Vaccination with COVID-19 mRNA-1273 is not associated with HIV-RNA blips among people with HIV on ART: a retrospective cohort study

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Abstract

Objectives: Viral blips (VB) have been reported in people with HIV (PWH) after COVID-19 vaccination. **Materials and Methods:** We performed a retrospective cohort study among virally suppressed PWH vaccinated against COVID-19 with mRNA-1273, comparing the occurrence of VB in the 12 months after the first vaccine shot with those recorded in the 12 months before. The association between several clinical and immunologic variables and VB have been evaluated through logistic regression. **Results:** Overall, 48 individuals were included in our analysis. No difference was recorded between VB incidence in the 12 months before and after vaccination [11/48 (23%) vs 15/48 (31.3%), p=0.42]. No significant association was detected between selected variables and VB occurrence after vaccination. In a *post hoc* analysis including also 8 PWH excluded for not reaching the definition of viral suppression, we observed 15 increases of HIV RNA out of 56 PWH (26.8%) before vaccination and 23 increases of HIV RNA out of 56 PWH after vaccination (41.1%). This difference in incidence remained not significant (p=0.10) but a strong association between increases of HIV RNA occurrence before and after vaccination [p=0.02, OR 4.3 (95% CI 1-22-15.17)] was found. **Conclusion:** Among virally suppressed PWH, COVID-19 vaccination with mRNA-1273 is not associated with increased occurrence of VB.

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Abstract

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Results: Overall, 48 individuals were included in our analysis. No difference was recorded between VB incidence in the 12 months before and after vaccination [11/48 (23%) vs 15/48 (31.3%), p=0.42]. No significant association was detected between selected variables and VB occurrence after vaccination. In a *post hoc* analysis including also 8 PWH excluded for not reaching the definition of viral suppression, we observed 15 increases of HIV RNA out of 56 PWH (26.8%) before vaccination and 23 increases of HIV RNA out of 56 PWH after vaccination (41.1%). This difference in incidence remained not significant (p=0.10) but a strong association between increases of HIV RNA occurrence before and after vaccination [p=0.02, OR 4.3 (95% CI 1-22-15.17)] was found.

Conclusion: Among virally suppressed PWH, COVID-19 vaccination with mRNA-1273 is not associated with increased occurrence of VB.

Introduction

Viral blips (VB) are transient fluctuations of plasma HIV RNA values above the detection limit occurring in people with HIV (PWH) who are receiving effective antiretroviral therapy (ART). Not an infrequent event, occurring in at least one-quarter of patients on stable ART with undetectable viral loads (VL), their clinical significance is still a matter of debate, with some studies linking VB with viral failure. The pathophysiology of this phenomenon is complex and has not been clearly elucidated, but research has associated VB with vaccinations. Indeed, vaccines are immunostimulatory agents by definition and it has been postulated that they can induce a generalized inflammatory response with cytokine production, activating bystander cells harbouring latent HIV and thus leading to HIV reactivation and virion production.

We have recently described the case of a PWH who, having previously achieved stable viral suppression, experienced a VB after receiving a dose of COVID-19 vaccine, despite strict adherence to ART and plasma drug levels within the therapeutic range. Similar findings have been provided by Levy et al. and Milano et al., who reported 3/143 (2.1%) and 20/228 (8.9%) episodes after the second shot of COVID-19 vaccine in their cohort of PWH, respectively. To better understand the real incidence of VB among PWH vaccinated against COVID-19 and evaluate the variables associated with this phenomenon we designed this retrospective cohort study, in which we compared the occurrence of VB in the 12 months after the first vaccine shot with those recorded in the 12 months before.

Materials and Methods

Study design and population

We performed a retrospective cohort study among PWH under routine follow-up at the Infectious Diseases Unit of the IRCCS Ospedale Maggiore Policlinico in Milan, Italy and enrolled in the Polimmune study, an analysis conducted in our centre about immunogenicity of COVID-19 vaccines. Inclusion criteria were age >18 years, immunization with mRNA-1273 vaccine according to the schedule of attendance in the context of the Italian national vaccination program (a primary schedule of 2 doses given 28 days apart followed by 1

booster dose around 10 months after the first shot) and being stable on ART for 18 months before vaccination achieving viral suppression, defined as VL <200 copies/mL. The main outcome was the occurrence of a VB, defined as a VL between 20-200 copies/mL, in the 12 months since the first vaccination shot. As a comparison, we evaluated the occurrence of VB among the same individual in the 12 months before vaccination.

Demographic and clinical data were extracted from electronic medical records. Participants had outpatient clinic visits every 4 months in agreement with the Italian guidelines, performing VL quantification and immunologic profiling at each time. Good adherence to ART was defined as taking >90% of the doses according to the patient self-report. Optimal immunologic response (OIR) was defined as CD4+ T/CD8+ T ratio [?]1 plus CD4+ T [?]500cells/μL plus CD4+ T [?]30%. Virologic failure was defined as the detection of HIV RNA >200 copies/mL in at least two separate blood samples. As part of the Polimmune study, enrolled individuals provided blood samples at T0 (enrolment), T1a (one month after the first dose), T1b (one month after the second dose), T3 (three months after the first dose), T6 (six months after the first dose), and T1c (one month after the first booster dose). SARS-CoV-2 anti-N antibodies were assessed at T0, T3, T6 and T1c whereas anti-S antibodies and antibody neutralisation activity (ND50) were assessed at T1b.

Laboratory procedures

HIV-1 quantitative test was performed employing the Cobas® HIV-1 kit (Roche Diagnostics, Mannheim, Germany) for RNA extraction from EDTA plasma followed by PCR amplification and detection with Cobas® 4800 System (Roche Diagnostics, Mannheim, Germany). Anti-SARS-CoV-2 nucleocapsid antibodies (total Ig) and anti-SARS-CoV-2 spike RBD (total Ig) were measured as previously described. To quantify the neutralizing potential of sera (ND50), we used an in vitro assay based on VSV-pseudoparticles bearing SARS-CoV-2 spike glycoprotein and human ACE-2 over-expressing HEK293TN cell line as previously described.

Statistical analysis

Quantitative variables were described as mean and standard deviation (SD) or medians and inter-quartile ranges (IQR). Categorical variables were presented as frequencies and percentages. McNemar's test was employed to compare the frequency of VB before and after vaccination among the same individuals. The association between selected variables and the occurrence of VB was investigated with logistic regression models, odds ratio (ORs) and 95% confidence interval (CIs) were calculated. Analyses were performed with Stata 17 (STATA Corp., College Station, TX).

Ethical issues

The study protocol (286_2021) was approved by the INMI "Lazzaro Spallanzani" Ethics Committee (Roma, Italy).

Results

Study flowchart (**Supplementary figure 1**) shows the selection process of PWH starting from the original Polimmune cohort. Overall, 48 individuals were included in our analysis. Most of them were male (39/48, 81.2%), with a mean age of 46.3 years and all were on ART, with integrase strand transfer inhibitors (34/48, 77.3%) and nucleoside reverse transcriptase inhibitors (42/48, 87.5%) representing the drugs most frequently administered. The mean CD4+ T cell count was 798 cells/μL with a mean percentage of 35.3% and a median ratio of 0.98. Overall, 19/48 (39.6%) patients had achieved the OIR and 11/48 (22.9%) had received an AIDS diagnosis. The mean VL at vaccination was <20 copies/mL in all but 9 patients who had, respectively, 85, 50, 47, 33, 31, 29, 28, 22 and 20 copies per mL, and were considered as VB occurring before vaccination. **Table 1** and **Table 2** show population characteristics at enrolment and VL and immunologic profile at follow-up visits, respectively. Overall, no difference was recorded between VB incidence before and after vaccination [11/48 (23%) vs 15/48 (31.3%), p=0.42]. **Figure 1** depicts the trend of viral load in all the enrolled patients, before and after vaccination.**Table 3** reports the association between selected variables and VB occurrence after vaccination, with no significant findings.

As *post hoc* analysis we included also 8 PWH previously excluded for not reaching the definition of viral suppression before vaccination. In this extended cohort, we observed 15 HIV RNA increase out of 56 PWH (26.8%) before vaccination and 23 HIV RNA increase out of 56 PWH after vaccination (41.1%). This difference in incidence among the two groups remained not significant (p=0.10) but a strong association between HIV RNA increase occurrence before and after vaccination [p=0.02, OR 4.3 (95% CI 1-22-15.17)] was found. Among the 8 PWH excluded, 4 (50%) had a HIV RNA increase before vaccination but all (100%) experienced a HIV RNA increase after vaccination.

Discussion

Overall, in our study we observed how, among virally suppressed PWH, COVID-19 vaccination with mRNA-1273 was not associated with increased occurrence of VB in the 12 months after the first vaccine shot, a time-period where two other vaccine doses, and thus several stimuli to the immune system, were provided.

Some studies, other than our previous case report, have reported VB after COVID-19 vaccination. In their cohort of 143 PLH vaccinated with BNT162b2 mRNA COVID-19 vaccine, Levy et al. observed how in 3 (2.1%) patients the viral load increased after the second dose from undetectable levels (<40 copies/mL) to 47, 52 and 92 copies/mL, whereas in 3 patients, who had low-level viraemia at baseline, the viral load did not change significantly following vaccination. Instead, Milano et al. assessed HIV RNA after the second BNT162b2 mRNA COVID-19 vaccine dose among 228 out of 697 enrolled PWH. They found 20 (8.9%) VB among patients who were previously undetectable and reported high adherence to ART. Overall, our study is the first one assessing VB occurrence in PWH vaccinated with mRNA-1273 and it is reporting an incidence, of 15/48 (31.3%), which is quite superior to that reported by other studies. It should be noted that we performed viral load assessment with higher frequency compared to the above-mentioned reports and the main outcome of our study was precisely to assess VB. Our incidence is in line with the data of Joya et al., which reported how 46% of ART recipients in a large American cohort had [?]1 detectable VL without meeting criteria for virologic failure, including both VB and sustained low-level viremia.

Our study has limitations related to its retrospective design. Notably, VL and immunologic profile assessments were performed concomitantly to outpatient clinic visits which leads to a non-homogenous sampling schedule after vaccination. Thus, VB may be underreported and a study with prespecified sampling time points could provide a better description of VB incidence. Nonetheless, having applied the same sampling schedule (every 4 months) before and after vaccination, we can assume *a priori* the same probability of detecting the occurrence of a VB in the two periods. Moreover, it can be argued that VB in the follow-up period could be related to other conditions than COVID-19 vaccination. The study design, with the evaluation of VB occurrence in an equal timeframe (12 months) before COVID-19 vaccination, should have led to a balance in the impact of these possible confounders. Moreover, anti-N antibody titres were detectable in 7 patients and 11 patients at baseline and T1c, respectively, suggesting very few cases of COVID-19 during the study follow-up and thus a small impact by this infection.

Overall, we can affirm that COVID-19 mRNA vaccination does not lead to an increased risk of VB occurrence, among virally suppressed PWH, with their frequency being comparable before and after vaccination. We can speculate that the immunostimulatory action elicited by mRNA-1273 is not sufficient to reactivate HIV transcription from its latent state. As expected, when we included in our analysis also the PWH previously excluded for not reaching the definition of viral suppression, thus with less efficient control of viral replication, we observed an HIV RNA increase in all of them. This confirms the assumption of vaccines as immunostimulatory agents, more prone to lead to increase viremia in those patients with inadequate viral control and possibly larger viral burden. Interestingly, despite not being significant, a trend toward a higher number of VB among individuals with lower anti-S titres and serum-neutralizing activity was observed. This finding suggests how an immune system able to respond adequately to immunogenic stimuli is less prone to develop VB, probably through a well-controlled HIV infection which led to a reduction in systemic inflammation and immune activation.

COVID-19 mRNA vaccination can be safely performed among PLWH without any issues related to the pos-

sible occurrence of VB. Patients concerned about this possibility should be informed that, among individuals who are adherent to ART, this event seems to occur with the same frequency before and after vaccination. Patients with inadequate viral control might experience a higher frequency of HIV RNA increase after vaccination, but this should not prevent them to receive a potential life-saving instrument. Further research is needed to confirm the results of our study in larger cohorts and validate them also with other vaccines.

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Conflict of interest

AL: Gilead Sciences Inc. All the other authors have nothing to declare.

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Contributors

AL, GB, AG, and AB conceived the study. AL, GB, GV, CA, VC, AM, SL and AB, take care of the patients and collected clinical data. SC, GMB, PB, FC and LM performed laboratory analyses. AL performed statistical analyses and wrote the first draft of the manuscript. All the authors revised the final version of the manuscript.

Data availability statement

Data will be provided by reasonable request.

References

Figures and tables

Figure 1. Viral load values in the 48 enrolled patients before vaccination, at vaccination, and during follow-up. (FU: follow-up)

Supplementary figure 1. Study flow-chart.

Table 1. Demographics and clinical characteristics of enrolled PWH at time of mRNA-1273 first shot. Data are expressed as n (%) or median (IQR) except when stated otherwise.

	PWH (n=48)
Sex	
Female	9 (18.8%)
Male	39 (81.2%)
Age, (years)*	46.3 (12.8)
On antiretroviral therapy	48 (100%)
InSTI	34 (77.3%)
NNRTI	7 (15.9%)
PI	9 (20.4%)
NRTI Backbone	42 (87.5%)
TDF-TAF/FTC-3TC	26 (54.2%)
ABC/FTC-3TC	9 (18.8%)
3TC-FTC	7 (14.6%)
Plasma HIV viral load (copies/mL)	<20·0[§]
CD4+ T cell count per μL*	798 (271)
<350 per μL	2 (4.17%)
350-500 per μL	5 (10.4%)

	PWH (n=48)
>500 per µL	41 (85.4%)
CD4+ T cell count percentage*	35.3 (14.35)
CD4+ T/CD8 cells ratio	0.98 (0.64)
CD4+ T/CD8 ratio >=1	23 (47.9%)
CD4+ T/CD8 ratio >=0·4	41 (85.4%)
OIR	19 (39.6%)
HCV-Ab positive	2 (4.2%)
HBsAg positive	2 (4.2%)
CMV IgG positive	27 (77.1%)
Years since diagnosis	6 (10)
CD4+ T cell count per µL at nadir*	254 (190)
Plasma HIV viral load at zenith	99692 (164664)
AIDS diagnosis	11 (22.9%)
SARS-CoV-2 anti-N Ig positive	7 (14.6%)
VB in the preceding 12 months	11 (22.9%)

PWH, people with HIV; NRTIs, nucleoside reverse transcriptase inhibitors; InSTIs, integrase strand transfer inhibitors; NNRTIs, non-nucleoside reverse transcriptase inhibitors; PIs, protease inhibitors; OIR, optimal immune response (CD4+ T/CD8 ratio[?]1 plus CD4+ T[?]500cells/µL plus CD4+ T%[?]30%); HCV, hepatitis C virus; HBsAg, hepatitis B surface antigen; CMV, cytomegalovirus; anti-N, anti-nucleocapsid protein; VB, viral blip.

*mean (standard deviation).

§All viral loads were undetectable except for 9 patients who had, respectively, 85, 50, 47, 33, 31, 29, 28, 22 and 20 copies per mL.

Supplementary table 2. Viral load and immunologic profile of enrolled patients in the 12 months after first vaccine shot. Data are expressed as n (%) or median (IQR) except when stated otherwise.

	Visit 1	**Visit 2**	**Visit 3**
Days after first shot	144 (73)	257 (69)	359 (46)
Plasma HIV viral load, (copies/mL)	< 50	<50	<50
CD4+ T cell count per µL*	799 (267)	807 (273)	884 (291)
<350 per µL	1 (2.1%)	1 (2.1%)	1 (2.1%)
350-500 per µL	3 (6.3%)	3 (6.3%)	2 (4.2%)
>500 per µL	44 (91.7%)	44 (91.7%)	45 (93.8%)
CD4+ T cell count percentage*	35.4 (9.6)	36.2 (9.8)	37.7 (10.7)
CD4+ T/CD8 cells ratio	0.93 (0.47)	0.95 (0.70)	1.1 (0.89)
CD4+ T/CD8 ratio >=1	20 (41.6%)	20 (41.6%)	20 (41.6%)
CD4+ T/CD8 ratio >=0·4	42 (87.5%)	42 (87.5%)	42 (87.5%)
ART changes	0 (0%)	0 (0%)	0 (0%)
Viral blips	12 (25%)	4 (8.3%)	6 (12.5%)
Plasma HIV quantification of VB, (copies/mL)	55.5 (63)	44.5 (12)	46.5 (73)

*mean (standard deviation).

Table 3. Variables associated with the occurrence of viral blips in the 12 months after first vaccine shot.

Variable	Viral blip (N= 15)	No viral blip (N= 33)	p	OR	95% CI
Gender (N, %)					
Female	2 (13.3 %)	7 (21.2%)	0.52	1	Reference
Male	13 (86.7%)	26 (78.8%)	-	1.75	0.32-9.64
CD4+ T cell count per µL at T0 (N, %)					
<500 per µL	2 (13.3%)	5 (15.2%)	0.87	1	Reference
>500 per µL	13 (86.7%)	28 (84.6%))	-	1.16	0.20-6.79
CD4+ T/CD8 ratio >=1 at T0 (N, %)					
No	7 (46.7%)	18 (54.5%)	0.61	1	Reference
Yes	8 (53.5%)	15 (45.5%)	-	1.37	0.40-4.66
CD4+ T/CD8 ratio >=0.4 at T0					
No	1 (6.7%)	6 (18.2%)	0.32	1	Reference
Yes	14 (93.3%)	27 (81.8%)	-	3.11	0.34-28.5
OIR at T0					
No	10 (66.7%)	19 (57.6%)	0.56	1	Reference
Yes	5 (33.3%)	14 (42.4%)	-	0.68	0.19-2.43
Positive anti-N T0					
No	13 (86.7%)	28 (84.6%)	0.87	1	Reference
Yes	2 (13.3%)	5 (15.2%)	-	0.87	0.15-5.04
Positive anti-N T3					
No	14 (93.3)	28 (84.6%)	0.42	1	Reference
Yes	1 (6.7%)	5 (15.2%)	-	0.4	0.04-3.76
Positive anti-N T6					
No	13 (86.7%)	27 (81.8%)	0.68	1	Reference
Yes	2 (13.3%)	6 (18.2%)	-	0.70	0.12-3.91
Positive anti-N T1c					
No	12 (80%)	25 (75.8%)	0.75	1	Reference
Yes	3 (20%)	8 (24.2%)	-	0.78	0.18-3.48
Anti-S titre T1b, U/mL					
< 2,500	8 (53.3%)	13 (39.4%)	0.59	1	Reference
2,500 - 7,500	5 (33.3%)	13 (39.4%%)	-	0.62	0.12-2.43
>7,500 - <12,500	1 (6.7%)	1 (3%)	-	1.62	0.09-29.78
>12,500	1 (6.7%)	6 (18.2%)	-	0.30	0.03-2.68
ND50 T1b					
<1000	4 (26.7%)	12 (36.4%)	0.82	1	Reference
1000-2500	7 (46.7%)	8 (24.2)	-	2.63	0.57-12
2500-5000	2 (13.3%)	5 (15.2%)	-	1.2	0.16-8.8
>5000	2 (13.3%)	8 (24.2%)	-	0.75	0.11-5.1
Blips before vaccination					
No	9 (60%)	28 (84.6%)	0.06	1	Reference
Yes	6 (40%)	5 (15.2%)	-	3.73	0.92-15.2

OIR, optimal immunologic response (CD4+ T/CD8 ratio[?]1 plus CD4+ T[?]500cells/µL plus CD4+ T%[?]30%); anti-N, anti-nucleocapsid protein; anti-S, anti-Spike protein; ND50, serum neutralizing activity.