Higher aspartate aminotransferase (AST) (44.5 [34.0;54.8]), alanine aminotransferase (ALT) (33.5 [18.2;63.0]), both with p<0.001, and GGT (47.0 [31.0;110.9]) levels (p<0.006).

HBsAg was positive in a higher percentage in the HBV DNA positive group than in the HBV DNA negative one, as described in Figure 1. Anti-HBsAg antibodies were present in higher proportion in the HBV DNA negative group, as described in Figure 2.

In HBV DNA positive participants the median period from the most recent positive HBV DNA evaluation was 262 days ([IQ] 32;475), while in HBV negative participants the median period from the most recent negative HBV DNA evaluation was 2528 days ([IQ] 997;3702).

### Methods

#### Study Participants

145 PLWH, HBsAg positive, followed as outpatients at San Raffaele Hospital with available HBV DNA-quantitation in years 2020-2021 and currently receiving ART, including TDF or TAF, were enrolled. Anonymized clinical and laboratory data were collected or extracted from the Infectious Diseases Department database (CSLHV Cohort). Written informed consent was obtained from all participants, and the study was approved by the ethics committee of the San Raffaele Hospital, Milan, Italy.

#### Virological Assays

HBV DNA and HIV RNA were measured by real-time PCR (Cobas 6800 system, Roche diagnostic, Italy). HBV DNA was classified as detectable ≥10 IU/mL or undetectable <10 IU/mL. HIV RNA was classified as detectable ≥200 copies/mL or undetectable ≤50 copies/mL.

Resistance mutations within the polymerase gene of HBV were detected by population sequencing.

#### Statistical Analysis

Results were reported as median (interquartile range, IQR) or frequency (%). Categorical variables were compared using the χ² test, or Fisher's exact test. Continuous variables were compared using the Mann–Whitney test. A p value < 0.05 was considered statistically significant. Analysis was completed using R software, version 4.1.2 (2021-11-01).

#### Results

Characteristics of PLWH under ART active on HBV/HBV co-infected patients responding to HBV DNA negativity or not are summarized in Table 1. Overall, most patients were males and the main route of transmission was the sexual route. The comparison of HBV DNA positive patients with those HBV DNA negative showed a different immune status profile:

- Higher CDR cells count in the HBV DNA positive group [1060 [886;1313]] vs [876 [538;1189]] with a trend towards significance (p=0.054).
- Lower CDR/CDB ratio in the HBV DNA positive group (0.32 [0.24;0.52] vs 0.81 [0.58;1.06]) p=0.003), while CDR cells count were similar in the two groups.

Concerning HIV load, HBV DNA positive participants with respect to HBV DNA negative participants had:

- Higher frequency of HIV- RNA positivity ≥5 copies/mL, 35.7%, compared to HBV DNA negative participants who had a 9.92% positivity (p=0.041).
- Higher HIV viral load (23.5 [5.42;73.5]) compared to HBV DNA negative participants (0.90 [0.09;26.02]) (p=0.017).

Concerning nonantiretroviral chemotherapy assessed by lamivudine and tegafur plus every 2 days, and lamivudine and entecavir, LAM and M204V, but none had resistance to TAF or TDF.

One participant previously reported to harbor a resistant strain to lamivudine had a reversion to wild type, although HBV DNA was positive at high levels (3580000 IU/mL). This patient was on ART including TAF 50 mg + FTC and treatment was changed to TDF + FTC with a dramatic reduction of HBV viremia (127,000 IU/mL). This case is described in Figure 4.

Concerning adherence to treatment, poor treatment adherence was recorded in 4/14 HBV DNA positive participants.

Antirectroviiral therapy, compared to the HBV DNA undetectable group, patients with detectable HBV DNA had:

More prescription of TAF10 and TDF.

Way less prescription of FTC.

As described in Figure 2.

#### Figure 1. Serology HBsAg in HBV DNA positive (A) and negative (B) participants.

The summary result of HBV DNA positive patients responding to anti-HBV ART active on both viruses we found that HBV DNA positive participants respect to the counterpart of HBV DNA negative high:

- A less preserved immune status (C/CD4/CD8 ratio).
- A higher frequency of viral load, viral load was positive in patients with ART included TDF or TAF.
- A more frequently HBV DNA positive or TAF and TDF were detected in tested specimens (9/14). However, 2/22.2% cases had resistance to lamivudine and entecavir. In one other case an interesting HBV mutational profile was detected with also viral load dynamic change in relation to ART change.

#### Discussion and conclusion

The finding that about 10% of participants had positive HBV viremia, 5 with concomitant positive HBV viremia suggests in some cases a poor adherence to treatment. Importantly none of PLWH had resistance to TDF or TAF, while in 0/2 cases was identified a dominant strain resistant to nucleoside analogs (NA) lamivudine and entecavir. Most of HBV DNA positive patients were on ART including TAF10 and TDF. Therefore, we could form the hypothesis of a better forgiveness for HBV (that is the difference between the duration of beneficial action after dosing and the prescribed dosing interval, F=1) of TAF 25 respect to TAF10 and TDF.

In conclusion, over than poor adherence, a relatively less preserved immune status, ART regimen resistance to AN lamivudine and entecavir, may have contributed to HBV DNA detectability.

### Summary

- A less preserved immune status (C/CD4/CD8 ratio).
- A higher frequency of viral load, viral load was positive in patients with ART included TDF or TAF.
- A more frequently HBV DNA positive or TAF and TDF were detected in tested specimens (9/14). However, 2/22.2% cases had resistance to lamivudine and entecavir. In one other case an interesting HBV mutational profile was detected with also viral load dynamic change in relation to ART change.