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Risk of COVID-19 re-infection and its predictors (CORES) – Study Protocol for a community based longitudinal cohort study

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Manuscripts

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3 **1 Study Title:**
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5 2 Risk of COVID-19 re-infection and its predictors (CORES) – Study Protocol for a
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8 3 community based longitudinal cohort study
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1
2
3 76 **Abstract**
4

5 77 **Introduction**
6

7 78 The incidence of SARS-CoV-2 re-infection has not been widely evaluated in Low and Middle-
8
9
10 79 Income Countries (LMICs). Understanding immune responses elicited by SARS-CoV-2
11
12 80 natural infection and factors that lead to re-infection in a community setting is important for
13
14 81 public health policy. We aim to investigate the risk of primary infection and re-infection among
15
16 82 those without and with evidence of prior infection as defined by the presence of antibodies to
17
18 83 SARS-CoV-2 spike protein.
19

20
21 84 **Methods and Analysis**
22

23
24 85 A baseline seroprevalence survey will test for SARS-CoV2 antibodies among 2000 randomly
25
26 86 selected healthy adults in Vellore, India. Based on an expected seropositivity rate of 50% in the
27
28 87 general population, with an annual attack rate of 12%, 6%, 4.8% and 4% among those
29
30 88 unvaccinated and seronegative, vaccinated and seronegative, unvaccinated seropositives, and
31
32 89 vaccinated seropositives respectively, we will recruit 1200 adults for follow up for a total of 24
33
34 90 months. Weekly self-collected saliva samples will be tested by RT-PCR to detect SARS-CoV2
35
36 91 infections, for a period of one year. For any person testing RT-PCR positive, blood samples
37
38 92 will be collected within 2 days of RT-PCR positivity and on days 30 and 90 to assess IgG
39
40 93 antibodies to the spike protein and for detailed immunogenicity to assess the kinetics and
41
42 94 longevity of the antibody responses, B cell memory as well as T cell function and persistence
43
44 95 post-infection. The data will be analyzed to estimate seroprevalence at baseline and over time,
45
46 96 the risk factors for infection, rates of primary infection and re-infection and provide a
47
48 97 comparison of the rates across groups based on infection and vaccination status.
49

50
51 98 **Ethics and dissemination**
52

53
54 99 The study has been approved by the institutional review board, (IRB No: 13585), Christian
55
56 100 Medical College, Vellore.
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60

101 **Trial Registration**

102 The trial has been registered with the Central Trial Registry of India (CTRI). The trial
103 registration number is CTRI/2020/11/029438.

104 **Strengths and limitations:**

- 105 • Following up the cohort for a period of two years will help to capture the re-infection
106 rates of SARS CoV 2 in the community.
- 107 • The use of saliva samples for SARS CoV 2 surveillance will be an acceptable alternate
108 as it is self-directed, non-invasive.
- 109 • The cross reactivity between the SARS CoV 2 and other beta coronavirus will help in
110 better understanding of the clinical outcome.
- 111 • The detailed immunogenicity following SARS CoV 2 infection will help in decision
112 making with regards to booster vaccination.
- 113 • Though there is a good concordance of saliva and nasopharyngeal swab for SARS CoV
114 2 surveillance, there could be some cases which may be missed with saliva sampling.

115 **Study protocol**

116 **Keywords**

117 COVID-19, Immunology, Public Health.

118 **Background**

119 Immune responses to SARS-CoV-2 infection, vaccination, and the immune correlates of
120 protection are areas of active investigation [1]. A few studies have shown that the development,
121 amount, kinetics of antibodies may correlate with the clinical outcome of SARS CoV 2
122 infections. [2-5]. The coordinated response between humoral and cellular immunity has been
123 hypothesized to be protective [6]. From a public health perspective, it is crucial to understand
124 the duration of protective immunity offered by natural infections and vaccination [1]. There are

1
2
3 125 re-infections in patients who have recovered from the disease [7, 8]. At the population level,
4
5 126 the incidence of re-infection over the long term (duration of one to two years) has not been
6
7 127 evaluated, and this may not be feasible, given the rapid pace of vaccination. Preliminary studies
8
9 128 suggest that antibody levels persist for at least seven to nine months or more post-infection [9-
10
11 129 10]. The rates of attrition of potential immune correlates like memory B and T cell responses,
12
13 130 and the association of these humoral and cellular immune parameters with subsequent re-
14
15 131 infections are unknown. The duration of protective immunity to SARS-CoV-2 is being
16
17 132 measured, but so far has largely been extrapolated from the data of phylogenetically related
18
19 133 viruses. Antibody responses to SARS-CoV-1 persist for two to three years [11] and memory T
20
21 134 cells persist for 11 years after infection [12]. In contrast, beta coronaviruses [β -CoV] that are
22
23 135 phylogenetically close to SARS-CoV-2 are known to re-infect humans throughout life [13],
24
25 136 suggesting shortlasting protective immunity. Human controlled infection models using
26
27 137 common cold associated beta coronaviruses (β -CoV) showed partial protection from antibodies
28
29 138 that persist for one year [14]. These findings suggest that similar protective immune
30
31 139 mechanisms could be operative in SARS-CoV-2 as well but would need detailed
32
33 140 characterization. Further, uninfected individuals could harbor antibodies and memory T cells
34
35 141 to other beta coronaviruses [15]. Such cross-reactive T cell responses [15] targeting several
36
37 142 epitopes on the surface proteins of SARS-CoV-2 [16], could potentially influence the course of
38
39 143 infection, or the clinical outcomes. The limited availability of data on SARS-CoV2 infections
40
41 144 in LMICs where exposure to other coronaviruses may differ warrant a detailed evaluation of
42
43 145 cross-reactive T cell and antibody landscapes in primary infections and re-infection outcomes
44
45 146 in the community.

53
54 147 This protocol describes a study to estimate the incidence of infection, re-infection and
55
56 148 vaccine breakthrough infections in a community in India. The study would also determine the
57
58
59
60

149 antibody profile, duration of antibody persistence as well the cellular immune responses
 150 following natural COVID-19 infection and re-infection.

151 **Objectives and Outcome:**

Objective 1: To estimate the seroprevalence of antibodies to SARS-CoV-2 spike protein in Vellore

Outcome:

- a. The proportion of individuals ≥ 18 years of age who are seropositive for antibodies to spike protein of SARS-CoV-2 in Vellore
- b. Prevalence of seropositivity across clusters (wards)

Objective 2: To measure the incidence of SARS-CoV-2 infection in a cohort of individuals ≥ 18 years in Vellore

Outcome:

- a. Incidence of SARS-CoV-2 infection among those without evidence of prior infection or vaccination
- b. Incidence of SARS-CoV-2 infection among those with evidence of prior SARS-CoV-2 infection
- c. Incidence of SARS-CoV-2 infection in those who have received at least one dose of COVID-19 vaccine

Objective 3: To track cellular and humoral immune correlates of COVID-19 infection, re-infection, and clinically significant disease

Outcome:

- a. Kinetics and longevity of antibody responses and immunological memory

b. Influence of baseline memory T and B levels (both SARS-CoV-2 specific as well as cross-reactive) on infections

152 **Methods**

153 **Study setting: Description of the site**

154 Vellore is a tier 2 city in northern Tamil Nadu with a population of close to 5,00,000. It is
155 divided into four zones and 60 administrative wards. The Vellore Health and Demographic
156 Surveillance System (VHDSS), established by the Christian Medical College, monitors a
157 population of 1,20,000 people across zones 3 and 4 of the city. This study area has a very high
158 population density predominantly belonging to the economically poorer section, and is largely
159 homogenous, with daily wage-earners being the largest sub-group of the population.

160 **Study design**

161 The study will have three components (1) serosurvey to estimate the seroprevalence of SARS-
162 CoV-2 spike protein antibodies in the study area, (2) prospective weekly follow-up to estimate
163 the infection and re-infection rates in a cohort of 1200 individuals, (3) intensive follow up of
164 incident SARS-COV-2 infections (both symptomatic and asymptomatic) to characterize
165 immunological and clinical features of infection in the cohort. The study flow chart is depicted
166 in Figure 1.

167 **Patient and Public involvement**

168 No patients or public involvement in the design or conduct or reporting or dissemination plans
169 of our research.

170 **Inclusion and exclusion criteria**

171 **Serosurvey**

172 Inclusion criteria:

- 173 1. Above the age of 18 years

- 1
2
3 174 2. Permanent residents of the selected wards
4
5 175 3. Only one member from each selected household will be enrolled
6
7

8 176 Exclusion criteria:

- 9
10 177 1. Participant refusal of consent
11
12 178 2. Pregnant women and immunocompromised patients will be excluded
13
14 179 3. Acute febrile illness in the participant at the time of the survey
15
16
17

18 180 **Longitudinal study**

19
20 181 Inclusion criteria:

- 21
22
23 182 1. Individuals with a history of clinical illness suggestive of COVID-19 or confirmed COVID-19
24
25 183 in the past, who are seropositive at baseline in the serosurvey (symptomatic seropositive).
26
27 184 2. Individuals seropositive at baseline, with no history of COVID-19 (asymptomatic seropositive).
28
29 185 3. Individuals seronegative at baseline, stratified by the ward of residence.
30
31
32

33 186 Exclusion criteria:

- 34
35 187 1. Participants who are not willing for intensive follow-up till the end of the study.
36
37 188 2. Participants with immunodeficiency states such as people living with HIV infection.
38
39 189 3. Active cancers or bleeding disorders
40
41
42

43 190 **Statistical Consideration.**

44 45 191 **Assumptions**

46
47 192 We make the following assumptions.

- 48
49 193 1. 50% of the population will be seropositive at baseline.
50
51 194 2. 40% will have received two doses of vaccine mid-way into the study.
52
53 195 3. The annual incidence of SARS-CoV-2 infection detected by the salivary PCR in those
54
55 **unvaccinated and have no detectable antibodies** (unexposed) at baseline will be
56
57 **12%.**
58
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- 1
2
3 198 4. The annual incidence of SARS-CoV-2 infection detected by the salivary PCR in those
4
5 199 **vaccinated and** unexposed at baseline will be **6%** (VE 50% against infection).
6
7
8 200 5. The annual incidence of SARS-CoV-2 infection detected by the salivary PCR in those
9
10 201 **unvaccinated and who have antibodies (exposed)** at baseline will be **4.8%**.
11
12 202 6. The annual incidence of SARS-CoV-2 infection detected by the salivary PCR in those
13
14 203 **vaccinated and exposed** at baseline will be **4%**.
15
16

17
18 204 Based on these assumptions, for 90% power to detect a 5% difference in the rate of re-infection
19
20 205 and primary infection in the cohort, a sample size of 1200 participants is proposed, assuming a
21
22 206 10% dropout rate.
23

24 207 **Key definitions**

25
26
27 208 **Seropositive** is defined as serum/plasma samples positive for IgG spike protein antibody to
28
29 209 SARS CoV2 identified by Chemiluminescence Immunoassay (CLIA) using DiaSorin's Liaison
30
31 210 XL.
32

33
34 211 **Past asymptomatic infection** refers to those who are seropositive (or documented RT-PCR
35
36 212 positive >1 month in the past) but are neither antigen or RTPCR positive at baseline assessment
37
38 213 AND have had no symptoms of COVID-19.
39

40
41 214 **Recent asymptomatic infection** refers to those who are seronegative AND are either RTPCR
42
43 215 or antigen positive AND have had no symptoms of COVID-19.
44

45
46 216 **Past symptomatic infection** refers to those who are seropositive (or documented RTPCR
47
48 217 positive >1 month in the past) but are neither antigen or RTPCR positive at assessment AND
49
50 218 have had symptoms of COVID19 in the past.
51

52
53 219 **Recent symptomatic infection** refers to those who are seronegative AND are either RTPCR
54
55 220 or antigen positive at assessment AND have symptoms of COVID19 within the past one month.
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3 **221 Study procedures**
4

5 **222 Baseline serology screening**
6
7

8 223 A baseline serosurvey, conducted on 2000 individuals in four urban clusters, is planned based
9
10 224 on population proportionate to size (PPS). The participants who satisfy the inclusion criteria
11
12 225 will be selected for the serosurvey from areas within the Vellore corporation limits after
13
14 226 obtaining written informed consent. The inclusion and exclusion criteria are detailed in the
15
16 227 earlier section. The baseline demographic information, along with details of any clinically
17
18 228 relevant illness in the past one month, will be documented. History of confirmed COVID-19
19
20 229 or COVID-like illnesses during the period of the pandemic will also be documented. A
21
22 230 peripheral blood sample (5 ml serum) will be collected.
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27 **231 Establishment of the cohort**
28

29 232 Based on the seroprevalence from the serosurvey, longitudinal follow-up will be initiated in the
30
31 233 Vellore health and demographic surveillance system (VHDSS) area. A total of 1200 residents
32
33 234 living in the densely populated wards of zone 3 and 4 of the Vellore corporation will be recruited
34
35 235 for the longitudinal follow-up. Those subjects who agree to the specific terms of the
36
37 236 longitudinal follow-up of 24 months will be recruited after informed consent. Each study
38
39 237 participant will be assigned a unique cohort ID used for reference during the follow-up period.
40
41 238 Upon recruitment, blood samples (15-30 ml) will be collected and stored appropriately.
42
43 239 Peripheral Blood Mononuclear Cells (PBMCs) will be isolated prior to storage to assess the
44
45 240 baseline T-cell and memory B cell profiles in the future.
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3 **241 Weekly follow up**
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6 **242** An assigned field research assistant (FRA) will contact the study participant every week, either
7
8 **243** by telephonic or direct visit and collects information regarding any COVID-like symptoms in
9
10 **244** the preceding week. The study participants will be trained to collect 2 ml of saliva in the
11
12 **245** universal sample container, early in the morning, on one designated day of the week. The
13
14 **246** participants will be asked to collect these samples as per the study protocol, prior to routine
15
16 **247** oral hygiene, and consumption of any food or drink. The samples will be transported to the
17
18 **248** lab in vaccine carriers with ice packs to maintain a temperature of 4°C. The samples once
19
20 **249** received in the lab will be aliquoted in two different vials. One vial will be retained at the
21
22 **250** Wellcome Trust Research Laboratory, Vellore. The other vial is sent to the National Centre for
23
24 **251** Biological Sciences, Bangalore (NCBS) for RT-PCR.

25
26
27
28
29
30 **252** If an individual tests positive for SARS-CoV2, the weekly salivary sample collection will be
31
32 **253** suspended for the next 90 days. The weekly contact, however, will be continued. The study
33
34 **254** participants will be requested to inform the study team if they experience any clinically
35
36 **255** significant febrile or respiratory distress. Symptomatic individuals will be advised to visit
37
38 **256** Christian Medical College Hospital, Vellore and get tested by nasopharyngeal RT-PCR, as
39
40 **257** deemed necessary, after clinical examination.

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42
43
44 **258** During the second year of the study, weekly follow up would be through telephonic interviews.
45
46 **259** Weekly salivary samples would not be collected, and home visits would be done for subjects
47
48 **260** with symptoms. Any incident infection will be followed up for detailed immunological testing.
49
50 **261** Once every six months, a blood sample (5 ml) will be collected for assessing the serostatus of
51
52 **262** the participants to identify any infection that was missed through the RT-PCR screening.
53
54 **263** Sequencing will be done on all positive samples to identify the genetic sequence of the virus
55
56 **264** at NCBS, Bangalore.
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3 265 **Detailed follow-up of COVID-19 infections**
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5 266 All COVID-19 infections, including symptomatic and asymptomatic will be followed up from
6
7 267 the day of the positive report (Day 0). Blood samples (30ml) are collected for PBMC isolation
8
9
10 268 and storage within 24 hours of identification of positives on (Day-0), Day-30 (+2 days) and
11
12 269 Day-90 (+7 days) post-infection.
13

14
15 270 **Sample collection**
16

17 271 **Blood sample -serology:** Five ml of peripheral blood will be collected (in serum tubes) from
18
19 272 2000 individuals during the baseline serosurvey, and once every six months from the 1200
20
21 273 study participants who are a part of the longitudinal cohort.
22

23
24 274 **Salivary sample:** Salivary samples will be self-collected, stored and transported to the NCBS
25
26 275 laboratory, Bengaluru, as per the Standard Operating Procedure, once a week during the first
27
28 276 year of the study. The results will be uploaded timely into the secure data entry portal designed
29
30 277 for the laboratory.
31

32
33 278 **Nasopharyngeal swab:** If any study participants report any clinically significant febrile illness
34
35 279 or respiratory distress, they will be offered a medical consultation, and when necessary, a
36
37 280 nasopharyngeal RT-PCR at CMC or in any institute of their choice.
38

39
40 281 **Blood sample (for PBMC):** 30 ml (minimum 15 mL) of blood will be collected (in 9 ml
41
42 282 heparin tubes) after recruitment into the longitudinal study and for confirmed SARS-CoV-2
43
44 283 infections on Day-0, Day-30 and Day-90. PBMCs will be separated by density gradient
45
46 284 centrifugation method and cryopreserved in liquid nitrogen.
47
48

49 285 **Laboratory procedures**
50

51
52 286 **Weekly salivary samples**
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56 287 Upon receipt and aliquoting, salivary samples will be pooled for testing on the same day. Ten
57
58 288 μ l of five samples each will be pooled in a single well of the PCR plate, and 6 μ l of proteinase
59
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1
2
3 289 K of 50 µg/µl concentration will be added to each well. Subsequently, the plates will be sealed
4
5 290 and heated at 95° Celsius for 5 minutes in a dry thermal bath. After heat inactivation, the plates
6
7 291 will be stored at minus 80°C. The pooled PCR plate and an aliquot of saliva will be transported
8
9 292 on dry ice to NCBS. RT-PCR will be performed on the pooled samples targeting the N gene,
10
11 293 E gene and RdRp gene of SARS CoV 2. If any pool turns out to be positive, RT-PCR will be
12
13 294 performed on individual samples. All positive samples will undergo sequencing.
14
15

16 295 **Blood samples**

17 296 **Serological assays**

18
19
20 297 The plasma or serum sample collected at different time points will be tested for IgG antibody
21
22 298 against spike protein using a high throughput automated platform. (Diasorin LiaisonXL)
23
24
25

26 299 **Immunophenotyping**

27
28
29 300 Quantitation of SARS-CoV-2 specific T cells will be done by flow cytometric detection of
30
31 301 cytokines and Activation Induced Marker (AIM) upregulation in T cells after stimulation with
32
33 302 peptide pools. PBMC stimulation will be done using a 10-mer peptide pool for CD8 and 20-
34
35 303 mer peptide-pools for CD4 T cells. Four peptide pools will be used, corresponding to the major
36
37 304 proteins of SARS-CoV-2 (Spike, Envelope, Membrane and Nucleoprotein). For all the
38
39 305 stimulation conditions, one well (vehicle-treated) will act as negative control. An additional
40
41 306 well of cytomegalovirus (CMV)-peptide-stimulated control (a mix of 10-mer and 15-mer CMV
42
43 307 peptides) will be kept as positive control for each sample. Baseline levels of cross-reactive T
44
45 308 cells to non-SARS-CoV-2 human Coronaviruses (hCoV) will be estimated using the same
46
47 309 methodology, using peptide pools derived from hCoV strains. Memory B cells will be detected
48
49 310 by flow cytometry after staining PBMCs with fluorophore-tagged viral proteins and memory
50
51 311 B cell markers.
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312 **Statistical Analysis**

313 Seroprevalence is estimated as a proportion and will be assumed to follow a binomial
314 distribution. The incidence of infection within the cohort is expected to follow a Poisson
315 distribution. We will permit repeated infections to be captured in analysis and account for the
316 same in the analysis. A time to event analysis using Prentice, Williams and Peterson models
317 comparing incidence in the exposed and unexposed cohorts will be performed. We will adjust
318 for background infection rates in each cluster (ward) and covariates such as age, SES,
319 vaccination status, per-capita floor space and occupation class.
320 The statistical analysis plan will detail the estimation of seroprevalence, its risk factors, the
321 incidence of primary and re-infection and a comparison of these rates.

322 **Key comparisons in the study**

323 We will make comparisons between

- 324 • Incidence rates of infection overall and in seropositive and seronegative subgroups.
- 325 • Incidence rates of infection among the vaccinated individuals in the cohorts
- 326 • Kinetics and longevity of memory B and T cells in infections occurring in the
327 seropositive and seronegative cohort
- 328 • Baseline cross-reactive T cells and antibodies to non-SARS-CoV-2 beta coronaviruses
329 between symptomatic infections vs asymptomatic infections vs uninfected individuals
330 in the seronegative cohort
- 331 • Baseline SARS-CoV-2 specific memory T and B cells and antibody levels between
332 infected individuals versus uninfected individuals in the seropositive cohort

333 **Data Management Plan**

334 All the Case Report Format (CRFs) will be in the electronic format (Redcap©), and the entry
335 platform will be connected to the Central database server. The Data management system is
336 responsible for the periodic validation process and quality of the data. Any further correction

1
2
3 337 in the database after the entry is 'saved' is accompanied by a duly completed "Data
4
5 338 Clarification form." The electronic data management system tracks key study progress
6
7
8 339 parameters on an access-restricted online dashboard. The weekly contact made by the field
9
10 340 research assistants will be independently validated by a field worker who calls 5% of all
11
12 341 individuals who were contacted that week.

14 342 **Discussion**

16
17 343 To our knowledge, this study is the first to follow up a cohort in an LMIC, for a period of two
18
19 344 years for COVID-19 infection and re-infection. In terms of surveillance of SARS-CoV-2
20
21 345 infection, though the nasopharyngeal swab has been the gold standard for diagnosis, the use of
22
23 346 saliva samples will be an acceptable alternate by the study participants as it is self-directed,
24
25 347 non-invasive and has a good concordance with the nasopharyngeal swab. The study aims to
26
27 348 address several gaps in the current scientific evidence of SARS-CoV-2 infection and immunity.
28
29 349 Firstly, there are a limited number of studies that investigate the long term follow up of
30
31 350 individuals for the rates of infection and re-infection in the community. Secondly, the study
32
33 351 aims to look at the kinetics of IgG antibodies following infection. The cross-reactivity between
34
35 352 SARS-CoV-2 and other human coronaviruses will support better understanding of
36
37 353 determinants of symptomatic infection. The T cell and B cell memory responses would help
38
39 354 in understanding the kinetics and longevity of immune responses in seropositive and
40
41 355 seronegative individuals and would help in decision making with regard to booster
42
43 356 vaccination.

44
45 357 To conclude, CORES will help in estimating the re-infection rates, detailed
46
47 358 immunogenicity amongst the COVID-19 positive individuals, establish the antibody kinetics
48
49 359 and characterise the breakthrough infections amongst the vaccinated individuals in the
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51 360 community.

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3 362 **Statements**
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5 363 **a. Contributorship statement:** The study design and concept were conceived by JJ and GK. RM will
6
7 364 conduct the study as part of her PhD under the supervision of GK, SB, SP and JJ. JSP and JJ
8
9 365 designed the process evaluation and wrote the statistical analysis plan and JJ, DK and JSP organise
10
11 366 data management and will oversee field operations. All authors provided edits and critiqued the
12
13 367 manuscript for the scientific content. All authors read and approved the final version of the
14
15 368 manuscript.

16
17
18 369 **b. Competing interests:** The authors declare that they have no competing interests.

19
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21
22 371 024915. The funders will have no role in the design of this study and will not have any
23
24 372 role during its execution, analyses, interpretation of the data, or decision to submit results.

25
26 373 **d. Data sharing statement:** No data are available.

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30 374 **References:**

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40 426 **Abbreviations:**

41	CAP	Chinnallapuram
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43	CMC	Christian Medical College, Vellore
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45	CMV	Cytomegalovirus
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47	COVID-19	Coronavirus Disease-2019
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49	CRF	Case Report Format
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51	FRA	Field Research Assistant
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53	hCoV	Human Coronavirus
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55	LMIC	Low and Middle Income Country
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3	NCBS	National Centre for Biological Sciences
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5	PBMC	Peripheral Blood Mononuclear Cells
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7	PPS	Probability proportionate to size
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10	RT-PCR	Reverse Transcription -Polymerase Chain Reaction
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12	SARS-COV-2	Severe acute respiratory syndrome coronavirus 2
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14	SES	Socio Economic Status
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17	β-CoV	Betacoronavirus
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19	VHDSS	Vellore Health & Demographic Surveillance System
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23 **427 Ethics approval and consent to participate:**

24
25 428 The study has been approved by the institutional review board, (IRB No: 13585), Christian
26
27 429 Medical College, Vellore. The study will adhere to the principles that govern biomedical
28
29 430 research involving human subjects. The Declaration of Helsinki will be followed to assure that
30
31 431 the rights, integrity, and confidentiality of study participants are protected, and that reported
32
33 432 results are credible and accurate. The privacy and confidentiality of all information collected,
34
35 433 including those derived from clinical specimens, will be ensured during and after the project.
36
37 434 Individuals will not be identified in any reports or publications based on the study. All
38
39 435 participant data will be computerized using password protection. The participants will be asked
40
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42
43
44 436 to provide written informed consent.

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47 437 **Consent for publication:** Not applicable.

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49
50 438 **Acknowledgements:** We would like to acknowledge the participants who are willing to
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52 439 participate in the current study and help us in understanding the current knowledge gaps in
53
54 440 COVID-19 infection and re-infections.
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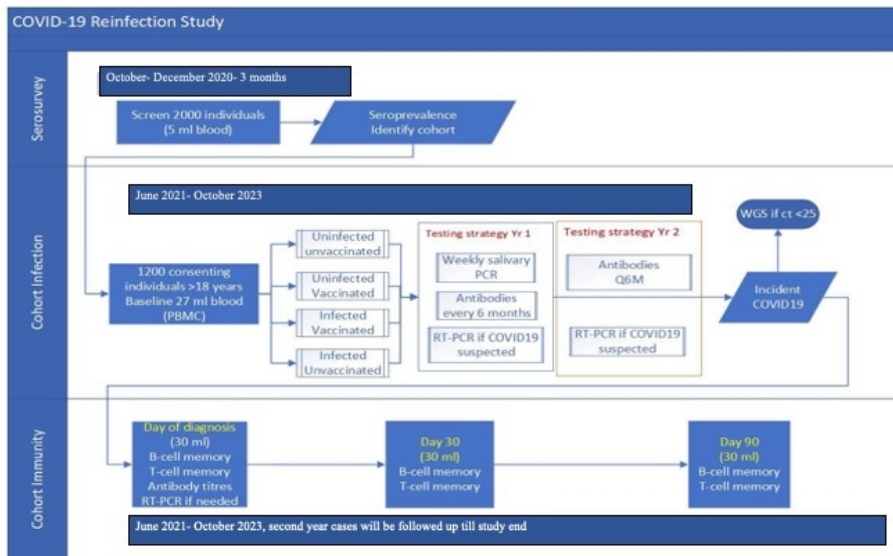
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2
3 441 We would like to thank the National Centre for Biological Sciences, Bangalore for helping us
4
5 442 in processing our weekly saliva samples.
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8 443 **Figure legend**
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10 444 Figure 1: CORES study flowchart
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For peer review only



CORES study flowchart

338x190mm (54 x 54 DPI)

BMJ Open

Risk of COVID-19 re-infection and its predictors (CORES): protocol for a community based longitudinal cohort study in Vellore, India

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Primary Subject Heading:	Public health
Secondary Subject Heading:	Epidemiology, Infectious diseases
Keywords:	COVID-19, IMMUNOLOGY, PUBLIC HEALTH

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Manuscripts

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3 1 **Risk of COVID-19 re-infection and its predictors (CORES): protocol for a community**
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5 2 **based longitudinal cohort study in Vellore, India**
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3 76
45 77 **Abstract**6
7
8 78 **Introduction**

9
10 79 The incidence of SARS-CoV-2 re-infection has not been widely evaluated in low-income and
11
12 80 middle-income countries (LMICs). Understanding immune responses elicited by SARS-CoV-
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14 81 2 natural infection and factors that lead to re-infection in a community setting is important for
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16 82 public health policy. We aim to investigate the risk of primary infection and re-infection among
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18 83 those without and with evidence of prior infection as defined by the presence of antibodies to
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20 84 SARS-CoV-2 spike protein.
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23
24 85 **Methods and analysis**

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26 86 A baseline seroprevalence survey will test for SARS-CoV2 antibodies among healthy adults
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28 87 in Vellore, India. Based on an expected seropositivity rate of 50% in the general population,
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30 88 with an annual attack rate of 12%, 6%, 4.8% and 4% among those unvaccinated and
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32 89 seronegative, vaccinated and seronegative, unvaccinated and seropositive, and vaccinated and
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34 90 seropositive respectively, we will recruit 1200 adults who will be followed up for a total of
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36 91 24 months. Weekly self-collected saliva samples will be tested by RT-PCR to detect SARS-
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38 92 CoV2 infections, for a period of one year. For any person testing RT-PCR positive, blood
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40 93 samples will be collected within 2 days of RT-PCR positivity and on days 30 and 90 to assess
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42 94 the kinetics and longevity of the antibody responses, B cell memory and T cell memory post-
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44 95 infection. The data will be analyzed to estimate seroprevalence at baseline and over time, the
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46 96 risk factors for infection, rates of primary infection and re-infection and provide a
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48 97 comparison of the rates across groups based on infection and vaccination status.
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3 **98 Ethics and dissemination**
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5 99 The study has been approved by the institutional review board (IRB No: 13585), Christian
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7
8 100 Medical College, Vellore. The results of the study will be made available through journal
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10 101 publications and conference presentations.
11

12 **102 Study registration**
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14 103 The study has been registered with the Central Trial Registry of India (CTRI; registration
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17 104 number CTRI/2020/11/029438).
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21 **106 Strengths and limitations of this study**
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24 107 • The use of saliva samples for SARS CoV 2 surveillance will be an acceptable alternate
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26 108 as it is self-directed and non-invasive.
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28 109 • Weekly salivary RT-PCR will serve as surveillance for SARS CoV 2 at the community
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31 110 level in Vellore.
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33 111 • The study involves analysis of both humoral and cellular immune responses in
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35 112 individuals with infections and re-infections.
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37 113 • The immunological profile following vaccine breakthrough infections will be studied
38
39 114 in detail.
40
41 115 • Though there is a good concordance of saliva and nasopharyngeal swab for SARS CoV
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43 116 2 surveillance, there could be some infections which may be missed with saliva
44
45 117 sampling.
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50 **118 Keywords:** COVID-19, Immunology, Public Health.
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122 Introduction

123 Immune responses to SARS-CoV-2 infection, vaccination, and the immune correlates of
124 protection are areas of active investigation [1–4]. A few studies have shown that the
125 development, amount, kinetics of antibodies may correlate with the clinical outcome of SARS
126 CoV 2 infections. [5–7]. The coordinated response between humoral and cellular immunity has
127 been hypothesized to be protective [8]. From a public health perspective, it is crucial to
128 understand the duration of protective immunity offered by natural infections and vaccination.
129 The reported duration of protection following a natural infection is around 8 months to 1 year.
130 [2–4]. Re-infections from a different strain have been documented in persons who have
131 recovered from a prior natural infection.[9,10] At the population level, the incidence of re-
132 infection over a longer term of one to two years due to various variants of concern (VOC) has
133 not been evaluated, and this is also affected by vaccination. Preliminary studies suggest that
134 antibodies persist for seven to nine months or more post-infection [11,12]. The rates of attrition
135 of potential immune correlates like memory B and T cell responses, and the association of these
136 humoral and cellular immune parameters with subsequent re-infections, particularly with VOCs
137 are unknown. The duration of protective immunity to SARS-CoV-2 is being measured, but so
138 far has largely been extrapolated from the data of phylogenetically related viruses. Antibody
139 responses to SARS-CoV-1 persist for two to three years [13] and memory T cells persist for 11
140 years after infection [14]. In contrast, beta coronaviruses [β -CoV] that are phylogenetically
141 close to SARS-CoV-2 are known to re-infect humans throughout life [15], suggesting short
142 lasting protective immunity. Human controlled infection models using common cold associated
143 beta coronaviruses (β -CoV) showed partial protection from antibodies that persist for one year
144 [16]. These findings suggest that similar protective immune mechanisms could be operative in
145 SARS-CoV-2 as well but need detailed characterization in populations with known viral
146 circulation. Further, uninfected individuals could harbor antibodies and memory T cells to other

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3 147 beta coronaviruses [17]. Such cross-reactive T cell responses [17] targeting several epitopes on
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5 148 the surface proteins of SARS-CoV-2, could potentially influence the course of infection, or the
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8 149 clinical outcomes. The limited availability of data on SARS-CoV2 infections in LMICs where
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10 150 exposure to other coronaviruses may differ, warrant a detailed evaluation of cross-reactive T
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12 151 cell and antibody landscapes in primary infections and re-infection outcomes in the community.

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15 152 This protocol describes a study to estimate the incidence of infection, re-infection and
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17 153 vaccine breakthrough infections in a community in India. The study would also determine the
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19 154 antibody profile, duration of antibody persistence as well the cellular immune responses
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21 155 following natural COVID-19 infection and re-infection.

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24 156 **Objectives and Expected outcomes**

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26 157 The CORES study has the objectives and outcomes as described in table 1.

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28 158 **Table 1: Objectives and outcomes of CORES study**

Objective 1: To estimate the seroprevalence of antibodies to SARS-CoV-2 spike protein in Vellore (May- October 2021)
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Outcome:

- a. The proportion of individuals ≥ 18 years of age who are seropositive for antibodies to spike protein of SARS-CoV-2 in Vellore
- b. Prevalence of seropositivity across clusters (wards)

Objective 2: To measure the incidence of SARS-CoV-2 infection in a cohort of individuals ≥ 18 years in Vellore (May 2021- October 2023)
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Outcome:

- a. Incidence of SARS-CoV-2 infection among those without evidence of prior infection or vaccination

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- b. Incidence of SARS-CoV-2 infection among those with evidence of prior SARS-CoV-2 infection
 - c. Incidence of SARS-CoV-2 infection in those who have received at least one dose of COVID-19 vaccine at least 14 days prior to infection.

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Objective 3: To track cellular and humoral immune correlates of COVID-19 infection, re-infection, and clinically significant disease (May 2021- October 2023)

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Outcome:

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- a. Kinetics and longevity of antibody responses and immunological memory
 - b. Influence of baseline memory T and B levels (both SARS-CoV-2 specific as well as cross-reactive) on infection

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159 **Methods and analysis**

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160 **Study setting**

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161 Vellore is a tier 2 city in northern Tamil Nadu with a population of close to 5,00,000. It is divided into four zones and 60 administrative wards. The Vellore Health and Demographic Surveillance System (VHDSS), established by the Christian Medical College, monitors a population of 1,20,000 people across zones 3 and 4 of the city. This study area has a very high population density predominantly belonging to the economically poorer section, and is largely homogenous, with daily wage-earners being the largest sub-group of the population.

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167 **Study design**

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168 The study will have three components (1) serosurvey to estimate the seroprevalence of SARS-CoV-2 spike protein antibodies in the study area (2) prospective weekly follow-up to estimate the infection and re-infection rates in a cohort of 1200 individuals, (3) intensive follow up of incident SARS-COV-2 infections (both symptomatic and asymptomatic) to characterize immunological and clinical features of infection in the cohort. The study flow is in Figure 1.

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3 174 **Study status and timeline**

4 175
5 176 The cohort recruitment started on 11th May 2021 and was completed on 28th October 2021. The
6
7 cohort will be followed up for a period of two years and data will be collected until October
8 177
9 2023.
10 178

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12 179 **Patient and public involvement**

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14 180 No patients or public were involved in the design or conduct of the study. We will report the
15 181 data in peer reviewed publications and share it with state health authorities. Participants will
16
17 be provided with study results and interpretation at a public meeting at the end of the study.
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22 183 **Inclusion and exclusion criteria**

23
24 184 Inclusion criteria:

- 25
26 185 1. Age 18 years and above
27
28 186 2. Permanent residents of the selected wards
29
30 187 3. Only one member from each selected household will be enrolled
31
32 188 4. Individuals with a history of clinical illness suggestive of COVID-19 or confirmed COVID-19
33
34 in the past, who are seropositive at baseline in the serosurvey (symptomatic seropositive).
35 189
36 190 5. Individuals seropositive at baseline, with no history of COVID-19 (asymptomatic seropositive).
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38 191 6. Individuals seronegative at baseline, stratified by the ward of residence.
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43 192 Exclusion criteria:

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45 193 1. Participant refusal of consent
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47 194 2. Pregnant women and immunocompromised patients
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49 195 3. Participants not willing for follow-up till the end of the study.
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51 196 4. Active cancers or bleeding disorders.
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55 197 **Statistical considerations**

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57 198 **Assumptions**
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199 We make the following assumptions. The assumptions were based on the early findings of the
200 Com-CoV study as there were no published data regarding vaccine efficacy and re-infections
201 prior to the start of the study.[18]

- 202 1. 50% of the population will be seropositive at baseline.
- 203 2. 40% will have received two doses of vaccine mid-way into the study.
- 204 3. The annual incidence of SARS-CoV-2 infection detected by the salivary PCR in those
205 **unvaccinated and have no detectable antibodies** (unexposed) at baseline will be
206 **12%.**
- 207 4. The annual incidence of SARS-CoV-2 infection detected by the salivary PCR in those
208 **vaccinated and unexposed** at baseline will be **6%** (VE 50% against infection).
- 209 5. The annual incidence of SARS-CoV-2 infection detected by the salivary PCR in those
210 **unvaccinated and who have antibodies (exposed)** at baseline will be **4.8%.**
- 211 6. The annual incidence of SARS-CoV-2 infection detected by the salivary PCR in those
212 **vaccinated and exposed** at baseline will be **4%.**

213 Based on these assumptions, for 90% power to detect a 5% difference in the rate of re-infection
214 and primary infection in the cohort, a sample size of 1200 participants is proposed, after
215 allowing for a 10% dropout rate.

216 **Key definitions**

217 **Seropositive** is defined as serum/plasma samples positive for IgG spike protein antibody to
218 SARS CoV2 identified by LIAISON® SARS-CoV-2 TrimericS IgG assay by Diasorin
219 platform. The cut off for seropositivity is more than or equal to 33.8 BAU/ml.

220 **Past asymptomatic infection** refers to those who are seropositive (or documented RT-PCR
221 positive >1 month in the past) but are neither antigen or RTPCR positive at baseline assessment
222 AND have had no symptoms of COVID-19.

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3 223 **Recent asymptomatic infection** refers to those who are seronegative AND are either RTPCR
4
5 224 or antigen positive AND have had no symptoms of COVID-19.

6
7 225 **Past symptomatic infection** refers to those who are seropositive (or documented RTPCR
8
9 226 positive >1 month in the past) but are neither antigen or RTPCR positive at assessment AND
10
11 227 have had symptoms of COVID19 in the past.

12
13 228 **Recent symptomatic infection** refers to those who are seronegative AND are either RTPCR
14
15 229 or antigen positive at assessment AND have symptoms of COVID19 within the past one month.

16
17 230 **Clinically significant disease** refers to those who develop symptoms due to SARS CoV 2 and
18
19 231 require hospitalisation or Intensive Care Unit admission.

20
21 232 **Re-positivity** refers to those who test positive within 90 days of the first RT-PCR results with
22
23 233 symptoms.

24
25 234 **Re-infection** refers to those who test positive after 90 days of the first RT-PCR results with or
26
27 235 without any symptoms.

28 29 236 Study procedures

30 31 237 **Baseline serology screening**

32
33 238 A baseline serosurvey, conducted on 2000 individuals in four urban clusters, is planned based
34
35 239 on population proportionate to size (PPS). The participants who satisfy the inclusion criteria
36
37 240 will be selected for the serosurvey from areas within the Vellore corporation limits after
38
39 241 obtaining written informed consent. The inclusion and exclusion criteria are detailed in the
40
41 242 earlier section. The baseline demographic information, along with details of any clinically
42
43 243 relevant illness in the past one month, will be documented. History of confirmed COVID-19
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45 244 or COVID-like illnesses during the period of the pandemic will also be documented. A
46
47 245 peripheral blood sample (5 ml serum) will be collected.

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4 **248 Establishment of the cohort**

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7 250 Based on the seroprevalence from the serosurvey, longitudinal follow-up will be initiated in the
8
9 251 Vellore health and demographic surveillance system (VHDSS) area. A total of 1200 residents
10
11 252 living in the densely populated wards of zone 3 and 4 of the Vellore corporation will be recruited
12
13 253 for the longitudinal follow-up. Those subjects who agree to the specific terms of the
14
15 254 longitudinal follow-up of 24 months will be recruited after informed consent. One member in
16
17 255 the household will be selected using simple random sampling. Each study participant will be
18
19 256 assigned a unique cohort ID. The final 1200 participants will be in any of the four groups based
20
21 257 on their vaccination and infection status with no specific distribution across these four groups.
22
23 258 The vaccination status will be obtained and recorded at the baseline and every 6 months for
24
25 259 those who were unvaccinated at enrolment. Details of precautionary or booster doses also will
26
27 260 be captured during the 6 monthly interview. The vaccination certificate would be verified for
28
29 261 confirmation of details (date, type of vaccine, number of doses etc). Upon recruitment, blood
30
31 262 samples (15-30 ml) will be collected, processed and stored as per standard protocol. Peripheral
32
33 263 Blood Mononuclear Cells (PBMCs) will be isolated prior to storage to assess the baseline T-
34
35 264 cell and memory B cell profiles in the future.

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41 **265 Intensive follow up phase**

42
43 266 The first year following recruitment of the cohort would be the intensive follow up phase during
44
45 267 which weekly follow up visits and saliva sampling is planned. An assigned field research
46
47 268 assistant (FRA) will contact the study participant every week, either by telephonic or direct
48
49 269 visit and collect information regarding any COVID-like symptoms in the preceding week. The
50
51 270 study participants will be trained to collect 2 ml of saliva in the universal sample container,
52
53 271 early in the morning, on one designated day of the week. The participants will be asked to
54
55 272 collect these samples as per the study protocol, prior to routine oral hygiene, and consumption
56
57 273 of any food or drink. The samples will be collected by the FRAs and transported to the lab in

1
2
3 274 vaccine carriers with ice packs to maintain a temperature of 4°C. The samples once received
4
5
6 275 in the lab will be aliquoted in two vials. One vial will be retained at the Wellcome Trust
7
8 276 Research Laboratory, Vellore. The other vial is sent to the National Centre for Biological
9
10 277 Sciences, Bangalore (NCBS) for RT-PCR.

11
12
13 278 If an individual tests positive for SARS-CoV2, the weekly salivary sample collection will be
14
15
16 279 suspended for the next 90 days. The weekly contact, however, will be continued. During the
17
18 280 weekly contact if a subject develops symptoms, their samples will be collected. If they are RT-
19
20 281 PCR positive within 90 days it will be considered as re-positive. The study participants will be
21
22 282 requested to inform the study team if they experience any clinically significant febrile or
23
24 283 respiratory distress. Symptomatic individuals will be advised to visit Christian Medical College
25
26 284 Hospital, Vellore and get tested by nasopharyngeal RT-PCR, as deemed necessary, after
27
28 285 clinical examination. Clinical symptoms, response to treatment and details of treatment during
29
30 286 hospitalization or during home management would be recorded on the Case Report Form
31
32 287 (CRF) for every participant who is positive by RT PCR.

33 34 35 36 37 288 **Follow up phase - second year**

38
39
40
41 289 During the second year of the study, weekly follow up would be through telephonic interviews.
42
43 290 Weekly salivary samples will not be collected, and home visits will be done only for subjects
44
45 291 with symptoms. Any incident infection will be followed up for detailed immunological testing.
46
47 292 Once every six months, a blood sample (5 ml) will be collected for assessing the serostatus of
48
49 293 the participants to identify any infection that was missed through the RT-PCR screening.
50
51 294 Sequencing will be done on all positive samples to identify the genetic sequence of the virus
52
53 295 at NCBS, Bangalore and help us determine which variant of concern was responsible for the
54
55 296 infections and re-infections. This will include samples classified as 're-positives'.
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3 297 **Detailed follow-up of COVID-19 infections**
4

5 298 All COVID-19 infections, including symptomatic and asymptomatic will be followed up from
6
7 299 the day of the positive report (Day 0). Blood samples (30ml) will be collected for PBMC
8
9
10 300 isolation and storage within 24 hours of identification of positives on (Day-0), Day-30 (+2
11
12 301 days) and Day-90 (+7 days) post-infection.
13

14
15 302 **Sample collection**

16
17 303 **Blood sample - serology:** Five ml of peripheral blood will be collected (in serum tubes) from
18
19 304 2000 individuals during the baseline sero-survey, and once every six months from the 1200
20
21 305 study participants who are a part of the longitudinal cohort.
22

23
24 306 **Salivary sample:** Salivary samples will be self-collected, stored and transported to the NCBS
25
26 307 laboratory, Bengaluru, as per the Standard Operating Procedure, once a week during the first
27
28 308 year of the study. The results will be uploaded into the secure data entry portal designed for
29
30 309 the laboratory.
31

32
33 310 **Nasopharyngeal swab:** If any study participants report any clinically significant febrile illness
34
35 311 or respiratory distress, they will be offered a medical consultation, and when necessary, a
36
37 312 nasopharyngeal RT-PCR at CMC or in any institute of their choice.
38

39
40 313 **Blood sample (for PBMC):** 30 ml (minimum 15 mL) of blood will be collected (in 9 ml
41
42 314 heparin tubes) after recruitment into the longitudinal study and for confirmed SARS-CoV-2
43
44 315 infections on Day-0, Day-30 and Day-90. PBMCs will be separated by density gradient
45
46 316 centrifugation method and cryopreserved in liquid nitrogen.
47
48

49 317 **Laboratory procedures**

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51
52 318 **Weekly salivary samples**
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55
56 319 Upon receipt and aliquoting, salivary samples will be pooled for testing on the same day. Ten
57
58 320 μ l of five samples each will be pooled in a single well of the PCR plate, and 6 μ l of proteinase
59
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1
2
3 321 K of 50 µg/µl concentration will be added to each well. Subsequently, the plates will be sealed
4
5 322 and heated at 95° Celsius for 5 minutes in a dry thermal bath. After heat inactivation, the plates
6
7 323 will be stored at minus 80°C. The pooled PCR plate and an aliquot of saliva will be transported
8
9 324 on dry ice to NCBS. RT-PCR will be performed on the pooled samples targeting the N gene,
10
11 325 E gene and RdRp gene of SARS CoV 2. The limit of detection of the commercial kit that is
12
13 326 used for testing is 100 copies/ ml and the sensitivity of detection in saliva samples is around
14
15 327 94% compared to NP swab. [19] If any pool turns out to be positive, RT-PCR will be performed
16
17 328 on individual samples. All positive samples will undergo sequencing.

21 329 **Blood samples**

24 330 **Serological assays**

25 331 The plasma or serum sample collected at different time points will be tested for IgG antibody
26
27 332 against spike protein using a high throughput automated platform (Diasorin LiaisonXL).

31 333 **Immunophenotyping**

32 334 Quantitation of SARS-CoV-2 specific T cells will be done by flow cytometric detection of
33
34 335 cytokines and Activation Induced Marker (AIM) upregulation in T cells after stimulation with
35
36 336 peptide pools. PBMC stimulation will be done using a 10-mer peptide pool for CD8 and 20-
37
38 337 mer peptide-pools for CD4 T cells. Four peptide pools will be used, corresponding to the major
39
40 338 proteins of SARS-CoV-2 (Spike, Envelope, Membrane and Nucleoprotein). For all the
41
42 339 stimulation conditions, one well (vehicle-treated) will act as negative control. An additional
43
44 340 well of cytomegalovirus (CMV)-peptide-stimulated control (a mix of 10-mer and 15-mer CMV
45
46 341 peptides) will be kept as positive control for each sample. Baseline levels of cross-reactive T
47
48 342 cells to non-SARS-CoV-2 human Coronaviruses (hCoV) will be estimated using the same
49
50 343 methodology, using peptide pools derived from hCoV strains. Memory B cells will be detected
51
52 344 by flow cytometry after staining PBMCs with fluorophore-tagged viral proteins and memory
53
54 345 B cell markers.

346 **Statistical analysis**

347 Seroprevalence is estimated as a proportion and will be assumed to follow a binomial
348 distribution. The incidence of infection within the cohort is expected to follow a Poisson
349 distribution. We will permit repeated infections to be captured in analysis and account for the
350 same in the analysis. A time to event analysis using Prentice, Williams and Peterson models
351 comparing incidence in the exposed and unexposed cohorts will be performed. We will adjust
352 for background infection rates in each cluster (ward) and covariates such as age, SES,
353 vaccination status, per-capita floor space and occupation class.

354 The statistical analysis plan will detail the estimation of seroprevalence, its risk factors, the
355 incidence of primary and re-infection and a comparison of these rates. Continuous variables
356 will be described using mean (SD) and median (IQR) where necessary. Categorical data will
357 be expressed as frequency (%). Incidence of infection and re-infection will be calculated per
358 thousand person years. Hazard ratios will be estimated to assess protection/risk conferred by
359 vaccination and previous infection.

360 **Key comparisons in the study**

361 We will make comparisons between:

- 362 • Incidence rates of infection overall and in seropositive and seronegative subgroups
- 363 • Incidence rates of infection among the vaccinated individuals in the cohorts
- 364 • Kinetics and longevity of memory B and T cells in infections occurring in the
365 seropositive and seronegative cohort
- 366 • Baseline cross-reactive T cells and antibodies to non-SARS-CoV-2 beta coronaviruses
367 between symptomatic infections vs asymptomatic infections vs uninfected individuals
368 in the seronegative cohort
- 369 • Baseline SARS-CoV-2 specific memory T and B cells and antibody levels between
370 infected individuals versus uninfected individuals in the seropositive cohort

371 **Data management plan**

372 All the Case Report Format (CRFs) will be in the electronic format (Redcap©), and the entry
373 platform will be connected to the Central database server. The Data management system is
374 responsible for the periodic validation process and quality of the data. Any further correction
375 in the database after the entry is 'saved' is accompanied by a duly completed "Data
376 Clarification form." The electronic data management system tracks key study progress
377 parameters on an access-restricted online dashboard. The weekly contact made by the FRAs
378 will be independently validated by a field worker who calls 5% of all individuals who were
379 contacted that week.

380 **Ethics and dissemination**

381 The study has been approved by the institutional review board (IRB No: 13585), Christian
382 Medical College, Vellore. The study will adhere to the principles that govern biomedical
383 research involving human subjects as required in India. The Declaration of Helsinki will be
384 followed to assure that the rights, integrity, and confidentiality of study participants are
385 protected, and that reported results are credible and accurate. The privacy and confidentiality
386 of all information collected, including those derived from clinical specimens, will be ensured
387 during and after the project. Individuals will not be identified in any reports or publications
388 based on the study. All participant data will be computerized using password protection. The
389 participants will be asked to provide written informed consent. The knowledge gained and the
390 results will be made available through journal publications and conference presentations.

391

392 **Discussion**

393 To our knowledge, this study is the first to follow up a cohort in India, for a period of two years
394 for COVID-19 infection and re-infection. In terms of surveillance of SARS-CoV-2 infection,
395 though the nasopharyngeal swab has been the gold standard for diagnosis, the use of saliva

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3 396 samples will be an acceptable alternate by the study participants as it is self-directed, non-
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5 397 invasive and has a good concordance with the nasopharyngeal swab. The study aims to address
6
7 398 several gaps in the current scientific evidence of SARS-CoV-2 infection and immunity. Firstly,
8
9 399 there are a limited number of studies that investigate the long term follow up of individuals for
10
11 400 the rates of infection and re-infection in the community. Secondly, the study aims to look at
12
13 401 the kinetics of IgG antibodies following infection. The cross-reactivity between SARS-CoV-2
14
15 402 and other human coronaviruses will support better understanding of determinants of
16
17 403 symptomatic infection. The T cell and B cell memory responses would help in understanding
18
19 404 the kinetics and longevity of immune responses in seropositive and seronegative individuals
20
21 405 and would help in decision making with regard to booster vaccination. By studying the
22
23 406 immunity and the risk of reinfection we can potentially understand the factors that contribute
24
25 407 to symptomatic COVID-19 infections. The study design also will allow the study of how the
26
27 408 various VOC contribute to re-infections. Large scale vaccination had begun by the time
28
29 409 enrolment had been completed. We anticipate that the majority of participants will be
30
31 410 vaccinated at the end of the study and would have a hybrid immunity resulting from past
32
33 411 infection and vaccine. In view of the one-year intensive follow up that requires weekly samples,
34
35 412 we have planned to use salivary RT PCR and only symptomatic individuals will receive
36
37 413 nasopharyngeal swab for RT PCR.

38
39 414 To conclude, CORES will help in estimating the re-infection rates, detailed immunogenicity
40
41 415 amongst the COVID-19 positive individuals, establish the antibody kinetics and characterise
42
43 416 the breakthrough infections amongst the vaccinated individuals in the community.
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55
56 419 **Contributors:** The study design and concept were conceived by JJ and GK. RM will conduct
57
58 420 the study as part of her PhD under the supervision of GK, SB, SP and JJ. JSP and JJ designed
59
60

1
2
3 421 the process evaluation and wrote the statistical analysis plan and JJ, DK and JSP organise data
4
5 422 management and will oversee field operations. PKH, RA, GSR performed the RT-PCR of the
6
7 423 weekly saliva samples. All authors provided edits and critiqued the manuscript for the scientific
8
9 424 content. All authors read and approved the final version of the manuscript.
10
11
12

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14
15

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17
18

19 427 The funders have no role in the design of this study and will not have any role during its
20
21 428 execution, analyses, interpretation of the data, or decision to submit results.
22
23

24 429 **Consent for publication:** Not applicable.
25
26
27

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29
30 431 current study and help us in understanding the current knowledge gaps in COVID-19
31
32 432 infection and re-infections. We also thank the National Centre for Biological Sciences,
33
34 433 Bangalore for helping us in processing our weekly saliva samples.
35
36

37 434 **Abbreviations**

39 CAP	Chinnallapuram
40	
41 CMC	Christian Medical College, Vellore
42	
43 CMV	Cytomegalovirus
44	
45 COVID-19	Coronavirus Disease-2019
46	
47 CRF	Case Report Format
48	
49 FRA	Field Research Assistant
50	
51 hCoV	Human Coronavirus
52	
53 LMIC	Low and Middle Income Country
54	
55 NCBS	National Centre for Biological Sciences
56	
57	
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60	

PBMC	Peripheral Blood Mononuclear Cells
PPS	Probability proportionate to size
RT-PCR	Reverse Transcription -Polymerase Chain Reaction
SARS-COV-2	Severe acute respiratory syndrome coronavirus 2
SES	Socio Economic Status
β-CoV	Betacoronavirus
VHDSS	Vellore Health & Demographic Surveillance System

435

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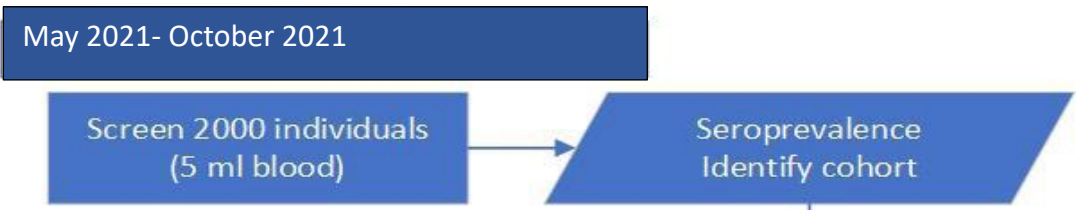
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49 491 **Figure title**

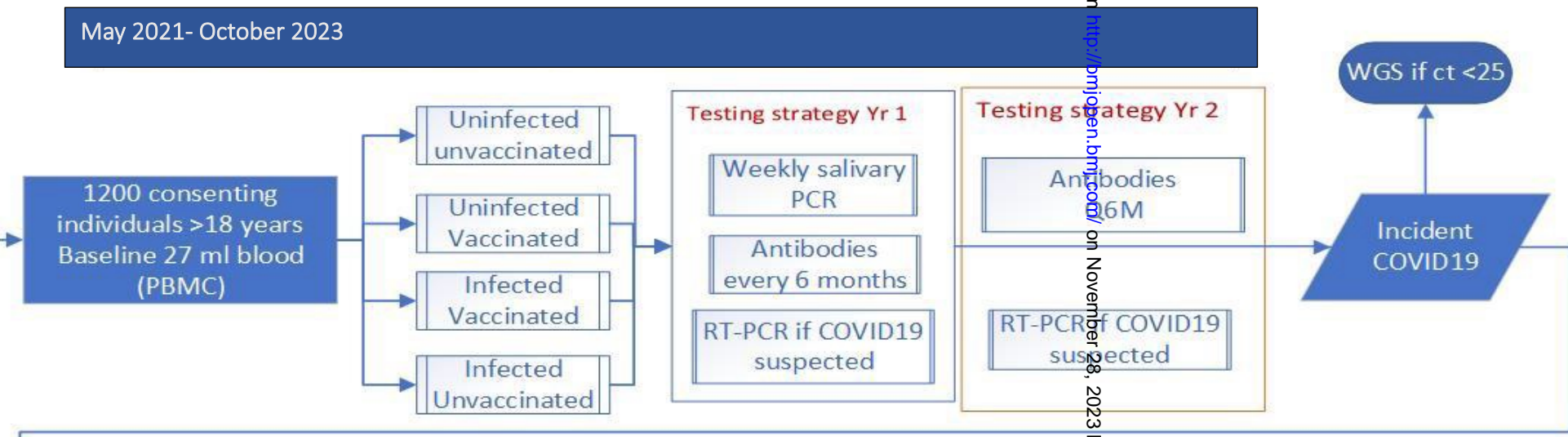
51 492 **Figure 1: CORES study flowchart**

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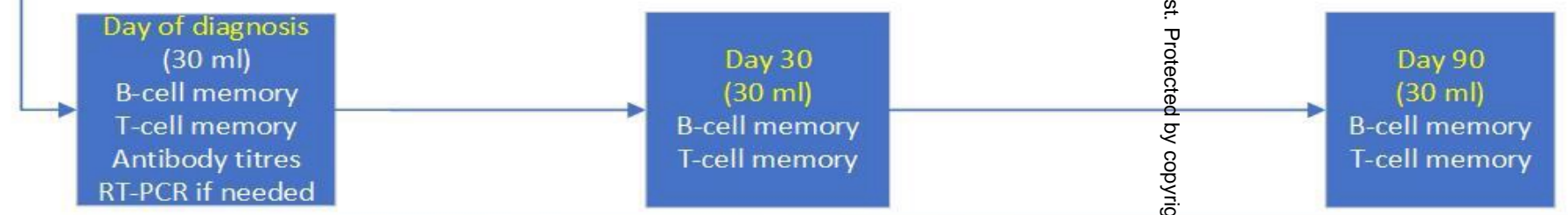
Serosurvey



Cohort Infection



Cohort Immunity



May 2021- October 2023, second year cases will be followed up till study end

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