AANVRAAGFORMULIER
UITGEWERKTE SUBSIDIEAANVRAAG
– BOTTOM-UP RONDE

COVID 19 programma
MKMD geoormerkt budget voor Proefdiervrije innovaties

Deadline voor indiening: 15 juni 2020 (14:00 u)

LEES ALSTUBLIEFT ALLE INSTRUCTIES IN BIJLAGE "TOELICHTING INDIENING SUBSIDIEAANVRAAG" VAN DE OPROEPTEKST ZORGVULDIG!

Wanneer u het formulier heeft ingevuld:

1. **Zet het formulier om naar een PDF file en controleer de details**
2. **Upload het complete formulier als een bijlage bij uw indiening in Projectnet**

ProjectNet: Aandachtsgebied 1 (voorspellende diagnostiek en behandeling)

BASISGEGEVENS (voorpagina)

NAAM VAN DE HOOFDAANVRAGER:

(10)(2e)

ORGANISATIE:

Sanquin

ENGELSE PROJECTTITEL:

Establishment and duration of protective immunity against SARS-CoV-2 in relation to severity of SARS-CoV-2 infection.

NEDERLANDSE PROJECTTITEL:

De opbouw en duur van beschermende immuniteit tegen SARS-CoV-2 in relatie tot de ernst van infectie

ONDERZOEKSVOORSTEL max 8 pagina's A4 (inclusief literatuurreferenties)	(voorpagina met basisgegevens niet meegerekend - font type Arial 10 pts)

1. PROBLEEMSTELLING, URGENTIE EN DOELSTELLING(EN)

Problem: There is great heterogeneity in disease symptoms in SARS-CoV-2 infected individuals, ranging from asymptomatic to severe or fatal disease. Nevertheless, most infected patients who clear the virus show an adaptive immune response as measured by antibodies and T cell responses. In analogy to many other viral infections, it is to be expected that this immune response confers immunity to (or decreases severity of) reinfections. However, it is presently not known how long-lasting and protective this immune response is, nor which markers can serve as correlates of protection. Furthermore, it has to be investigated to which extent establishment and maintenance of protective immunity varies between individuals and depends on severity of disease upon primary infection.
Urgency: Knowledge on the duration and effectiveness of immunity after a SARS-CoV-2 infection (especially after asymptomatic and mild disease) is of high relevance to guide societal measures in the next stages of this pandemic. If correlates of protection can be defined, one could employ the strategy of shielding immunity. In that strategy all individuals who are immune can move freely. The availability at our blood bank of large numbers of blood samples, collected longitudinally before and after the emergence of SARS-CoV2, provides the unique ability to study the acquisition, maintenance and protective capacity of adaptive immunity to this virus, not only in individuals with detectable disease, but even in those that remain asymptomatic.
Aims: Therefore, the aims of this project are to:
1) Investigate the quality and dynamics of the adaptive immune response in longitudinal cohorts of in total 500 SARS-CoV-2 infected individuals during 2 years, in relation to disease symptoms at primary infection.
2) Elucidate the degree of protection to reinfection after seroconversion in 2000 prospectively followed seroconverted donors and among 300.000 blood donors within 2 years and compare to matched non-seroconverted donors.

2. LOPEND ONDERZOEK

Viral infections induce adaptive immune responses. Such adaptive immune responses generate immunological memory, which ensures faster and more effective immune protection upon subsequent encounter of the same virus. For some viruses, such as measles, this memory provides life-long protection, whereas for others protection lasts shorter. The absence of pre-existing immunological memory to SARS-CoV-2 has greatly contributed to the rapid spread of SARS-CoV-2 infections. Adaptive immune response, in the shape of SARS-CoV-2 specific antibodies and T cell reactivity, are elicited in most individuals infected with the virus.[1] That at least short term immune protection develops is suggested by the finding that rhesus macaques are protected against rechallenge after natural infection[2] as well as after vaccination.[3,4] It still remains to be established whether immune responses against SARS-CoV-2 always result in protective immunity in humans and how long protection persists. For the related Corona viruses SARS and MERS, reinfection has not been observed, but how long protection is maintained cannot be determined, as these viruses have been eradicated or largely contained. Adults experience yearly reinfection with one of the four endemic human coronaviruses, but whether this is due to decay of specific immunity or caused by subsequent infections with genetically distinct strains is not known.[5] Small-scale experimental infection studies in humans suggest that protection after these coronavirus infections may last 1 or 2 years.[6,7]

Humoral immune response

In vitro neutralizing antibodies (nAbs) can block viral entry, and are therefore thought to correlate to antibody mediated immunity and used as the gold standard to evaluate the efficacy of a vaccine. For SARS-CoV-2, antibodies against the spike protein can exhibit *in vitro* neutralizing activity. The most potent nAbs interfere with the interaction between the receptor binding domain (RBD) of the spike protein and the human ACE2 entry receptor.[8] However, also antibodies that bind outside the RBD domain can exhibit neutralizing activity, probably by interfering with the conformational change of the S1 domain or S2-mediated membrane fusion.[9] Recombinant *in vitro* nAbs have been shown to confer protection to SARS-CoV-2 reinfection in a Syrian hamster model.[10] In addition to directly neutralizing the infectivity of the virus, other antibody-mediated pathways may also play a role in protection, such as antibody-dependent cellular cytotoxicity, phagocytosis and/or complement activation.[11] For other viruses, it has indeed been shown that *in vivo* protection might be conferred by non-nAbs,[12] suggesting that antibodies against other SARS-CoV-2 protein domains might also be protective. Next to the spike protein, the nucleocapsid protein (NP) of SARS-CoV-2 is highly immunogenic,[13] and high titers of antibodies against ORF8 have also been measured.[14] Our results in sera from PCR-confirmed- recovered COVID-19 individuals demonstrate a wide

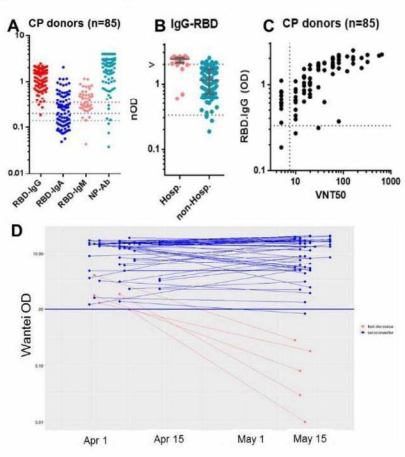

Figure 1. **Anti-SARS-CoV-2 antibody levels**. **A**: Large variability between antibody levels **B**: Higher levels in previously hospitalized patients **C**: RBD-IgG correlates with neutralizing titers **D**: 52 seroconverted blood donors measured twice within 6 wks show loss of anti-RBDantibodies in 5 donors

variability in antibody titers, isotype and antigen-specificity of the SARS-CoV-2-specific antibody response (Fig.1a). Most individuals are positive if tested in our in-house developed sensitive screening assay for total antibody to RBD (Fig. 1b). While>90% of the individuals had detectable IgG to both RBD and NP, about 60% had detectable IgA, and about 40% IgM to RBD. The predominant subclass was IgG1 and variable amounts of IgG3 were observed in up to 40% of patients. Importantly, large variation in antibody levels were observed. Antibody titers were significantly higher in hospitalized patients than in PCR-confirmed donors who were non-hospitalized (Fig 1b). Anti-RBD IgG levels correlated with neutralizing titers (Fig 1c). Neutralizing titers in our convalescent plasma (CP) donors varied from 1:10 to 1:640, and in 10% no nAbs were detected (Fig 1c). Likewise, Wu and colleagues could not detect neutralizing antibodies in 10 out of 175 recovered COVID-19 patients.[15] A similarly broad variation in antibody levels was observed in our first sero-prevalence study examining 7361blood donors, in which we detected 200 seroconverted cases with in 25% low antibodylevels.[16] This resembles results in mild cases of MERS, where low antibody titers and absence of neutralizing antibodies were reported.[17]

One of the most important questions is how long the SARS-CoV-2 antibody response is maintained. Currently, longitudinal studies on antibody responses have not been reported and, four months into the epidemic, these studies are badly needed. Antibody titers to most endemic coronaviruses wane within months.[5] In contrast, for SARS-CoV-1 (causative agent of SARS and most similar to SARS-CoV-2), antibody levels were stable in the first year and neutralizing antibodies in SARS survivors can be observed 9–15 years after the initial infection.[18] Recently, we performed a serosurvey among a second series of 7154 blooddonors. As shown in figure 1D we observed that in 5 out 57 blood donors who were tested twice anti-RBD antibodies became undetectable within 6 weeks.

In conclusion, the anti-SARS CoV-2 antibody response is variable between individuals upon primary infections, and might relate to severity of primary disease. Whether this large heterogeneity in titer and specificity of the antibody repertoire reflects differences in protection is unknown. Moreover, it is not known how long (neutralizing) SARS-CoV-2 antibodies are maintained after viral clearance, nor whether this duration relates to severity of primary disease and protection.

Cellular immune response

Serum antibody levels are maintained by long-lived plasma cells. Immunological memory is also sustained through memory B cells and T cells, even when antibodies are not present in serum. Upon reinfection, memory B cells can (with help of memory CD4+ T cells) rapidly expand and differentiate into antibody secreting cells. Memory CD8+ T cells can target virus-infected cells upon reactivation.

SARS-CoV-2- specific memory B cells

The memory B cell pool in SARS-CoV-2 has hardly been studied. Several groups successfully isolated anti-SARS-CoV-2 B cell receptor sequences from memory B cells of COVID-19 patients using multimerized SARS-CoV-2 proteins to select antigen-specific B cells. Our groups have established SARS-CoV-2-specific IgG ELISPOT assays (Fig 3A) and in collaboration with van Gils (AMC) flow cytometry procedures for identification of SARS-CoV-2 positive B cells (Fig. 3b).[9] This now allows us to follow SARS-CoV-2 reactive B cell responses in time and correlate functional phenotypes with the duration of the B cell, T cell and antibody responses and with the degree of protection against reinfection.

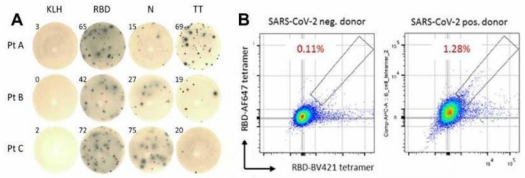

Figure 3. **Identification of SARS-CoV-2 reactive B cells in PBMCs** . **A.** PBMCs stimulated with R848 and rIL-2 for 6 days and SARS-COV 2 Ag-specific IgG antibody-secreting cells were detected by RBD and N-specific ELISpot assay. **B.** B cells were stained directly ex vivo in PBMCs using multimerized RBD domain, labelled with two different fluorophores

SARS-CoV-2 reactive B cell responses are induced in primary infection. The number, phenotype and duration of the SARS-CoV-2 positive B cell responses and relation of these factors to disease severity remain to be established.

SARS-CoV-2-specific T-cell responses

CD8+ T cells are important for directly attacking and killing virus-infected cells, whereas CD4+ T cells execute various effector functions, depending of the T helper (Th) type induced. They may aid CD8+ T cells, and promote the differentiation of those B cells that yield long-lived, class-switched and affinity-matured antibody responses. Limited data are available on T cell immune responses in COVID-19. We detected SARS-CoV-2-specific CD4+ T cells in the blood of 64 individuals who experienced a SARS-CoV-2 infection with different severities. In accordance with recent publications, [19,20] responses against Spike (S) were the most prevalent with a 100% response rate, followed by those against Nucleocapsid (N) and membrane glycoprotein (M/VME1), which were also recognized in the majority of patients. ORF3A (AP3A) and a pool of ORF antigens were recognized by CD4+ T cells in a subset of these donors, whereas responses against the envelope protein (E/VEMP) were not detected (Fig 4A,B). This CD4 T cell response pattern against SARS-CoV2 is apparently much broader than that against SARS-CoV.[21]The SARS-CoV2–specific CD4 T cell response was predominantly of the Th1 type: IFNγ and TNFα were produced, but hardly any Th2 cytokines (data not shown). Similar results were obtained by Grifoni et al.[19] A small population of Tfh (IL-21) cells was observed, which seemed to be smaller in more severe patients (not shown). Intriguingly, the frequency of SARS-CoV-2-specific CD4+ cells was higher in hospitalized patients than in CP donors with mild symptoms, whereas the lowest frequency was observed in ICU cases (Fig 4D,E). The frequency of S- specific CD4+ cells in infected donors experiencing mild symptoms was correlated with time after onset of disease, with higher frequencies over time. In these donors also a positive correlation with anti-RBD antibody level was observed (P<0.0001). Strikingly, CD4+ T cell responses against SARS-CoV-2 were also observed in healthy donors (cells isolated before the present pandemics). The frequency of these cells was much lower than in SARS-CoV-2 experienced cases and similar to that seen against the spike proteins of the common corona viruses 299E and OC43 (Fig 4C). This suggests that low frequency cross-reactivity exists, as has also been observed by Braun et al (ref). The impact of these pre-existing T cells is unknown. Only limited information is available on SARS-CoV-2 specific CD8+ T cells, only Grifoni et al. described their existence in 7 out of 10 tested donors, and in 4 out of 10 unexposed individuals.[19]

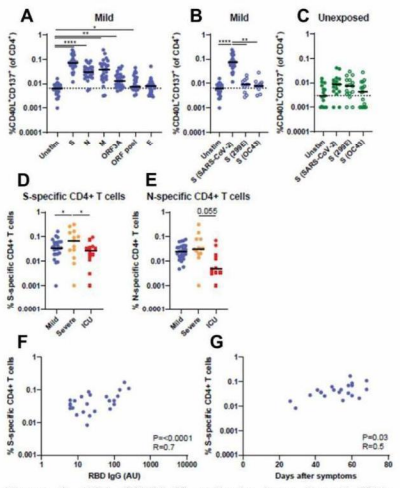

Figure 4. Mild COVID-19 patients have broad CD4+ responses to SARS-CoV-2 peptides but low frequencies of cross-reactive T cells. A. Frequencies of CD4+ T cells specific for Spike (S), Nucleocapsid (N), Membrane (M), ORF3A, pool of 7 ORF peptide pools, Envelope (E), determined by upregulation of CD40L and CD137 upon peptide stimulation and compared to unstimulated in mild Covid-19 patients. **B,C** :Cross-reactivity against spike antigen from other coronavirus strains in mild Covid-19 patients (n=16) and unexposed individuals (n=23. **D,E :** Frequencies of S- and N-specific CD4+ T cells were compared between the mild (n=23), severe (n=14) and ICU cohorts (n=14). **F:** frequency of S-specific CD4+ T cells and RBD IgG titers and **G:** frequency of S-specific CD4+ T cells and days after symptoms were assessed in mild patients.

In conclusion, SARS-CoV2 specific CD4+ and CD8+ T cells are detectable early after infection. It remains to be established whether such cells are present in all infected individuals (including those undergoing asymptomatic infection), to what degree their frequency correlates with disease severity and how long such cells persist. Moreover, it remains to be determined whether cross-reactive CD4+ T cells elicited by related Corona viruses contribute to resistance against SARS-CoV2

3. PLAN VAN AANPAK (ONDERBOUW KEUZES)

THEORETISCHE EN/OF EMPIRISCHE ONDERBOUWING

Our preliminary results reveal that immune responses against SARS-CoV2 are widely variable between individuals, and correlate to some extent with severity of disease. Moreover, the chance of reinfection has become low due highly effective social distancing measures. To determine the maintenance and quality of SARS-CoV-2 immunity over time, therefore, large numbers of individuals, having experienced different degrees of primary disease severity, must be sampled longitudinally and analyzed for SARS-CoV-2-specific immunity (aim 1) and clinical signs of reinfection (aim 2). Protective immunity can be maintained by

circulating neutralizing antibodies or by anamnestic responses of memory B and T cells. These 3 lines of defense will be studied in this project.

DESIGN

Aim 1. Quality and Dynamics of immune response in relation to severity of primary disease

We have built 4 complementary COVID-19 cohorts in which infected individuals can be followed over time:

1. *Asymptomatic* persons identified in our seroprevalence donor study (n=150)
2. CP donors and persons from our seroprevalence studies who experienced *mild* symptoms (n=250)
3. CP donors who recovered after hospitalization (*severe* disease) (n=50)
4. CP donors or patients from the control arm of convalescent plasma trial (COV-PLAS) who recovered after ICU admission (*critically ill*). (n= 50)

These cohorts will be analyzed for:

Antibodies. In the first year, IgG antibody levels against RBD will be measured in all donors from these cohorts at each donation. In addition, we will measure (by ELISA) concentrations of isotypes, IgM and IgA against RBD and N proteins, as well as IgG against total S, N, ORF8, and ORF3b proteins. In 50 donors from each group, nAb titers will be measured at inclusion and 3-monthly in the first year. Antibodies to the endemic coronaviruses HCoV-229E, -NL63, -OC43, and -HKU1 will be measured using commercially available assays.[22] In the second year, the donors will be tested at 16, 20 and 24 months for all above mentioned parameters. All required ELISAs (except for ORF8 and ORF3b) have already been developed and validated for sensitivity and specificity. Recombinant antibodies have been obtained against RBD and S proteins[9] and isotype variants and subclasses of these are under construction (using established pipelines) to serve as standards for quantification in ELISA. Neutralizing titers will be measured using a pseudoviral entry assay, developed by our collaborators at the AMC.[9]

T and B cell responses. Extra blood will be drawn (group 1: 30 donors, group 2 : 30 donors, group 3: 15 donors, group 4 :15 donors) to investigate the magnitude and functional phenotype of SARS-CoV-2-specific CD8+ T cells (activation and induction of cytotoxic phenotype) and CD4+ T cells (activation and induction of Th1/Th2/Tfh) upon in vitro recall at 3-monthly intervals in the first year, and 6-monthly in the second year. Recall stimulation will be performed with a predefined small set of SARS-CoV-2-specific immunodominant peptides. CD8 T cell responses will be monitored using a selected set of epitopes in a predefined MHC-class-I tetramer panel. SARS-CoV-2-specific B cells and plasmablasts will be detected by flow cytometry using fluorescently labeled multimerized S, RBD and N proteins. Frequencies and functional phenotypes will be measured directly after isolation or upon *in vitro* recall and related to SARS-CoV-2-specific serum antibody responses. CD4 T cell assays on peptide pools are operational, and CD8 T cell assay will be developed. Biotinylated S, RBD and N proteins have already been produced.

Together, these data will show how SARS-CoV-2-specific antibody levels and T and B cell responses are maintained over time, and how these features are related to severity of primary disease, age and gender.

Aim 2. Protection to reinfection after seroconversion

In addition to detection of SARS-CoV-2-specific immune parameters, the ultimate proof of protective immunity will require analysis of actual protection against reinfection. Large longitudinal cohort studies are needed, and Sanquin is able to perform a prospective as well as a retrospective study.

Prospectively, we will examine between Sept 2020 until July 2022 the rate of (re)infection among 2000 sero-converted donors identified in the seroprevalence studies until September 2020. These 2000 donors will be collected from the random donors of our serosurvey cohorts and supplemented with CP donors. A similarly sized matched group of seronegative donors will be included as controls. To this end, we will check whether they indicate health problems and/or fever since their previous attendance on the standard donor health questionnaire that precedes each donation. If so, an electronic questionnaire is offered, on possible COVID-19 symptoms and risks for SARS-CoV-2 infection, since the previous donation. In case of clinical symptoms, the two last samples will be tested for changes in anti-SARS-CoV-2 antibody titers as well as those directed against endemic human CoV, as described above. Electronic questionnaires are already in place on a widely used and secure platform (Qualtrics) and approved by the EAR of Sanquin.

Retrospectively, we will examine stored plasma samples from patients that develop COVID19 in the next two years. To this end, we will arrange with the Municipal Health Service (GGD'en) to ask all cases with a positive COVID-19 PCR test result between September 2020 and July 2022 whether they (i) have symptoms of COVID-19 disease at time of PCR (ii) have donated blood or blood components in the previous two years and (iii) are willing to give consent to look back for the presence of SARS-CoV-2 antibodies in archived plasma samples. The frequency of antibody positivity in the archived plasma samples of the newly infected cases will be compared with that of a similarly sized PCR-negative control group (for power calculations we used seroconversion around July 2020). This comparison will allow an estimation of the level of protection. For each newly PCR-positive case, all longitudinal archived samples will be analyzed for antibody profiles and compared to the patterns obtained under aim 1. Sanquin has to archive plasma samples from all donations for 2 years. We already have approval of the Responsible Person (VP) of Sanquin Blood Bank to thaw plasma samples for this study. Definitive arrangements with the GGD'en will be made upon granting of the proposal.

Together, these studies will give insight whether COVID-19 recovered individuals are protected against reinfection, and might reveal biomarkers of protection.

STUDIE POPULATIE/DATABRONNEN

Aim 1: The 4 cohorts described above will be obtained from the following groups of donors/patients

1) Seroprevalence studies on blood donors. Sanquin has tested 2 cohorts of each >7000 donors in April 2020 and May 2020 (3 and 5.5% seroprevalence, resp). In the coming year each week 2000 donors will be tested for seroconversion. Questionnaires on COVID-19 related disease symptoms reveal that over 90% of seroconverted donors report some symptoms and 10% are asymptomatic. Only 54% of seroconverted donors suspected to have had COVID-19, while 12% of seronegative donors did so.
2) Convalescent plasma donors. Sanquin presently has 1137 donors enrolled into the COVID-19 convalescent plasma program, while over 5000 donors have signed up and will enroll soon. Based on questionnaires on COVID-19 disease, 82% has mild symptoms, 18 % was hospitalized and whom 6,5% (1,1% of all CP) at the ICU.
3) Sanquin is responsible for the laboratory evaluation of COVID-19 patients treated with CP in the randomized trial with CP of the LUMC (COV-PLAS). Hospitalized patients will be recruited from 203 patients in the control arm, of which about 18 % will be treated at the ICU and survive.

Aim 2:

1) Within Sanquin, more than 300.000 blood donors, ± 3% of the Dutch population between 18-78 yrs, regularly donate blood or plasma. From each donation a plasma sample is archived for 2 years.
2) Individuals who are found PCR positive from Sept 2020 – July 2022 and are blood donors (3%) and will have donated blood between April 2020 – Sept 2020, and a similarly sized and matched control group tested PCR negative.

VERWACHTE UITKOMST

Aim 1: The dynamics in quality and quantity of anti-SARS-CoV-2 antibodies, and of memory B and T cells after SARS-CoV-2 infections will give an indication about the duration of SARS-CoV2-specific immunological memory in the first 24 months after infection and reveal the variation between individuals.

Aim 2: The risk of reinfection within 24 months after a primary SARS-CoV-2 infection will be determined and compared to this risk in naïve individuals, and correlates of protection will be defined.

DATA-ANALYSE Apart from descriptive analyses, logistic regression analyses will be performed to assess risks of reinfection and -if possible and relevant- survival analyses (Cox regression or similar) to compare times until events in the prospective study.

POWERBEREKENING At time of writing this proposal it is not known how the pandemic will evolve. For the prospective study on 2000 seroconverted donors, we will be able to find statistically significant differences assuming at least 1% incident SARS-CoV-2 infections in seronegative donors between now and April '22, and 0.29% or less in the seroconverted group (ß of .80 and α of .05). The incidence of PCR-proven COVID-19 infections will determine the power to determine immunity to reinfection. If we assume that 50% of individuals with a positive PCR test will participate, that for 40% of the PCR-tested blood donors we will have a plasma sample from around July 2020, and that 6 and 1% of PCR-negative and -positive cases show seroconversion in July '20, we need 35167 positive PCR tests nationwide to find a significant difference. Duration of protection will be calculated in real time between Sept 2020 and April '22.

4. PLAN VAN AANPAK (ONDERBOUW KEUZES)

TIJDSSCHEMA At the start of the project in July 2020 we will have access to seroconverted donors from ongoing studies (seroprevalence monitoring study, CP donors). We will select from these groups donors with high donation frequency, representing the different disease categories.

July-September 2020 Arrangements with RIVM/GGD and METC approval for accruing of COVID-19 PCR tested. ICT implementation to follow seroconverted donors at Sanquin and to identify COVID-19 PCR tested blood donors at GGD/RIVM. July 2020 – April 2022 Longitudinal measurement of cases. October 2020- April 2022 Measurement of archived blood samples for aim 2.

MOTIVATIE HAALBAARHEID

Aim 1 Within Sanquin we can rely on large cohorts, covering the full range of disease severities from critically ill to asymptomatic and allowing comparisons between pre- and post infection within the same individuals.The study is highly feasible, because no extra blood drawings are needed as studies will be done with regular plasma donors, who are allowed to donate every 2 weeks. **At the end of May**, we have already banked from **450 CP donors** 3-5 ampoules with > $8x10^6$ cells/donor, and 2 ml plasma from 1200 different donations from 790 CP donors. Sanquin blood bank aims to enroll at least 5000 extra CP donors in the coming months. All donors completed questionnaires on COVID-19 disease symptoms. 82% had mild symptoms, 18% were hospitalized, of which 6,5% at the ICU. At the end of May, **652 seroconverted blood bank donors** have been identified, from whom plasma is stored and PBMCs will be obtained during subsequent donations. Preliminary analyses show that about 10% of seropositive donors were asymptomatic, and 46% did not suspect to have had COVID-19. In the coming year, each week 2000 donors will be screened for antibodies, and we expect to identify at least 100 seroconverted donors/week.

We have already developed and validated almost all assays needed for the project (see 2 .LOPEND ONDERZOEK). The majority of the labwork consists of ELISA assays, which are fully automated, enabling high throughput.

In conclusion, the 500 donors/patients with different disease severities, that are needed for the longitudinal studies can easily be selected from the CP donors and serosurveys among blood donors.

Aim 2 At time of writing it is not known how the pandemic will evolve, and the power of the study will be dependent on the infection rate. In our view, the assumptions on infection risk and protection rate for the power calculations are rather conservative and therefore the proposed study is feasible with the included numbers. For the prospective study we used for the power calculation a percentage of 1% extra infections, and at least a 2/3 decreased risk of reinfection in seroconverted persons. By June 11th 2020, at very low infection risk, still 184 new individuals with positive PCR are detected per day. At this rate, within 191 days 35167 positive PCRs will be collected, needed for the retrospective study to show that the risk that PCR-pos individuals are reinfected is lower than 1% with an estimated 6% seropositivity in PCR-neg individuals.

RECRUTERINGSSTRATEGIE: For aim 1 we have almost completed at the start of the study the intake of donors that will be longitudinally followed. If not enough critically ill patients can be included we will use samples from the AMC COVID-19 Biobank. For aim 2 we will recruit PCR-converted individuals with help of the GGD as outlined in 2.

5. RELEVANTIE

1) *Impact on pandemic*: Insight in protective immunity has important implications for epidemiologic modeling and for decisions on scaling up or down of population-based interventions.
2) *Why in the Netherlands*: Other blood establishments (BE) may do similar epidemiological studies, but Sanquin is one of the few with a national screening lab and housing a research institute. This is exemplified by being the first in the world to perform a SARS CoV-2 seroprevalence study among 7000 blood donors.
3) *Not elsewhere*: The access to prospective longitudinal blood samples of large cohorts of seroconverted persons, as well to archived longitudinal plasma samples of 300.000 donors is unique in the Netherlands.
4) *Public funding necessary*: The recruitment of donors for the proposed study will already be completed at start of this project. We request only funding to be able to perform immunological follow-up studies, as well as the prospective and retrospective studies.
5) *Generalizable knowledge*: Results will be published via open access routes and are relevant for future outbreaks of other SARS viruses. The knowledge is relevant to directly evaluate vaccines on long-lasting protective immunity.
6) It is a *nationwide* application.
7) *Collaboration of different disciplines:* Immunologists, virologist, epidemiologist, blood bankers, public health care workers, medical doctors, policy makers. For stakeholders see 5.
8) *Added value*: If we find that antibody titers wane, this should be considered in seroprevalence studies quantifying incidence of new infections in the population.
9) *Effect on societal and economic issues*: As soon as we are able to identify correlates of protection, the "shield immunity" approach, which works synergistically with social distancing, could reduce the length and overall societal and economic burden of the current outbreak and be employed to protect vulnerable persons in our society.

AANVULLENDE RELEVANTIE

All experiments will be done with human derived materials. By using sophisticated analysis techniques and capitalizing on large existent cohorts of human samples at the blood bank, we can study immune parameters directly in humans. The effect of gender on immune protection will be analyzed. Socio-economic status of blood donors will be taken into account.

6. PROJECTGROEPLEDEN EN HUN ROLLEN

(10)(2e) Professor in Experimental Immunohematology. Her research focuses on the immune response against blood group antigens, which closely resembles the immune response to enveloped viruses. Role: Project leader and responsible for coordination of the complete project

(10)(2e) **Epidemiologist**. Role: Co-projectleader and responsible for epidemiological modelling and coordination of follow-up on donors (aim 2)

(10)(2e) Professor in Biological **Immunology** Role: Co-projectleader and responsible for evaluation of the SARS-CoV-2 specific immune response wet labwork

(10)(2e) (10)(2e) Role: Co-projectleader and responsible for collection of **blood donor** material

(10)(2e) Professor in **Virology**, member of National Corona Serology Task force. Role: Co-applicant and responsible for interpretation serological data and virus neutralising titers, contact with RIVM

(10)(2e): Immunobiologist, .Role: Co-applicant and responsible for antibody characterization

(10)(2e) Immunologist, Role: Co-applicant and responsible for biobanking and B-T cell assays

(10)(2e) T cell immunologist Role: Co-applicant and responsible for T cell assays

7. KENNISOVERDRACHT, IMPLEMENTATIE, BESTENDIGING

Our data are shared with the Ministry of health and RIVM to be used for adaptation of societal measures during the different stages of the pandemic.
Our research will be published in peer-reviewed journals, while also guaranteeing open access availability. Our data will be shared with layman public to inform the public with new information on SARS-CoV-2 via Sanquin website and press releases, which will find their way to newspapers, television and social media, thus contributing to societal confidence in science and national measures.

8. A) DEELNAME VAN DE STAKEHOLDER(S)/EINDDOELGROEPEN

Results of our studies will be regularly shared with the RIVM and via (10)(2e) with the Corona Serological Task Force, so that they can be used to advice the Government. Sanquin Blood Bank and all blood donors, active participation will be organized with our Donor Council. Donors will be informed via regular donor communication channels (social media, 'Bloedverwant' and/or 'Gul' donor magazines, Sanquin website, press releases). Active participation of GGD for COVID-19 PCR tested individuals. Academic hospitals for collaboration in COV-PLAS trial.

9. LITERATUURREFERENTIES:

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