer for the current SARS-COV2 vaccination(s) and the SARS-CoV-2 booster vaccination. The vaccines will be administered by their own GPs or GGD, not as part of this study.

The risk of blood via finger pricks sampling is considered low.

Group relatedness

SARS-COV2 infection is a disease with a high disease burden in the elderly. The results of the study may contribute to adaptations in vaccination schedules for risk groups and thereby to a better control of SARS-COV2 disease in older age groups on a population level in the future.

11.5 Incidental findings

Incidental findings are unexpected observations or test results that have a potential clinical significance for the subject's health or the health of his relatives. The study, includes (in-depth) analysis of a lot of different components of the immune system and general health related parameters (e.g. CRP, cystatin C, creatinine). All incidental (expected and unexpected) findings that might indicate potential treatable health problems will be communicated with the subject's general practitioner, who will be asked to discuss the finding with his patient.

11.6 Compensation for injury

The METC has decided that participating in the study is without risks, and has granted the RIVM dispensation from the compulsory participants' indemnification as laid down in article 7, paragraph 5 of the WMO. According to a Ministerial Order, RIVM is

Vaccine responsiveness to SARS-CoV2 in aged persons

excluded from compulsory liability insurance for clinical research as determined by the Dutch law on Medical Investigations (WMO, section 7, paragraph 10). Any liability claims should be directed to the RIVM.

11.7 Incentives

After T4 of this study, the participants will receive a newsletter and a gift with a value of about 5.1.2b

Subjects participating in the SARS-CoV-2 booster vaccination part will receive a gift with a value of about 5.1.2b after B3.

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 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. The identification key will be stored at the RIVM. The principal investigator of the study or a designated representative will be responsible for the key to the code. Pseudonymization is already 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. Full anonymization of data of individual data subjects will not be feasible in this project since we will combine data from this study with the data previously collected in the Doetinchem Cohort Study. Data will be accessible to authorised, trained persons only.

Clinical Data Management is a crucial part of a clinical study and includes, Case Record Forms (CRF), 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 study.

All protocol required information collected during the study samplings will be recorded directly on (electronic) Case Record forms, these entries will be considered source data, as these recordings will be made during the visits. Personal identifiers will not be recorded on the CRF. CRF and questionnaire data will be digitalized, if not already digital, and stored on a secure national server. 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 in the

Vaccine responsiveness to SARS-CoV2 in aged persons

Netherlands 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

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 the RIVM 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 study lead investigators.

After project closure, all data (taking into account the requirements for data minimization) is stored on a secured network for at least 20 years. An exact retention period is not possible to be provided. The RIVM is obliged to keep (at least part of) the data for an indefinite period of time on the basis of the Archives Act as this research will probably be included in the COVID-19 file and will be kept in the archive longer than other studies.

12.1 Handling and storage of samples

All collected samples will be coded as described in section 8 and stored at the RIVM for 25 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. 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 as mentioned in the protocol/PIF. These subjects are; scientific research on infectious diseases, vaccination, the immune system and ageing.

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.

Vaccine responsiveness to SARS-CoV2 in aged persons

12.2 Monitoring and Quality Assurance

A detailed risk based monitoring plan will be written by the sponsor detailing the monitoring activities and frequencies. After the initiation visit, the subject recruitment can start and the first monitoring visit will be scheduled after the first subjects have been enrolled in the study to 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, monitoring visits will be performed, as described in the monitoring plan. 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 as described 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.

12.3 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 study; or
- the scientific value of the study; or
- the conduct or management of the study or
- the quality or safety of any intervention used in the study.

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 sponsor.

Vaccine responsiveness to SARS-CoV2 in aged persons

12.4 Annual progress report

The sponsor/investigator will submit a summary of the progress of the study 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 study, serious adverse events/ serious adverse reactions, other problems, and amendments.

12.5 Temporary halt and (prematurely) end of study report

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

The sponsor 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 sponsor 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 study, the investigator/sponsor will submit a final study report with the results of the study, including any publications/abstracts of the study, to the accredited METC and the Competent Authority.

12.6 Public disclosure and publication policy

The study results will be reported in progress reports and submitted for publication in peer-reviewed journals. Dissemination of the results will be as soon as possible in an open access journal after a review and approval process of the partners.

Vaccine responsiveness to SARS-CoV2 in aged persons

13. STRUCTURED RISK ANALYSIS**13.1 Potential issues of concern**

Not applicable.

13.2 Synthesis

Not applicable

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Vaccine responsiveness to SARS-CoV2 in aged persons

15. Amendment 1

15.1 Goal of amendment

This amendment concerns:

Submission of the VIDO study as drug research at the request of the METC.

Vaccine responsiveness to SARS-CoV2 in aged persons

16. Amendment 2

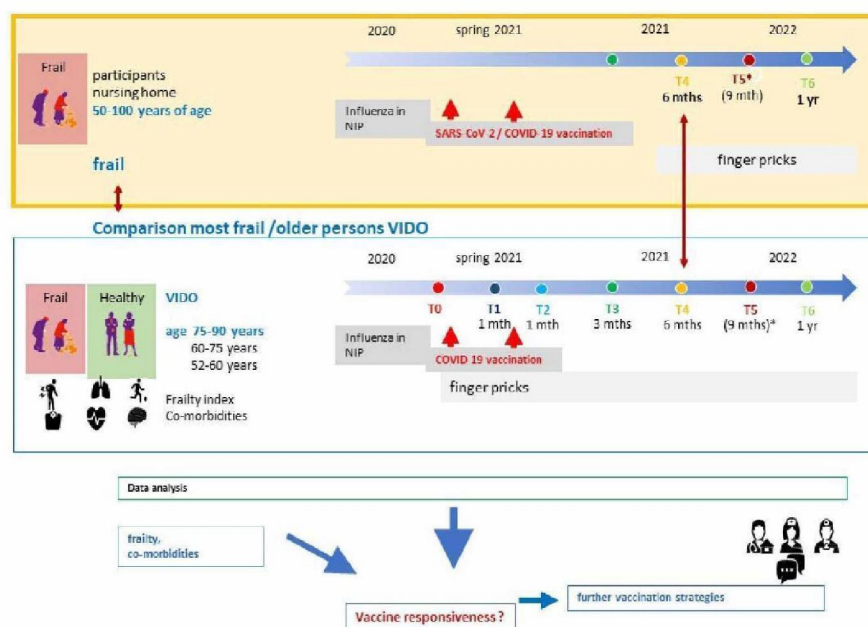
This amendment concerns:

Addition of a group of the most frail persons, people living in a nursing home, to assess COVID-19 vaccination induced antibody responses in this extremely frail group of people.

Early recognition of potential unfavorable vaccine responsiveness may be useful in conceiving approaches for further vaccination strategies for risk groups to be able to prevent possible SARS-CoV-2 infection in these frail older persons

Persons aged 50-100 years of age (N=400-800) living in nursing homes and capable of signing an ICF will be invited for participation in the current study from T4 (6 months post-vaccination) onwards. The blood collections by finger prick will be performed by a study nurse at location. Antibody responses to SARS-CoV-2 in this sub cohort of frail elderly will be determined from 6 months onwards after the last vaccination and related to those of age-matched frail persons in the DCS. Persons living at nursing homes will be categorized as being most/more frail related to the frail participants of the DCS cohort.

Nursing home participants being vaccinated with a COVID-19 vaccine and able to sign an informed consent will be invited by an information letter and receive additional study information. After sending the return card, persons are approached to make an appointment for blood sampling. At the first appointment ICF is signed. The blood collections by fingerprick will be performed by a study nurse at location. The nursing home practitioner or GP will be contacted for further information on vaccination background, possible SARS-CoV-2 related illness background and important health questions.



Vaccine responsiveness to SARS-CoV2 in aged persons

17. Amendment 3

17.1 Goal of amendment

- Follow the immune responses after SARS-CoV-2 booster vaccination, similar to the scheme as originally planned.
- Recruitment of extra nursing home participants for booster part of the study
- Administration of 3th SARS-CoV-2 vaccination.

17.2 Justification for amendment

- In case the Government decides to advice SARS-CoV-2 booster vaccination in this age group, amendment 3 will go into place. In that case we will follow the SARS-CoV-2 booster vaccination, in those participants that take the SARS-CoV-2 booster vaccination. This will result in extra timepoints; Pre booster (B0), at 28 days (-7 till +14 days) (B1) post, 6 months (+/- 4 weeks) (B2) and 12 months (+/- 4 weeks) (B3) post booster vaccination. At these timepoint vaccine-specific (functional) antibody levels pre and post vaccination will be measured. Depending on the timing of the SARS-CoV-2 booster vaccination original study timepoints can be skipped. At all timepoints fingerprick blood will be sampled for antibody responses, as well as questionnaires.
- In September 108 nursing home participants were recruited for the VIDO study. Because we expect more withdrawal in this group during the 12 months for the booster part of the study therefore we would like to recruit more participant for the booster part of the study.
- Some patients with an immune disorder will be offered a 3th SARS-CoV-2 vaccination. We don't know if participant of this study will be offered this 3th vaccination. Since this information is relevant to the study results questions about a 3th vaccination will be added to the questionnaire for timepoint T5 or B0 if this timepoint replaces T5.

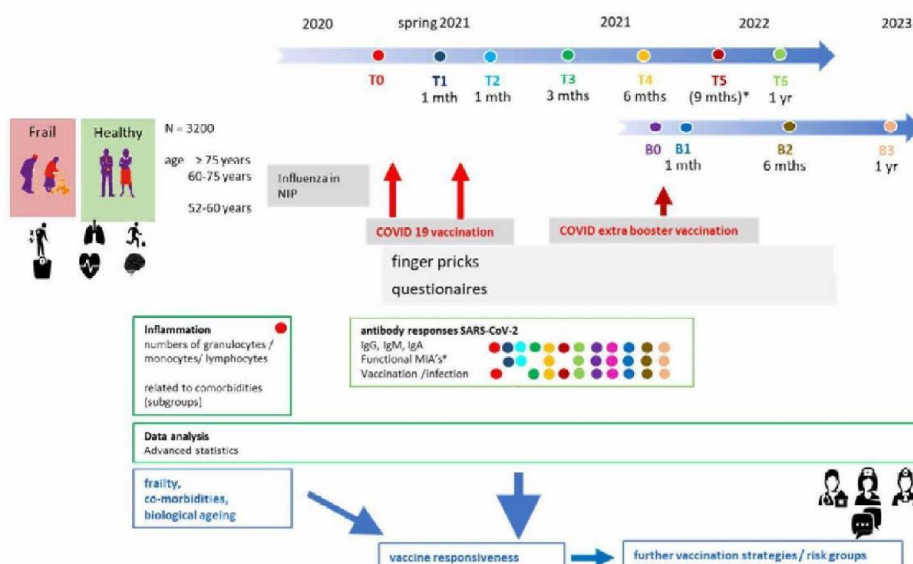


Figure 1. Study design. Persons of middle and older age, 52-90 years, participating in the Doetinchem cohort study, for assessing SARS-CoV-2 antibody responses post vaccination in relation the biological ageing. *= optional time point
In case of a booster vaccination participants can continue with B0-B3. Depending on the timing of the booster vaccination time points of the original study can be skipped. #= Functional MIA's; optional, will not be tested at all timepoints.

Vaccine responsiveness to SARS-CoV2 in aged persons

18. Amendment 4**18.1 Goal of amendment**

- a. Follow the immune responses after the second and following SARS-CoV-2 booster vaccination, similar to the scheme as originally planned.

18.2 Justification for amendment

- a. In February 2022 the minister decided to introduce a second SARS-CoV-2 booster vaccination for persons above 70+ years of age. We will follow this booster, and any following, SARS-CoV-2 booster vaccination given according to the Dutch COVID-19 vaccination program, in those participants that take the SARS-CoV-2 booster vaccination and approve follow up participation.
This will result in extra timepoints for blood sampling; pre booster, at 28 days (-7 till +14 days) post, 6 months (+/- 4 weeks) and 12 months (+/- 4 weeks) post booster vaccination. At these timepoint vaccine-specific (functional) antibody levels pre and post vaccination will be measured. Depending on the timing of the SARS-CoV-2 booster vaccination original study timepoints will be skipped or pushed forward. At all timepoints fingerprick blood will be sampled for antibody responses, and questionnaires will be filled in.

Vaccine responsiveness to SARS-CoV2 in aged persons

Appendix A**Supplementary Table 1.** Components of the frailty index that was used and compiled in the Doetinchem Study

Variable name	Deficit category	Description	Cutoff value
1	Chronic conditions	Systolic blood pressure	RR < 160 = 0 RR ≥ 160 = 1
2	Cardiologic RR Disease	One or more of the following conditions: myocardial infarction, bypass-surgery, balloon dilatation, cardiac catheterization, pacemaker implantation, large blood vessel surgery, hospitalization due to cardiac failure	No = 0 One/more = 1
3	Diabetes	Prevalence of Diabetes	No = 0 Yes = 1
4	Hearing	Ability to maintain a conversation in a group of 3 or more persons (with hearing aid if needed)	Yes or some effort = 0 No or great effort = 1
5	Malignancy	(History of) any form of malignancy	No = 0 Yes = 1
6	Joint Inflammation	Chronic joint inflammation in the past year	No = 0 Yes = 1
7	Osteoporosis	Osteoporosis diagnosed by a medical doctor, in the past year	No = 0 Yes = 1
8	Lower Back Pain	Severe lower back complaints in the past year (including lumbar herniated nucleus pulposus)	No = 0 Yes = 1
9	CVA	(History of) stroke	No = 0 Yes = 1
10	Migraine	Migraine prevalence in the past year	No = 0 Yes = 1
11	Neurologic Disease	One or more of the following neurological diseases, diagnosed by a medical doctor: m. Parkinson, Multiple Sclerosis, epilepsy	No = 0 Yes = 1
12	Asthma	Asthma diagnosed by a doctor and one or more asthma attacks in the past year	No = 0 Yes = 1
13	Spirometry Ratio	Spirometry measurements: first second of forced expiration divided by the forced vital capacity (FEV1/FVC)	FEV1/FVC ≤ 0,70 = 0 FEV1/FVC > 0,70 = 1
14	Digestive Tract	Severe bowel disorders in the past year, diagnosed by a medical doctor	No = 0 Yes = 1
15	Vertigo	Vertigo with falling the past 12 months	No vertigo = 0, some vertigo = 0,5, severe vertigo = 1

Vaccine responsiveness to SARS-CoV2 in aged persons

16	BMI	Chronic conditions	Body Mass Index	18.5 < BMI ≤ 30 = 0 BMI ≤ 18.5 or BMI > 30 = 1
17	Pain	Chronic conditions	Limited in daily activities due to pain	No limitation = 0 Some limitation = 0.5 Severe limitation = 1
18	Incontinence	Chronic conditions	Unintentional urine incontinence past 12 months	No = 0 Yes = 1
19	Ankle Brachial Index	Chronic conditions	Ratio of the systolic blood pressure in the ankle to the blood pressure in the arm	ABI > 0.9 = 0 ABI ≤ 0.9 = 1
20	Health Perception	Chronic conditions	Subjective health	No/some = 0 Severe impairment = 1
21	Eyesight	Chronic conditions	Bad eyesight perception, facial recognition within 4 meters (with glasses/ contact lenses if needed)	No = 0 Yes = 1
22	Renal Function	Chronic conditions	Renal function (estimated Glomerular Filtration Rate (eGFR), calculated with plasma creatinine concentrations) ⁶	eGFR ≥ 60 = 0 eGFR < 60 = 1
23	Cognitive Speed	Cognitive	Z-scores corrected for measurements per person and for education level. Scores derived from the Stroop Color-Word Test and the Letter-Digit Substitution Test, as described previously ⁷	10% lowest cohort = 1 Otherwise = 0
24	Cognitive Memory	Cognitive	Z-scores corrected for measurements per person and for education level. Scores derived from the Verbal Learning Test, as described previously ⁷	10% lowest rt = 1 Otherwise = 0
25	Cognitive Flexibility	Cognitive	Z-scores corrected for measurements per person and education level. Scores derived from the Stroop Color-Word Test, as described previously ⁷	10 % lowest = 1 Otherwise = 0
26	Physical Inactive	Physical	Not meeting the Dutch healthy exercise norm. ⁸ In addition: belonging to the 25th lowest percentile of walking activity and the 10th percentile lowest low/medium/high intensive activities in the Doetinchem cohort	No = 0 Yes = 1
27	ADL	Physical	Limited in washing and dressing due to health	No limitation = 0 Some limitation = 0.5 Severe limitation = 1
28	Household	Physical	Limited in daily activities (cooking, cleaning) due to health	No limitation = 0 Some limitation = 0.5 Severe limitation = 1
29	Walking	Physical	Limited in walking 100 meters	No limitation = 0 Some limitation = 0.5 Severe limitation = 1
30	Lifting	Physical	Limited in lifting or carrying groceries due to health	No limitation = 0 Some limitation = 0.5

Vaccine responsiveness to SARS-CoV2 in aged persons

31	Walking Stairs	Physical	Limited in climbing stairs	Severe limitation = 1 No limitation = 0 Some limitation = 0.5 Severe limitation = 1
32	Grip Strength	Physical	Measurement: grip strength (cutoff points as described previously) ⁹	Males: BMI ≤ 24 >29 = 0 ≤29 = 1 24 < BMI ≤ 26 >30 = 0 ≤30 = 1 26 < BMI ≤ 28 >30 = 0 ≤30 = 1 BMI > 28 >32 = 0 ≤32 = 1 Females: BMI ≤ 24 >17 = 0 ≤17 = 1 23 < BMI ≤ 26 >17.3 = 0 ≤17.3 = 1 26 < BMI ≤ 29 >18 = 0 ≤18 = 1 BMI > 29 >21 = 0 ≤21 = 1
33	Depressed	Psychological	Feeling depressed the past week	No limitation = 0 Some limitation = 0.5 Severe limitation = 1
34	Happiness	Psychological	Feeling happy the past 4 weeks	No limitation = 0 Some limitation = 0.5 Severe limitation = 1
35	Mental Effort	Psychological	Feeling as if every activity costs effort during the past week	No limitation = 0 Some limitation = 0.5 Severe limitation = 1
36	Getting Going	Psychological	Not being able to get going the past week	No limitation = 0 Some limitation = 0.5 Severe limitation = 1

Health deficits were either dichotomized or trichotomized, with 0= total absence of the deficit, 0.5= partial presence of the deficit, and 1= full presence of the deficit. For instance, the deficit 'limitations in walking 100 meters' was obtained from a self-reported questionnaire and was divided in no limitation (0), some limitation (0.5), and severe limitation (1). The frailty index is calculated by dividing the number deficits per individual by the total number of deficits investigated (n=36). We have analyzed the frailty index distribution per sex and the associations between frailty and all-cause mortality for further validation (Samson et al, 2019, ref 10)