IMmuno-Phenotyping Assessment of a Cohort To Vaccination (IMPACT V)

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Severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) manifests as the disease COVID-19 and causes a range of clinical outcomes, varying from asymptomatic infection to severe acute respiratory distress and death [1]. The pandemic has resulted in immense pressure on the health-care systems delivering care for COVID-19 and non-COVID-19 patients with major impact on the global economy. In a major race to save lives and tackle the pandemic more than 100 vaccine programs were developed globally [2] with the most successful ones deployed a few weeks ago supported by favourable phase 1 and phase 2 safety/efficacy data and phase 3 clinical trial outcomes. The vaccines by Pfizer-BioNTech, Moderna in the USA and Oxford-Astrazeneca in the UK are being deployed at the moment amongst others. The Indian version of the Oxford-Astrazeneca vaccine, Covishield and Covaxin are being deployed in India.

Coronaviruses are enveloped, positive sense single-stranded RNA viruses with a glycoprotein spike on the surface, which mediates receptor binding and cell entry during infection. The roles of the spike protein in receptor binding and membrane fusion make it an attractive vaccine antigen. The ChAdOx1 nCoV-19 vaccine (AZD1222) was developed at Oxford University [3] and consists of a replication-deficient chimpanzee adenoviral vector ChAdOx1, containing the SARS-CoV-2 full-length structural surface glycoprotein antigen (spike protein; nCoV-19) gene with a tissue plasminogen activator leader sequence. ChAdOx1 nCoV-19 expresses a codon-optimised coding sequence for the spike protein (GenBank accession number MN908947). Immunisation with ChAdOx1 nCoV-19 results in development of neutralising antibodies against SARS-CoV-2 in almost 100% of participants including older adults without severe comorbidities, with higher levels in boosted compared with non-boosted groups. An ideal vaccine against SARS-CoV-2 would be effective after one or two vaccinations; would protect target populations such as older adults and those with comorbidities, including immunocompromised individuals; would confer protection for a minimum of 6 months; and would reduce onward transmission of the virus to contacts. ChAdOx1 nCoV-19 is effective in preventing damage to the lungs after challenging Non-human primates (NHPs) with a high dose of SARS-CoV-2 to both upper and lower respiratory tract, and a prime–boost regimen significantly increased humoral immune responses.

India has been among the most affected countries in the pandemic, has gained approval for emergency use based on early results for the Oxford-Astrazeneca's version of the vaccine Covishield and ICMR-NIV-Bharat Biotech manufactured Covaxin. Both are being deployed at the moment. One of the key responses of the adaptive immune system to infection is the production of pathogen-specific antibodies by B cells. As the nation awaits immunization at a large scale, however, there are many open ended questions about the induction, specificity and kinetics of antibody response to the vaccine in addition to long term efficacy. Whether it is approval for emergency use or for regular use, the fast-track process of vaccine development has left some gaps in our knowledge which have public health significance. The gaps in our knowledge can be highlighted based on the following questions.

1. Will one dose of vaccine be enough to provide adequate immune response lasting for at least a year?
2. What are the types of antibodies produced and what is the level of total antibody generated following vaccination?
3. What is the level of neutralising antibody generated?
4. What is the efficiency of the antibodies directed against the vaccine?

The main goal/vision of the proposal is Immunophenotyping of individuals post vaccination to delineate the immune response across a longitudinal cohort and develop a database of these responses.

We plan to generate comprehensive profile data sets that will allow use to reconcile and analyze humoral immune response in individuals post vaccination. Longitudinal sampling in cohorts will answer important epidemiologic questions.
We plan to:

1. Identify and sample a cohort of individuals post-vaccination for long term impact of the vaccine
2. Identify and sample a cohort of individuals for antibody assessment of convalescent plasma from infected patients 2 months post infection
3. Monitor longitudinally anti-SARS-CoV-2 humoral immune response through qualitative and quantitative assessment of antibodies in the cohort
4. Develop and maintain a cryorepository of post-vaccination and convalescent plasma biological samples for future retrospective analysis

Our proposal if implemented will investigate these issues at the population level in vaccinated individuals in comparison to SARS-CoV2 infected and recovered individuals. The results can provide valuable inputs for planning further phases of the vaccination campaign, and will also support confidence-building for vaccination. The results will represent the largest post vaccination immune response study so far in the country. The implementation of this program will thus result in a significant milestone in the delineating the antibody response to SARS-CoV2 in India and its application to COVID 19 pandemic and human infectious disease.

STUDY DESIGN AND METHODS

Aim 1: Identify and sample a cohort of individuals post-vaccination for long term impact of the vaccine. The detailed study plan is described below.

a) The cohort recruited with informed consent will include volunteers from government health workers, who will both be among the first to receive vaccines and will understand the importance of the proposed study.
b) For each vaccine, three groups of volunteers will be followed. One group would be people below 50 years of age without any co-morbidities, a second group would be people below fifty years of age with one or more co-morbidities, and a third group would be people above fifty years of age with or without co-morbidities.
c) Total people recruited in each group should consist of at least 100-125 individuals, with equal numbers of men and women in each.
d) Each individual would need to provide a 10-ml blood sample seven times: one before the first vaccine dose, the second before the second vaccine dose, the third one month after the second vaccine dose, and the remaining subsequently at three-month intervals. Samples to be cryo-stored at each time-point.
e) A mobile application-based collection of data on demographic and background information and prospective symptomatology if any, to be self-reported.

Aim 2: Identify and sample a cohort of individuals for assessment of convalescent plasma from infected patients 2 months post infection. The detailed study plan is described below.

a) The cohort recruited with informed consent will include infected individuals
b) Three groups of volunteers will be followed similar to the post-vaccine immune monitoring program. One group would be people below 50 years of age without any co-morbidities, a second group would be people below fifty years of age with one or more co-morbidities, and a third group would be people above fifty years of age with or without co-morbidities.
c) Each group should consist of at least 40-50 individuals, with equal numbers of men and women in each.
d) Each individual would need to provide a 10-ml blood sample two times: once 15-20 days after infection, the second after 60-70 days. The longitudinal cohort of samples to be cryo-stored at each time-point. Clinical and background data of this cohort will be collected and stored in a database.

Aim 3: Monitor longitudinally anti-SARS-CoV-2 humoral immune response through qualitative and quantitative assessment of antibodies in the cohort

The detailed study plan is described below.

a) ELISA based estimation (qualitative and quantitative) of anti-SARS-CoV2 spike protein (at all 7 timepoints)
b) The ELISA tests to be performed on each serum sample will measure: (SARS-CoV-2 spike protein RBD-specific IgM, IgG and IgA antibody levels for all 7 time points. A 5 point calibration will be set up to accurately quantitate the antibody level and response.
c) Measurement of strength/avidity of binding of antibodies obtained in (b)
d) Relative contribution of IgG/IgA subtypes wherever applicable.
e) Virus Receptor-Neutralizing antibody (nAb) content of serum will be measured by a surrogate in vitro assay for all subjects at each time-point.
f) Randomly selected sera samples amounting to around 5% of all sero-positive samples at each time-point from each center to be sent for cell culture-based virus neutralization assays at CSIR-CCMB.

Aim 4: Develop and maintain a cryorepository of post-vaccination and convalescent plasma biological samples for future retrospective analysis. The detailed study plan is described below.

Samples will be cryo-stored at each time-point from each recruited subject:

a) Cryo-stored PBMC (in FBS with 10% DMSO)
b) Plasma isolated from EDTA blood samples
c) Serum
d) Genomic DNA isolated from granulocytoic precipitate while isolating PBMC
e) Total RNA from PBMC
f) RNA isolated from nasopharyngeal swabs (only from the convalescent plasma study cohort)

The project IMPACT V initiative will work with a network of 70 hospitals and the Pune Municipal Corporation. Hospitals that will partner with this initiative included but are not limited to Deenanath Mangeshkar Hospital, Sassoon Hospital etc.

IMPACT V aims to set up a unique program to monitor immune response in health-care workers and other individuals being immunized in the first phase of vaccination against COVID19. The core team will be CSIR-NCL, IISER Pune, PKC and PMC. CSIR-NCL and IISER-Pune with their strong base in biological and clinical outreach will provide the framework for sample collection, repository creation and antibody analysis. We will set up a team comprising of scientists, data analysts and co-ordinators.
Each research institution will contribute their unique expertise to collectively elucidate the humoral immune response to COVID-19 infection. The synergistic coalition of researchers will work closely and share data to maximize the impact of cohort samples. The overall goal is to identify immunological and virological correlates in the population. The comparisons with convalescent plasma will give baseline comparisons of differential antibody response in vaccinations and infection.

SOPs for informed consent, sample collection and processing will be uniform across all clinical centers. All specimen storage and analysis will be performed at the research institutes mentioned above. As immunizations in the first phase are targeted to healthcare workers followed by others, the pilot program will target recipients of the vaccine from not only healthworkers but recipients of Covishield vaccine.

Based on the above the industry can contribute CSR in the form of domain expertise, partner networks to facilitate the implementation of various activities including sample collection agencies, reagents for immune monitoring, and contributing equipment for the above studies.

Impact

With the largest vaccine roll-out intended in the country, our preparedness lies in evidence based strategies for a graded vaccination program. One of the strategies is to prioritize within the high risk groups, those who do not harbor any neutralizing antibody levels. The understanding of the differential antibody response in vaccinated cohort and the convalescent sera will help answer epidemiological questions.

Funding requirement
While the expertise and some of the infrastructure already exists, there will be a need to set up some of these functions from the ground up. An immune response tracking project at this scale requires high volume of molecular reagents, enzymes, kits and automation.

Establishing IMPACT V facility for rapid, accurate and high throughput analysis will cost approximately INR 5.5 Crores. This figure includes the following infrastructure and operations costs: ELISA plate readers equipment, automation and liquid handlers for accuracy, assays for detection of antibodies of SARS CoV-2, quantitation of antibodies, sub typing of immune responses, cloud-based bioinformatics services for data analysis and reporting, recruitment and training of multidisciplinary manpower, implementation research team with project management experience, necessary fixed/mobile infrastructure and other consumables.

Workstream	Budget Item	Unit Cost	Quantity	Total cost
Overall project management	Human Resources (senior research associates)	52,000	2	12,48,000
	Other administrative costs (local transport, database security etc)	10,00,000	1	5,00,000
Sample collection and other costs	Set-up of infrastructure lab for post-vaccination study -80C Freezers Liquid nitrogen tanks -20C Freezers ELISA plate readers and accessories Liquid Handling system			100,00,000
	Set-up of sample collection workflows for post-vaccination and post infection convalescent plasma studies			
Collecting and analyzing research data	ELISA based serology tests in pre-vaccination studies (10,000 people over 6 months)	2500	17500	4,40,00,000
	Personnel for on-site laboratory and survey activities			
	Data storage costs			
	Sample storage (cryorepositories)			
Grand Total			1	5,53,00,000

References:
[1] Folegatti, P. M. et al. Safety and immunogenicity of the ChAdOx1 nCoV-19 vaccine against SARS-CoV-2: a preliminary report of a phase 1/2, single-blind, randomised controlled trial. Lancet 396, 467–478 (2020)
[2] Draft landscape of COVID-19 candidate vaccines. https://www.who.int/publications/m/item/draft-landscape-of-covid-19-candidate-vaccines (World Health Organization, 2020).
[3] Ewer, K.J., Barrett, J.R., Belij-Rammerstorfer, S. et al. T cell and antibody responses induced by a single dose of ChAdOx1 nCoV-19 (AZD1222) vaccine in a phase 1/2 clinical trial. Nat Med (2020).