Hybrid Immunization In COVID-19: Time Matters

Laura Sánchez-de Prada (laura.sanchez.prada@uva.es)
University of Valladolid

Ana María Martínez-García
Hospital Clínico Universitario de Valladolid

Belén González-Fernández
Hospital Clínico Universitario de Valladolid

Javier Gutiérrez-Ballesteros
Hospital Clínico Universitario de Valladolid

Silvia Rojo-Rello
Hospital Clínico Universitario de Valladolid

Sonsoles Garcinuño-Pérez
Hospital Clínico Universitario de Valladolid

Alejandro Álvaro-Meca
Rey Juan Carlos University

Raúl Ortiz de Lejarazu
National influenza Center of Valladolid

Iván Sanz-Muñoz
National influenza Center of Valladolid

José María Eiros
University of Valladolid

Article

**Keywords:** COVID-19, vaccines, hybrid immunity, breakthrough infection, booster

**Posted Date:** June 12th, 2023

**DOI:** https://doi.org/10.21203/rs.3.rs-3008644/v1

**License:** © This work is licensed under a Creative Commons Attribution 4.0 International License. Read Full License
Abstract

**Purpose:** SARS-CoV-2 reinfections have been frequent, even among those vaccinated. The aim of this study is to know if hybrid immunity (infection+vaccination) is affected by the moment of vaccination and the number of doses received.

**Methods:** We conducted a retrospective study in 745 patients with a history of COVID-19 reinfection and recovered the dates of infection and reinfection and vaccination status (date and number of doses). To assess differences in the time to reinfection ($t_{RI}$) between unvaccinated, vaccinated before 6 months and later, and comparing one, two or three doses (incomplete, complete and booster regime) we performed the log-rank test of the cumulative incidence calculated as 1 minus the Kaplan-Meier estimator.

**Results:** The $t_{RI}$ was significantly higher in those vaccinated vs. non-vaccinated ($q<0.001$). However, an early incomplete regime (1 dose) protects similar time than not receiving a vaccine. Vaccination before 6 months after infection showed a lower $t_{RI}$ compared to those vaccinated later with the same regime ($q<0.001$). Actually, early vaccination with complete (2 doses) and booster regimes (3 doses) provided lower length of protection compared to vaccinating later with incomplete (1 dose) and complete regime (2 doses), respectively. Vaccination with complete and booster regimes significantly increases the $t_{RI}$ ($q<0.001$).

**Conclusion:** Vaccination increases the time it takes for a person to become reinfected with SARS-CoV-2. Increasing the time from infection to vaccination increases the time in which a person could be reinfected. Booster doses increase the time to reinfection. Those results emphasize the role of vaccines and boosters during the pandemic and can guide strategies on future vaccination policy.

Introduction

Until mid-November 2022, the COVID-19 pandemic has been responsible for more than 600 million cases and 6.5 million deaths [1]. Different vaccine approaches against COVID-19 arrived and evolved along with the virus through the pandemic [2–4]. The fast vaccine development has made possible for developed countries to reach a considerable vaccine coverage in an amazing short period of time [5]. However, concerns related to side effects and changes in commercialization authorizations, have caused delays in the administration of second doses, heterologous vaccination, and infections at the time of vaccination. In addition, the continuous raise of variants of concern (VOC) and their spread across the world, [6] have led to a variable viral immunoescape to antibodies elicited by vaccines. Thus, viral evolution has led to breakthrough with VOCs in vaccinated populations [7–9]. Those factors have led to some individuals being infected and reinfected to present different immune status that has been called hybrid immunity.

It has been postulated that a more robust immune response is obtained by vaccination before or after SARS-CoV2 infection [10]. As VOC keep emerging and vaccines evolving more concerns about immune escape after infection or vaccination with the original strain arise [11]. Actually, vaccine breakthrough has been documented since early stages of the pandemic and have surged, especially after the emergence of some variants [12,13], becoming more often detected by the National Influenza Centers (NICs) as a part of the GISRS integration of COVID-19 and Influenza surveillance.

Different circumstances influencing breakthrough after a first infection and subsequent vaccination, so called hybrid immunity breakthrough infection (HIBI), have been explored, particularly those involving time of vaccination. The results obtained in this study show different protection patterns of hybrid immunization associated to different vaccine schedules, boosters, and time of vaccination, and help guide future strategies on COVID-19 vaccination.

Methods

**Study design and materials**

A retrospective observational study was performed by the National Influenza Centre (NIC) in collaboration with the Microbiology department at the Hospital Clínic Universitario of Valladolid, Spain. Data was extracted from the laboratory database of 346,846 RT-PCR tests to confirm infections between March 2020 and April 30th, 2022. Due to scarce availability of tests at the beginning of pandemic and other circumstances, different PCR tests were employed, namely Roche (Switzerland), Vircell (Spain), Vitro (Spain), Cepheid (USA)m Menarini (Italy), and Thermofisher (USA). All of them detected at least two genes and were considered positive following manufacturer criteria. By looking for HIBI, a total of 2,886 patients with history of COVID-19 reinfection were initially selected from 36,965 positive tests. As initial exclusion criteria, patients whose laboratory confirmed infections were less than three months apart were discarded and considered to be the same process [14] (figure 1). Dates of first and second infection were collected, as well as vaccination status and if such, date of vaccination of correspondent doses and vaccine used. Then, other exclusion criteria were applied: patients with no vaccine details in their medical records, patients who had the infection at the time of vaccination or patients with no prior history of COVID-19 infection before immunization. At the beginning of 2021, 4 different vaccines were authorized in all EU countries. Three vaccines in which two doses were required for full immunization: Comirnaty (Pfizer®), Spikevax (Moderna®) and Vaxzevria (AstraZeneca®) with intervals between doses of 3 weeks, 4 weeks, and 8-12 weeks, respectively. And one vaccine in which only one dose was required: COVID-19 vaccine Janssen (Janssen®), therefore individuals receiving that vaccine were discarded.
A total of 745 cases with history of repeated COVID-19 infection were included and 559 of them had HI BI. The 186 individuals who did not receive any vaccine but suffered reinfection were considered as controls. From patients that presented HI BI, individuals were classified according to their vaccine status: 276 had received one dose of vaccine (incomplete vaccine regime), 114 two doses (complete vaccine regime), and 169 three doses (booster vaccine regime). Different vaccine regimes schedules were due to changes in vaccine guidelines as the pandemic evolved [15] (Figure 1).

This research was performed according to the Declaration of Helsinki. The data base was anonymized and the clinical data for this observational study was performed under the strict fulfillment of the Spanish Organic Law 41/2002 for regulation of the patient’s autonomy and his rights and obligations in matters of information and clinical documentation (BOE nº274 of 15th November 2002). This research was approved by the Ethics Committee of East-Valladolid health area under the code PI 22-2920 and informed consent was waived due to the retrospective nature of the study and the anonymous nature of the dataset.

Parameters and definitions

Three different parameters to assess reinfection and HI BI were considered. First, the time elapsed between first COVID-19 infection and first dose of vaccination ($t_1$) which was used to divide the different regime cohorts in to two (early and late) (Figure 2). Then, $t_2$ represents the time elapsed between the last dose of vaccine received and the second COVID-19 infection (HI BI). And finally, $t_{RI}$ or time of reinfection represents the time elapsed between first infection and reinfection, independently of receiving a vaccine in the meantime. Time was measured in months to help perform the analysis.

Statistical analysis

Initially, $t_2$ and $t_{RI}$ were calculated as median (interquartile range, IQR). Differences between vaccinated and non-vaccinated, as well as, vaccinated early and vaccinated late were assessed using the two-tailed Mann-Whitney U-test. The Cumulative Incidence of Covid-19 reinfection of different cohorts was calculated as 1 minus the Kaplan-Meier estimator. Difference between cumulative incidence was assessed by log-rank test. Additionally, individual comparisons between group were calculated with the same test correcting for multiple comparisons with the False Discovery rate. GraphPad Prism Version 9 (GraphPad Software, San Diego, CA, USA) and R software, version 4.2.1 (GNU- General Public License, the R Core Team, R, 2022). p values < 0.05 were considered statistically significant.

Results

Profile of patients who suffered COVID-19 reinfection.

Patients who suffered reinfections (745) were divided in four groups according to vaccine status: non-vaccinated (used as controls) (n=186, 25.0 %), reinfected after incomplete vaccine regime (n=276, 37.0%), reinfected after a complete vaccine regime (n=114, 15.3%) and reinfected after a booster regime (n=169, 22.7%). In addition, vaccinated individuals with all regimes were then divided depending on when the vaccine was received: in the first five months post-infection or after/equal six months. The characteristics are described in table 1.

Globally 84.15% of the doses were Comirnaty (Pfizer®), 12.35% were Spikevax (Moderna®), and 3.5% were Vaxzevria (AstraZeneca®). Only, 69 patients received a heterologous schedule combining more than one vaccine in any of the subsequent doses.

Vaccination protects longer time from reinfection. We next analyzed the different times of reinfection between vaccinated population and non-vaccinated population. The median time from infection to reinfection ($t_{RI}$) in non-vaccinated population was 6 months (IQR: 4-10), being significantly lower compared to 14 months (IQR: 9-16) in individuals that received either one, two or three doses of vaccine after their first infection (p<0.001) (Figure 3).

“If you wait, the longer you are protected”. Here the time of HI BI after the last vaccine dose ($t_2$) and the total time between both first infection and the reinfection ($t_{RI}$) were analyzed. Based on $t_1$ being under or equal to six months, we considered those who received an early or a late first dose. Significantly higher $t_2$ was found in those who received a late first those with a median of 5 months (IQR:3-6) compared to 4 months (IQR: 2-6) in those vaccinated earlier ($p=0.034$). Again, higher $t_{RI}$ was found in those who waited with a median of 15 months (IQR: 13-17) compared to 12 months (IQR: 8-15) in those vaccinated earlier ($p<0.001$). Then, based on that $t_1$, incomplete, complete and booster regime cohorts were divided into early one dose, late one dose, early two doses, late two doses, early three doses, and late three doses. Then, to explore the differences between incomplete, complete and booster regimes and their schedules, cumulative incidences were calculated, and individual differences were computed as well (Figure 4).

First, the highest $t_{RI}$ was found in individuals who received a booster regime after the initial two doses, the first one of them received at or after six months from COVID-19 infection ($q<0.001$). The lowest value was found in individuals that received an early incomplete regime which was similar to
that of non-vaccinated individuals, with a median $t_{RI}$ of 6 months ($q=0.849$). The rest of the cohorts showed significantly higher $t_{RI}$ compared to non-vaccinated individuals ($q<0.001$) (figure 4a).

Then, the comparison regarding $t_1$ in each regime (incomplete, complete and booster) showed that receiving the first dose at a minimum of six months post-infection provided a significantly higher $t_{RI}$ (median time 14.16 and 21 months) compared to receiving it earlier (median time 6.11 and 15 months) ($q<0.001$ for all three comparisons) (figure 4A). Interestingly, the time from last dose of vaccine until the HIBI event ($t_2$) was not significantly different when comparing schedules of the same regime (figure 4b).

**Three better than two, two better than one, even after infection.** Next, we decided to compare the differences in the time parameters analyzed between the people vaccinated with different regimes. In those who have received the vaccine earlier after infection, higher $t_{RI}$ was found in the booster regime with a median time of 15 months (IQR: 14-17) compared to the other two ($q<0.001$). Additionally, $t_{RI}$ in the early complete regime group (11 months, IQR: 9-14) was higher than the early incomplete one (6 months, IQR: 5-9) ($q<0.001$). Similar results were found in those who receive a late dose post-infection. The highest $t_{RI}$ was found in the booster regime (21 months, IQR 20-22) which was significantly higher than complete (16 months, IQR 15-17) and incomplete (14 months, IQR 13-15) regimes ($q<0.001$). Also, complete regime showed higher $t_{RI}$ than incomplete regime ($q=0.001$). Remarkably, $t_{RI}$ in individuals receiving a late 1 dose (late incomplete) and late 2 doses (late complete) were significantly higher when compared to early 2 doses (early complete) and early 3 doses (early booster), respectively (figure 4).

Regarding $t_2$, a higher value was found in early 2 doses group when compared to early doses of the incomplete regime ($p=0.001$) and the booster regime groups ($p<0.001$). Additionally, when comparing late doses, $t_2$ was lower in the booster regime compared to both, the complete ($q<0.001$) and incomplete regime ($q<0.001$). Although median times were similar, in the case of the late incomplete regime, the cumulative incidence is much higher than in the complete one, from the fifth month on ($q=0.014$).

**Discussion**

As far as we know, this is the first study to analyze deeply HIBI in Spain, and although there are several COVID-19 reinfection publications [16–18], none of them have yet described HIBI based on immunization regimes and schedules. This study integrates laboratory testing and immunization registry and reinfection data since the beginning of the COVID-19 pandemic along different variants emerged in Spain and so in Europe.

Three important points arise from the results of this study. First, vaccination after infection offers more protection than not getting vaccinated after infection, except for an early incomplete regime. Our results show that natural infection prevents significantly reinfection during a median period of 6 months. Secondly, complete and booster vaccine regimes in individuals that have previously been infected indeed confers a significant benefit by prolonging the time of reinfection compared to individuals with an incomplete regime. Third, data showed that vaccination too close after infection (minor than six months), despite the regime used, decreases significantly the time of reinfection.

Pre-existing immunity against SARS-CoV-2 through vaccination or infection is characterized by robust immune responses that had previously been associated with protection against infection or severe disease. However, as countries have reached significant vaccine coverage among their populations, infections in vaccinated individuals have been observed, leading to the concept of hybrid immunity [19–21]. A kind of immune response characterized by vaccination plus natural infection of virus variants. This is a very important aspect when a new virus of wide diffusion emerges into a naive population and imprints their immune system, driving the future evolution of the virus in populations according to their vaccine coverage rates. The results showed that reinfection takes place earlier in non-vaccinated individuals compared to vaccinated, and vaccination itself increases protection against reinfection up to 14 months compare to a median of 6-month protection provided by the first infection. Our results are aligned with previous studies indicating that hybrid immunity seems to protect against reinfection longer than just natural acquired immunity [10,22,23]. In respiratory transmitted infections by variable viruses, this global effect of hybrid immunity has not been previously considered and could change future vaccination schedules based on natural exposure.

Initially, studies showed that population previously infected by SARS-CoV-2 tend to mount intense immune responses with a single shot reaching levels equal or greater than naïve individuals with two doses [24–26]. Based on that, vaccine recommendations on these patients were to have only one dose in the urge to have more doses to vaccinate and reach herd immunity [15]. Our results show that having an incomplete vaccine regime does not provide longer protection against reinfection. Interestingly, despite the current debate about boosters, in previously infected individuals, claiming they could be unnecessary [27], our results prove the protection benefit acquired after a complete and a third-booster regime even after natural infection. Returning to the herd immunity concept we must consider that, similarly to other non-viremic respiratory infections that do confer low or no long protection against reinfection [28], a different epidemiological concept view of herd immunity is applied. This is reaching the level of immunity in a population where no additional prophylactic measures add significant protection. However, with the spread of omicron subvariants the debate still goes on [29]. In Spain as well as in other European countries a third dose or booster regime was initially recommended for patients at higher risk, starting with institutionalized in September 2021, followed by the elderly and sanitary workers through autumn that year. When Omicron variant was introduced in Spain in December 2021, its circulation was predominant by the end of the month and later through January 2022, with an exponential increase of cases [30]. Our results show individuals infected after three doses, despite having a longer time to reinfection, have lower times from the last protecting event. Many factors affected this. First, many individuals did not have the right amount of time to mount appropriate responses.
Second, omicron variants are known to evade the immune system to a certain extent. Although booster regimes have shown to increase protection, it wanes faster compared to previous variants[31]. In fact, that time has been estimated to be three months, similar to our results[31]. Finally, booster doses were based on the initial wild-type SARS-CoV-2 variant that emerged in Wuhan, which would mount antibodies with a reduced neutralizing capacity to omicron variants [30]. Actually, it has been suggested that an original antigenic sin effect, similar to that found in influenza virus[32], might shape humoral immune responses to new variants by eliciting greater responses to those first variants encountered in life[33]. Hence, vaccine updates might be periodically needed to improve responses to mutated variants rising.

Other factor influencing HIBI is the time interval between infection and vaccination, regardless of the number of doses received. Initial vaccination guidelines moved from disregarding time to vaccine after infection, to recommending waiting a minimum of five to six months [15]. Previous studies of vaccines against different pathogens revealed spacing between doses positively influence vaccine responses. Actually, schedules with longer intervals tend to lead to increased immune responses than accelerated schedules [34]. In this way, our work confirms that vaccination too close to the infection negatively affect the immune response by shortening the time of reinfection. Thus, the protection time provided by vaccination after a minimum of six months infection was significantly higher than vaccination in the first five months after infection. This occurred independently of receiving an incomplete, complete or booster vaccination regime. Furthermore, late doses in incomplete and complete regimes provided longer protection compared to early doses in complete and booster regimes, respectively. Negative interference and reaching a “ceiling effect” could explain the above observation[35]. If a vaccine antigen antigenically close is introduced in an experienced individual, the higher level of pre-existing immunity against it, would rapidly clear the antigen instead of mounting new specific immune responses [35]. On the other hand, heterologous vaccination in a minimum number of individuals does not allow this work to detect differences between them.

Our study has some limitations. This study is based on RT-PCR testing to confirm infection and reinfection. We do not have information about the variants causing the infection or reinfection or the severity of the infection. For that reason, it is unknown to what extent viral variability and immune escape could acts as a factor related to time of reinfection.

In summary, vaccination against COVID-19 after infection increases the time of protection against reinfection, highlighting the importance of vaccination, even in individuals previously infected. In those cases, time of reinfection relies upon two factors. First, number of doses received after infection, and. second, spacing out doses disregarding number of them, increases the time of reinfection.

The importance of this study lies in two main circumstances. First, this virus is here to stay circulating, probably for a while. Second, fully protective immune responses against infection tend to wane over time. Different and complex patterns of immunity are taking place in individuals and population; therefore, the results of this work can help design future vaccine strategies.

**Declarations**

**Data sharing**

The dataset can be made available upon reasonable request to the corresponding author.

**Ethical statement**

This research was approved by the Ethics Committee of East-Valladolid health area under the code PI 22-2920 and informed consent was waived due to the retrospective nature of the study and the anonymous nature of the dataset.

**Funding/Acknowledgements**

We thank the department of Microbiology at Hospital Clínico Universitario de Valladolid for their excellent work with RT-PCR testing during this pandemic.

This article was supported by Instituto de Salud Carlos III in Spain (Co-founded by European Regional Development Fund/European Social Fund “A way to make Europe” / "Investing in your future"). Río Hortega contract awarded to Laura Sánchez-de Prada (grant number CM20/00138).

**Competing interests**

The authors have no relevant financial or non-financial interests to disclose.

**References**


### Tables

**Table 1.** Patient characteristics and time of reinfection ($t_{RI}$) and time from the last dose ($t_2$).

<table>
<thead>
<tr>
<th></th>
<th>Total</th>
<th>Non vaccinated</th>
<th>Incomplete regime</th>
<th>Complete regime</th>
<th>Booster regime</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Early 1 dose</td>
<td>Late 1 dose</td>
<td>Early 2 doses</td>
<td>Late 2 doses</td>
</tr>
<tr>
<td><strong>No</strong></td>
<td>746</td>
<td>65</td>
<td>211</td>
<td>57</td>
<td>57</td>
</tr>
<tr>
<td><strong>Sex (male)</strong></td>
<td>305</td>
<td>28 (43.1)</td>
<td>90 (42.7)</td>
<td>16 (28.1)</td>
<td>26 (45.6)</td>
</tr>
<tr>
<td></td>
<td>42.0</td>
<td>21.1</td>
<td>24.0</td>
<td>24.0</td>
<td>24.0</td>
</tr>
<tr>
<td><strong>Age (years)</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>Time since first infection (months)</strong></td>
<td>14.0</td>
<td>6.0</td>
<td>10.0</td>
<td>0.0</td>
<td>0.0</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>Time after last dose (months)</strong></td>
<td>4.0</td>
<td>3.0</td>
<td>5.0</td>
<td>7.0</td>
<td>5.0</td>
</tr>
<tr>
<td><strong>Clinical characteristics</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Hypertension</td>
<td>151</td>
<td>25 (13.4)</td>
<td>0 (0.0)</td>
<td>15 (26.3)</td>
<td>8 (14.0)</td>
</tr>
<tr>
<td>Heart Disease</td>
<td>70</td>
<td>10 (5.4)</td>
<td>1 (1.5)</td>
<td>6 (2.8)</td>
<td>4 (7.0)</td>
</tr>
<tr>
<td>Immunosuppression</td>
<td>74</td>
<td>20 (10.8)</td>
<td>3 (4.6)</td>
<td>6 (10.5)</td>
<td>6 (10.5)</td>
</tr>
<tr>
<td>Lung disease</td>
<td>81</td>
<td>21 (11.3)</td>
<td>4 (6.2)</td>
<td>20 (9.5)</td>
<td>5 (8.8)</td>
</tr>
<tr>
<td>Diabetes</td>
<td>56</td>
<td>8 (4.3)</td>
<td>0 (0.0)</td>
<td>8 (3.8)</td>
<td>7 (12.3)</td>
</tr>
</tbody>
</table>

### Figures
Figure 1
Diagram of selection criteria for individuals in the study.

Figure 2
Diagram of the different time parameters analyzed in the study of reinfection.

t_{RI} : time between first and second COVID-19 infections

t_1 : time between infection and first dose

t_2 : time between last dose and reinfection
Figure 3

Time of reinfection ($t_{RI}$). The time elapsed between first and second COVID-19 infection and the comparison between vaccinated (with either incomplete, complete or booster regime) and non-vaccinated is represented. The two-tailed p-value was calculated by applying Mann-Whitney U-test; ***$P<0.001$. 
**Page 9/10**
Figure 4

(a) The Cumulative Incidence of time between both Covid-19 infections (t_{RI}) of different cohorts was represented as 1 minus the Kaplan-Meier estimator. Difference between cumulative incidence was assessed by log-rank test. (b) The Cumulative Incidence of time between last dose and reinfection (t_{2}) of different cohorts was represented as 1 minus the Kaplan-Meier estimator. Difference between cumulative incidence was assessed by log-rank test.