Association between markers of Hepatitis B Virus infection and risk of virological rebound in people with HIV receiving antiretroviral therapy.

Vincenzo MALAGNINO*1, Alessandro COZZI-LEPRI 2, Valentina SVICHER 3, Enrico GIRARDI 4, Carlo Federico PERNO 5, Annalisa SARACINO 6, Gianluca CUOMO 7, Stefano RUSCONI 8, Massimo PUOTI 9, Antonella D'ARMINIO MONFORTE 10, Massimo ANDREONI 1, Loredana SARMATI 1 for the ICONA Foundation Study Group§

1 Department of Systems Medicine, University of Rome Tor Vergata, Rome, Italy

2 Institute for Global Health, Centre for Clinical Research, Epidemiology, Modelling and Evaluation, London, UK

3 Department of Biology, University of Tor Vergata, Rome

4 National Institute for Infectious Diseases Lazzaro Spallanzani, Scientific Hospitaller and Care Institutions, Scientific Direction, Rome, Italy

5 Microbiology and Diagnostic Immunology Unit, Department of Diagnostic and Laboratory Medicine, Bambino Gesù Children's Hospital, IRCCS, Rome, Italy.

6 Clinic of Infectious Diseases, Department of Precision and Regenerative Medicine and Ionian Area, University of Bari, Policlinic of Bari

7 Clinic of Infectious Diseases, Azienda Ospedaliero-Universitaria Di Modena, Modena, Italy

8 Infectious Diseases Unit, Legnano General Hospital, ASST Ovest Milanese. Università degli studi Di Milano - Legnano

9 Department of Infectious Diseases, Grande Ospedale Metropolitano Niguarda, Milan, Italy

10 ASST Santi Paolo e Carlo, Department of Health Sciences, University of Milan, Milan, Italy

Running title : HIV viral rebound and coinfection with HBV

Key words: HIV/HBV, HBcAb, Occult hepatitis B infection, ART, HIV rebound

Abstract

Objectives: The aim of this analysis was to investigate the impact of Hepatitis B Virus (HBV) coinfection on the risk of HIV viral rebound (VR) after achieving suppression for the first time after starting antiretroviral therapy (ART) in the real-world setting.

Design: Patients living with HIV (PLWH), enrolled in the ICONA Foundation Study cohort, achieving viral suppression ≤50 copies/mL for the first time after starting ART, were prospectively evaluated and divided in 3 exposure groups according to serology test results: (A) HIV mono infected; (B) HIV+/HBcAb+/HBsAg-; (C) HIV+/HBsAg+. The occurrence of VR, defined as the time of two consecutive HIV-RNA values > 50 cp / mL after achieving viral suppression for the first time (baseline), was investigated.

Methods: Standard survival analysis by means of KM curves and Cox regression analysis with the serology exposure fitted as a time-fixed covariate measured at baseline was employed after controlling for key confounding factors.

Results: Of a total of 5657 patients included, 4,090 (72%) resulted HIV mono-infected, 1,342 (23.7%) HBcAb+, and 225 (3.9%) HbsAg + co-infected. Overall, 654 (11.5%) PLWH experienced VR>50 copies/mL over follow-up. After controlling for all sources of measured confounding, co-infected PLWH showed an increased risk of experiencing VR compared to HIV mono-infected. In particular, the strongest associations were seen for the HIV/HBsAg+ participants (aHR 1.56 [CI1.03-2.38], p=0.037) but an excess of risk was also seen in the HIV+/HBcAb+/HBsAg- (aHR=1.25 [CI 95% 1.00 – 1.55], p=0.047)

Conclusions: Coinfection with HBV seems to have an impact on the probability of maintaining HIV viral suppression achieved for the first time after ART initiation. Of note, even PLWH positive for HBcAb, a marker of not active HBV infection, appeared to be at higher risk of VR than HIV-mono infected and their HIV-RNA should be carefully monitored.

Introduction

A growing body of evidence suggests that co-infections (e.g., malaria, tuberculosis, herpes simplex virus type 2, cytomegalovirus) may promote HIV replication (1) and that HIV replication and coinfections may contribute to the pathogenesis of immune activation (2) and to the maintenance of the HIV DNA reservoir also during antiretroviral therapy (ART) (3,4,5).

An increased immune activation, as measured by CD38 expression on CD4+ and CD8+ T lymphocytes, has been observed in patients living with HIV (PLWH) and co-infection with hepatitis C (HCV) and hepatitis B (HBV) viruses (6,7,8), and this mechanism probably underlies the major risk of chronic liver damage and progression of HIV infection showed in coinfected subjects (9,10). Regarding HIV / HBV coinfection, Platt et coll. (10) recently demonstrated that 7.4% (IQR 1.4% - 15.7%) of a representative sample of PLWHs was HBsAg-positive, in line with estimates from italian data (prevalence of HbsAg-positive PLWH: 6.6%) (11) and other previous studies (12,13). In addition to the condition of chronic infection (HBsAg positivity), a proportion of PLWH HBsAg-negative have antibodies against the HBV core antigen (HBcAb), as a sign of previous infection, in the absence of anti-HBs antibodies. A negative value of HBsAg does not mean clearance of HBV DNA and many of these PLWH may have an occult HBV infection (OBI). OBI is defined as the presence of replication competent HBV DNA (i.e. covalently closed circular HBV DNA) in the liver and / or HBV DNA in the blood of HBsAg-negative people (14). According to the update of the Taormina declaration, the detection of HBcAb in the blood can be used as a surrogate marker to identify OBI in subjects with an immunosuppressive condition (14). Among patients with OBI, HBV can replicate at low levels for a long time with detectable or undetectable HBV DNA in serum and liver. The prevalence of OBI varies widely around the world and several studies have reported a large variability in prevalence, from 1 to 87%, depending on specific characteristics of the population studied (risk factors, HBV genotype, etc.) and to the sensitivity of the test. Higher rates of OBI were found in individuals co-infected with HCV, people who inject drugs, people on dialysis and PLWH. In the PLWH population, prevalence rates of OBI ranging from 10% to 45% have been reported (14,15).

OBI in PLWH has been associated with worse evolution of both HBV liver disease and HIV infection. Detection of HBcAb in PLWH at diagnosis of HIV infection has been associated with high levels of CD8 T cell counts for up to 24 months after initiation of ART (16), cryptic HBV replication (17), a delay in achieving undetectable HIV viremia after ART initiation and a significantly greater presence of viral rebound during effective ART (18).

The aim of this study was to evaluate whether the presence of HBV infection (intended as HBsAg positivity and/or HBcAb positivity), could influence the probability of maintaining HIV suppression during effective ART. The risk of viral rebound (2 consecutive detections of HIV-RNA> 50 copies / ml) after achieving viral suppression for the first time after starting ART, was evaluated in HBcAb and HBsAg co-infected patients and compared to that seen for HIV-monoinfected subjects.

Material and Methods

Study population

This analysis includes prospectively collected data of PLWH enrolled in the ICONA Foundation Study cohort. ICONA is an observational cohort of PLWH who were antiretroviral- naïve at the time of enrolment. This cohort was set up in January 1997 and currently consists of > 19,000 patients from 50 Italian infectious disease units. Demographic and socio-behavioural data, initiation and discontinuation dates of each antiretroviral drug, HIV viral load and CD4+ cell count every 3 ± 6 months, AIDS-defining diseases according to the Centres for Disease Control and Prevention (CDC) criteria, and non-HIV-related diseases and death were recorded for all enrolled participants. Hemato-chemical data, including liver function parameters, were also available at 3 ± 6-month intervals. Further details are available at http://www. fondazioneicona.org/.

Endpoint and inclusion criteria

Participants of the ICONA Foundation Study Cohort with evidence of HIV Virological Success (VS, defined as two consecutive HIV-RNA ≤50 cp/ mL achieved for the first time after starting ART) were enrolled in the study. The date of the achievement of VS (the second HIV-RNA ≤50 copies/mL) for the first time after ART initiation was defined as the baseline [BL] for the analysis. Participants were prospectively evaluated to investigate the influence of HBV co-infection on the risk of occurrence of HIV Virological Rebound (VR, defined as two consecutive HIV-RNA values ≥ 50 cp/ml) after BL. Participants who were never tested for viral hepatitis or never achieved confirmed suppression on ART were excluded. A sensitivity analysis was also performed using the HIV-RNA threshold of 200 copies/mL to define VR.

Three groups of co-infected PLWH were defined according to the results of their HBV serology tests at BL: (A) HIV+/HBsAg-/HBcAb-; (B) HIV+/HBcAb+/HBsAg-; (C) HIV+/HBsAg+.

Statistical Analysis

The characteristics of the study population were described and compared across the 3 exposure groups (A-C) with the Kruskal-Wallis or Pearson chi-square test as appropriate. Time to experience VR was analysed by means of standard survival analysis techniques such as Kaplan-Meier curves and Cox regression models with time- fixed covariates measured at BL. Our assumptions regarding the causal relationship between variables have been described by means of a direct acyclic graph (DAG), which was built using DAGitty, version 2.3 (http://www.daggity.net/). According to our DAG, there were a number of adjustment sets that were sufficient to block all the backdoor confounding pathways from the main exposure of interest to outcome (Figure 1 in Supplementary Materials). We have chosen to report the results for three of these sets and commented in the text using the set including the following factors: AIDS diagnosis, duration of HIV viral load (VL) suppression, mode of HIV transmission, VL at start of cART, CD4+ cells nadir, history of previous ART failure (i.e. suppression not obtained on first-line ART), nationality and anchor drug in baseline ART. In a sub analysis to evaluate the potential effect of antibodies HBsAb on VR occurrence, the same analyses were conducted using the alternative 3-group categorisation for the exposure: (A) HIV+/HBcAb-/HBsAb-; (C1) HIV+/HBcAb+/HBsAb- and (C2) HIV+/HBcAb+/HBsAb+.

Participants Consent Statement

The ICONA study has been approved by the institutional review boards of all the participating centres. Participants who agree to participate have signed consent forms, in accordance with the ethical standards of the Committee on Human Experimentation and the Helsinki Declaration (1983 revision). Demographic, clinical, and laboratory data and information on therapies are retrospectively collected and recorded in pseudo-anonymous form in accordance with Recital 26 (5) of the General Data Protection Regulation (GDPR).

Results

Overall Description of the Study Population

Among the 5,657 included participants (Table 1) with viral hepatitis serologic test available and evidence of sustained HIV-RNA ≤50 copies/mL achieved for the first time after starting ART, 1,141 (20.2%) were female, the median age of the study population was 40 years old (IQR 33-48) and around one fifth of the participants had foreign nationality (21.2%). The vast majority of the participants achieved viral suppression on ART for the first time after 2010 (87.9%), thus using modern antiretroviral regimens. Most of the participants acquired HIV through sexual contacts (48.8% men who have sex with men [MSM] and 43.5% heterosexual), while only 112 participants (2%) through intravenous drug use. Regarding the immunological parameters at BL, median CD4+ cell/count was 528 cell/mm^3 (IQR 351-728), while the CD4+ count nadir was 299 cell/ mm^3 (IQR 160-435). Overall, 683 (12.1%) participants had been diagnosed with AIDS prior to BL. At BL, the distribution of cART regimens used was as follows: 1,768 participants (31.2%) received Protease Inhibitors (PIs)- containing regimen, while 1,720 (30.4%) or with a Non-Nucleoside Transcripase Inhibitors (NNRTI)–regimen and 1,438 (25.4%) with an Integrase Inhibitors (INIs)-regimen. Only a total of 272 participants (4.8%) had a history of previous virologic failure prior to eventually achieving VS and entering this analysis (Supplementary Figure 2).

Study Population Stratified by viral hepatitis infection profiles

Table 1 shows the number and characteristics of the study population stratified by baseline exposure groups: 4,090 participants (64%) were in the HIV mono-infected (group A); 1,342 (23.7%) were HIV+/HBcAb+/HBsAg- (group B); and finally 225 (3.9%) were HIV+/HBsAg+ (group C). Overall, participants of group B and C were older than HIV-monoinfected ($p<0.001$) and achieved viral suppression at earlier calendar years $p < 0.001$). There was also a tendency for an association between mode of HIV transmission and HBV serology ($p=0.05$) although with small differences in the prevalence of PWID by group (2.0% vs. 1.7% vs. 3.6%, respectively).

Regarding the immunological status, HIV monoinfected group (group A) had a significantly higher median CD4+ cell count respect to HBcAb+ (group B) and HBsAg+ (group C) coinfected participants ($p < 0.001$), consequently, the proportion of those with a CD4+ cell count <200 cell/ mm^3 at baseline in these groups was also higher than in HIV-mono-infected group ($p < .001$) and the prevalence of AIDS diagnosis at baseline was also higher in the co-infected groups than in HIV-monoinfected group (group B 192 [14.3%], group C 34 [15.1%] vs. group A 457 [11.2%]; p=0.003). No evidence for a difference between groups was found with respect to BL CD8 cell counts and duration of follow up.

Risk of HIV Virological Rebound (VR) during follow up.

Figure 1 describes the cumulative risk of HIV VR>50 copies/mL in the different categories of co-infected groups as well as in HIV mono-infected participants. Overall, 654/5657 (11.5%) experienced the main outcome of confirmed VR>50 copies/mL over the follow-up period after BL, and the crude incidence risks of VR calculated in each of the exposure group were as follows: group A 439/4090 (10.7%), group B 179/1342 (13.3%), group C 36/225 (16%). After estimating the cumulative incidence risk (CIR) of VR using the Kaplan-Meier method, the groups of coinfected PLWH, with or without HBsAg (B and C), showed higher CIR of VR after BL (group B CIR by 24 months 9.22 [CI 95% 7.59-10.85] and group C CR by 24 months 9.44 [CI 95% 5.37-13.52]) than that seen for the HIV monoinfected (group A CR by 24 months 7.71 [CI 95% 6.84-8.57] .

These results were confirmed in an analysis which controlled for potential confounding factors. Indeed, from fitting a Cox regression model after controlling for AIDS diagnosis, duration of HIV viral load (VL) suppression, mode of HIV transmission, VL at start of ART, CD4+ cells nadir, history of previous ART failure (i.e. suppression not obtained on first-line ART), nationality and anchor drug in baseline ART, there was still strong evidence for a difference in the risk of VR comparing the co-infected groups with the HIV-monoinfected (Table 2). In a multivariable model, adjusted for duration

of VL suppression, mode of HIV transmission, VL at cART introduction and anchor drug in baseline ART, using group A as the comparator, PLWH HBcAb+ and HBsAg+ were found to have an increased risk of experiencing VR after BL. In particular, compared to HIV monoinfected, HIV+/HBcAb+ participants had a 25% increased risk of VR (HR 1.25 [955 CI 1.00-1.55]; p=0.047), while HIV+/HBsAg+ a 56% higher risk (HR 1.56 [95% CI 1.03-2.38]; p=0.037) Of note, results were similar when we used two alternative adjustments sets which, according to our assumptions, were also able to block all confounding backdoor pathways. The most important confounding factor appeared to be the history of virological failure prior to baseline, although the degree of attenuation was generally similar for each of the confounders when examined separately. We also investigated the impact of HBV coinfection on the risk of occurrence of VR using the HIV-RNA threshold of >200 copies/mL to define VR and results were similar, although the difference in risk was attenuated (see Supplementary Materials: Figure 3).

Potential protective role of HBsAb in the HBcAb-positive participants

In the subset of HBsAg-/HBcAb+ participants (group B n=1,342), we specifically investigated whether the detection of antibodies directed against the S antigen (HBsAb) might be associated with protection against the risk of VR. Data on HbsAb were available for 1338 group B participants. Table 3 shows participants' main characteristics at BL stratified by HBcAb+/HBsAb+ (group B1) vs. the HBcAb+/HBsAb- (group B2). At BL, median CD4+ cell count and CD4+ cell nadir was lower in HBcAb+/HBsAb-neg than in HBcAb+/HBsAb+ individuals (475 cell/ mm^3 [306-624] vs. 504 cell/ mm^3 [328-719], p=0.007; and 248 cell/mm^3 [103-368] vs. 286 cell/ mm^3 [141-427], p<0.001, respectively), HBcAb+/HBsAb- subjects were also more likely to have a previous diagnosis of AIDS (HBcAb+/HBsAb- 65 [19.3%] vs. 127 [12.7%] in HIV/HBcAb+/HBsAb+, p=0.003).

In the unadjusted analysis, there was a trend in the cumulative risk of VR>50 copies/mL in HBcAb+/HbsAb+ group B1 compared to HBcAb+/HbsAb- group B2 with a not significant difference between the two groups at the conventional level of 5% (p = 0.081) (Fig.2). The difference

in risk between the two groups was further attenuated and not significant in the multivariate Cox analysis after controlling for the same set of confounders used in the main analysis (aHR 0.79 [95% CI 0.52-1.20], p = 0.269) (Tab 4).

Discussion

The results of our analysis show that overt co-infection with HBV, but also HBcAb+ positivity in absence of s antigen, is associated with an increased risk of VR (defined as two consecutive observations of HIV-RNA> 50 cp / ml after achieving viral suppression for the first time during ART) compared to HIV mono-infection, after controlling for key confounders. Co-infected participants HIV+/HBcAb+ and HIV/HbsAg+ had, respectively, a 25% and 56% greater risk of VR than the HIV monoinfected group.

Achieving a persistent HIV-RNA suppression remains the main goal of ART and sustained viral suppression below 50 copies/ml was associated with a series of beneficial effects as reduction of morbidity, mortality, sexual HIV transmission (19, 20) and progressive immunological recovery (21). Transient viral blips as well as virus detectable below the detection limits of the tests (very low level viremia [VLLV]) are reported in ART treated patients, suggesting the possible presence of persistent HIV replication. Pathogenesis of this residual HIV replication is not fully understood; however, several papers have associated viral blips and VLLV with HIV viral rebound above 50 copies / ml and possibly virological failure (21,22,23,24, 25, 26).

A series of articles and two systematic reviews have explored the correlation between persistent and recurrent infections and HIV burden, and some studies found a benefit in terms of HIV viraemia reduction when treating co-infections (1, 27). Herpesvirus replication, and in particular detectable CMV-DNA and EBV-DNA has been associated with high level of HIV-DNA (28) and subclinical CMV and EBV shedding has been shown to contribute to the dynamics of the HIV DNA reservoir during suppressive ART (29).

Our results show an increased risk of HIV VR in HBV coinfected compared to HIV monoinfected subjects, which is consistent with previous published data showing that AIDS defining conditions and death were more frequent in HIV/HBV coinfected patients than mono-infected (30,31).

Regarding our exposure group B , it is known that HBcAb-positive status in HIV subjects is a marker of OBI, condition characterized by possible HBV replication which can also potentially interfere with HIV replication. OBI is prevalent in cohorts of PLWH. Morsica and colleagues (32) had found detectable HBV-DNA in approximately 15% of ICONA participants, and more recently, using a digital droplet PCR, with a sensitivity of 1–2 IU/mL, we found HBV cryptic replication in 40% of a population of HIV-HBcAb patients (17). Moreover, in a small cohort of HIV patients (33) we showed that HBcAb-positive status was a risk factor for delayed achievement of HIV < 50 copies/mL after ART initiation and related to the appearance of VR in HIV/HBV ART treated patients. In addition, the presence of HBsAb has been shown to be correlated with better control of HBV replication in immunocompromised patient populations (16) however, although we found a trend towards a higher cumulative risk of VR in HIV + / HBcAb + / HBsAb- compared to HIV + / HBcAb + / HBsAb + participants, results did not achieve statistical significance. However, there was a non-negligible reduction of 20% in the risk of VR and the analysis might be underpowered. .

The results of our study support the theory that HBV coinfection and replication could contribute to the poor control of HIV suppression during ART, even if the underlying mechanisms of this phenomenon remain unclear. A persistent viral replication is a known driver of immune activation, and an increased immune activation has been documented in HCV/ and HBV/HIV coinfected patients (7, 8, 34). A state of immune dysfunction and increase inflammation can persist despite ART and presence of co-infections are considered as possible contributors in its pathogenesis (2). Moreover, some elegant in vitro models have shown how other infections, such as tuberculosis, can stimulate HIV replication in vitro and how this correlates with immune activation levels (35). High levels of $CD4^+$ $HLADR^+$ $CD38^+$, $CD8^+$ $HLADR^+$ $CD38^+$ as sCD163 and sCD14 were correlated with the evolution of liver damage and fibrosis both in HIV/HBV and HIV/HCV populations (8, 36) and we recently demonstrated an increased risk of FIB-4 elevation in the group of HCV/HBV (HBcAb-positive) coinfected patients enrolled in the ICONA cohort (37). Unfortunately, we had not data directly measuring the level of inflammation in our cohort.

Before drawing final conclusions, some limitations of our analysis need to be further discussed. . First, collection of HBV-DNA data is very sparse in our cohort even in the group of HBsAg+ for whom monitoring is recommended at least once a year and were not used in this analysis. Future prospective studies that include periodic control of HBV viremia will be needed to demonstrate a correlation between HBV Occult infection or overt HBV infection and HIV rebound. Second, although we only included participants who eventually achieved viral suppression on ART, treatment adherence is not collected in our cohort and we cannot rule out that the difference in risk might be due to residual confounding by adherence. Overall, 25% of participants achieved viral suppression with a INSTI-based regimen which is seen also in non-100% adherent patients. Nevertheless, after controlling for type of regimens used at baseline, history of previous virological failure and duration of viral suppression (which might be used as a proxy of adherence to ART prior to baseline) results were similar. . Furthermore, our results cannot be applied to PLWH with HCV coinfection, because this population has been excluded at the outset. The main reason for this exclusion was the lack of detailed HCV-RNA data post baseline which could have led to misclassification of the exposure because of HCV cure. Finally, immune activation and inflammation parameters were also not available for analysis so we could not evaluate whether the observed higher risk of VR in co-infected participants might be mediated by an increase in inflammation.

Despite these limitations, our results provide important insights regarding connections between HIV/HBV coinfection and HIV VR in course of effective ART. Further studies are needed to identify the possible mechanisms at the basis of this observation.

References

1) Barnabas, R. V., Webb, E. L., Weiss, H. A. & Wasserheit, J. N. The role of coinfections in HIV epidemic trajectory and positive prevention: a systematic review and meta-analysis. *AIDS* **25**, 1559–1573 (2011).

2) Cai, C. W. & Sereti, I. Residual immune dysfunction under antiretroviral therapy. *Semin Immunol* **51**, 101471 (2021).

3) Hunt, P. W. HIV and inflammation: mechanisms and consequences. *Curr HIV/AIDS Rep* **9**, 139–147 (2012).

4) Hatano, H. Immune activation and HIV persistence: considerations for novel therapeutic interventions. *Curr Opin HIV AIDS* **8**, 211–216 (2013).

5) Massanella, M., Gianella, S., Lada, S. M., Richman, D. D. & Strain, M. C. Quantification of Total and 2-LTR (Long terminal repeat) HIV DNA, HIV RNA and Herpesvirus DNA in PBMCs. *Bio Protoc* **5**, (2015).

6) Gonzalez, V. D. *et al.* High levels of chronic immune activation in the T-cell compartments of patients coinfected with hepatitis C virus and human immunodeficiency virus type 1 and on highly active antiretroviral therapy are reverted by alpha interferon and ribavirin treatment. *J Virol* **83**, 11407–11411 (2009).

7) Demosthenes, J. P. *et al.* Chronic Immune Activation Among Treatment Naïve HIV/ HBV Coinfected Individuals From Southern India. *Curr HIV Res* **19**, 332–341 (2021).

8) He, Y. *et al.* Persistent chronic immune activation in HIV/HBV-coinfected patients after antiretroviral therapy. *J Viral Hepat* **28**, 1355–1361 (2021).

9) Chun HM, Roediger MP, Hullsiek KH, Thio CL, Agan BK, Bradley WP, Peel SA et all. Hepatitis B virus coinfection negatively impacts HIV outcomes in HIV seroconverters. J. Infect Dis 2012 Jan 15; 205(2) 185-193.

10) Platt L, French CE, McGowan CR, Sabin K, Gower E, Trickey A, et al. Prevalence and burden of HBV co-infection among people living with HIV: A global systematic review and meta-analysis. Journal of Viral Hepatitis. 2020 Mar;27(3):294–315.

11) Fasano M, Poliseno MC, Fiore JR, Lo Caputo S, D'arminio Monforte A, Santantonio TA. Management of Chronic Hepatitis B in HIV-Coinfected Patients. Viruses 2022 Sep 13;14(9):2022.

12) Thio CL. Hepatitis B and human immunodeficiency virus coinfection. Hepatology. 2009 May;49(5 Suppl):S138-145.

13) Basnayake SK, Easterbrook PJ. Wide variation in estimates of global prevalence and burden of chronic hepatitis B and C infection cited in published literature. J Viral Hepat. 2016;23(7):545–59.

14) Raimondo G, Locarnini S, Pollicino T, Levrero M, Zoulim F, Lok AS, et al. Update of the statements on biology and clinical impact of occult hepatitis B virus infection. J Hepatol. 2019;71(2):397–408.

15) Soriano V, Aguilera A, Gonzalez R, Gomez-Gallego F, Barea L, Treviño M, et al. Occult hepatitis B and HIV infection: European Journal of Gastroenterology & Hepatology. 2019 Apr;1.

16) Malagnino, V. *et al.* Poor CD4/CD8 ratio recovery in HBcAb-positive HIV patients with worse immune status is associated with significantly higher CD8 cell numbers. *Sci Rep* **11**, 3965 (2021).

17) Salpini R, Malagnino V, Piermatteo L, Mulas T, Alkhatib M, Scutari R, et al. Cryptic HBV Replicative Activity Is Frequently Revealed in Anti-HBc-Positive/HBsAg-Negative Patients with HIV Infection by Highly Sensitive Molecular Assays, and Can Be Predicted by Integrating Classical and Novel Serological HBV Markers. Microorganisms. 2020 Nov 18;8(11).

18) Malagnino, V. *et al.* HBcAb Positivity Is a Risk Factor for an Increased Detectability of HIV RNA after Switching to a Two-Drug Regimen Lamivudine-Based (2DR-3TC-Based) Treatment: Analysis of a Multicenter Italian Cohort. *Microorganisms* **9**, (2021).

19) Montaner, J. S. G. Treatment as prevention--a double hat-trick. *Lancet* **378**, 208–209 (2011).

20) Montaner, J. S. G. *et al.* Association of highly active antiretroviral therapy coverage, population viral load, and yearly new HIV diagnoses in British Columbia, Canada: a population-based study. *Lancet* **376**, 532–539 (2010).

21) Battegay, M., Nüesch, R., Hirschel, B. & Kaufmann, G. R. Immunological recovery and antiretroviral therapy in HIV-1 infection. *Lancet Infect Dis* **6**, 280–287 (2006).

22) Doyle, T. *et al.* Plasma HIV-1 RNA detection below 50 copies/ml and risk of virologic rebound in patients receiving highly active antiretroviral therapy. *Clin Infect Dis* **54**, 724–732 (2012).

23) Maggiolo, F. *et al.* Ultrasensitive assessment of residual low-level HIV viremia in HAART-treated patients and risk of virological failure. *J Acquir Immune Defic Syndr* **60**, 473–482 (2012).

24) Charpentier, C. *et al.* Persistent low-level HIV-1 RNA between 20 and 50 copies/mL in antiretroviral-treated patients: associated factors and virological outcome. *J Antimicrob Chemother* **67**, 2231–2235 (2012).

25) Doyle, T. & Geretti, A. M. Low-level viraemia on HAART: significance and management. *Curr Opin Infect Dis* **25**, 17–25 (2012).

26) Henrich, T. J., Wood, B. R. & Kuritzkes, D. R. Increased risk of virologic rebound in patients on antiviral therapy with a detectable HIV load <48 copies/mL. *PLoS One* **7**, e50065 (2012).

27) Modjarrad, K. & Vermund, S. H. Effect of treating co-infections on HIV-1 viral load: a systematic review. *Lancet Infect Dis* **10**, 455–463 (2010).

28) Gianella, S. *et al.* Replication of Human Herpesviruses Is Associated with Higher HIV DNA Levels during Antiretroviral Therapy Started at Early Phases of HIV Infection. *J Virol* **90**, 3944–3952 (2016).

29) Chaillon, A. *et al.* Subclinical Cytomegalovirus and Epstein-Barr Virus Shedding Is Associated with Increasing HIV DNA Molecular Diversity in Peripheral Blood during Suppressive Antiretroviral Therapy. *J Virol* **94**, (2020).

30) Chun HM, Mesner O, Thio CL, Bebu I, Macalino G, Agan BK, et al. HIV outcomes in Hepatitis B virus coinfected individuals on HAART. J Acquir Immune Defic Syndr. 2014 Jun 1;66(2):197–205.

31) Kouamé GM, Boyd A, Moh R, Badje A, Gabillard D, Ouattara E, et al. Higher Mortality Despite Early Antiretroviral Therapy in Human Immunodeficiency Virus and Hepatitis B Virus (HBV)-Coinfected Patients With High HBV Replication. Clin Infect Dis. 2018 Jan 6;66(1):112–20.

32) Morsica G, Ancarani F, Bagaglio S, Maracci M, Cicconi P, Cozzi Lepri A, et al. Occult hepatitis B virus infection in a cohort of HIV-positive patients: correlation with hepatitis C virus coinfection, virological and immunological features. Infection. 2009 Oct;37(5):445–9.

33) Malagnino V, Cerva C, Maffongelli G, Teti E, Foroghi Biland L, Cesta N, et al. HBcAb seropositivity is correlated with poor HIV viremia control in an Italian cohort of HIV/HBV-coinfected patients on first-line therapy. Sci Rep. 2019 16;9(1):11942.

34) Gonzalez, V. D. *et al.* High levels of chronic immune activation in the T-cell compartments of patients coinfected with hepatitis C virus and human immunodeficiency virus type 1 and on highly active antiretroviral therapy are reverted by alpha interferon and ribavirin treatment. *J Virol* **83**, 11407–11411 (2009).

35) Goletti, D. *et al.* Effect of Mycobacterium tuberculosis on HIV replication. Role of immune activation. *J Immunol* **157**, 1271–1278 (1996).

36) Lidofsky, A. *et al.* Macrophage Activation Marker Soluble CD163 Is a Dynamic Marker of Liver Fibrogenesis in Human Immunodeficiency Virus/Hepatitis C Virus Coinfection. *J Infect Dis* **218**, 1394–1403 (2018).

37) Malagnino V, Cerva C, Cingolani A, Ceccherini-Silberstein F, Vergori A, Cuomo G, et al. HBcAb Positivity Increases the Risk of Severe Hepatic Fibrosis Development in HIV/HCV-Positive Subjects From the ICONA Italian Cohort of HIV-Infected Patients. Open Forum Infect Dis. 2021 Jan;8(1):ofaa566.

ACKNOWLEDGEMENTS

Icona Foundation Study Group. BOARD OF DIRECTORS: A d'Arminio Monforte (President), A Antinori (Vice-President), S Antinori, A Castagna, R Cauda, G Di Perri, E Girardi, R Iardino, A Lazzarin, GC Marchetti, C Mussini, E Quiros-Roldan, L Sarmati, B Suligoi, F von Schloesser, P Viale. SCIENTIFIC SECRETARY: A d'Arminio Monforte, A Antinori, A Castagna, F Ceccherini-Silberstein, A Cingolani, A Cozzi-Lepri, E Girardi, A Gori, S Lo Caputo, G Marchetti, F Maggiolo, C Mussini, M Puoti, CF Perno. STEERING COMMITTEE: A Antinori, F Bai, A Bandera, S Bonora, A Calcagno, D Canetti, A Castagna, F Ceccherini-Silberstein, A Cervo, A Cingolani, P Cinque, A Cozzi-Lepri, A d'Arminio Monforte, A Di Biagio, R Gagliardini, A Giacomelli, E21 Girardi, N Gianotti, A Gori, G Guaraldi, S Lanini, G Lapadula, M Lichtner, A Lai, S Lo Caputo, G Madeddu, F Maggiolo, V Malagnino, G Marchetti, C Mussini, S Nozza, CF Perno, S Piconi, C Pinnetti, M Puoti, E Quiros Roldan, R Rossotti, S Rusconi, MM Santoro, A Saracino, L Sarmati, V Spagnuolo, N Squillace, V Svicher, L Taramasso, A Vergori. STATISTICAL AND MONITORING TEAM: F Bovis, A Cozzi-Lepri, S De Benedittis, I Fanti, A Rodano', M Ponzano, A Tavelli. COMMUNITY ADVISORY BOARD: A Bove, M Cernuschi, L Cosmaro, M Errico, A Perziano, V Calvino. BIOLOGICAL BANK INMI AND SAN PAOLO: S Carrara, S Graziano, G Prota, S Truffa, D Vincenti, Y D'Errico, R Rovito. PARTICIPATING PHYSICIANS AND CENTERS: Italy A Giacometti, A Costantini, V Barocci (Ancona); A Saracino, C Santoro, E Milano (Bari); L Comi, C Suardi (Bergamo); P Viale, L Badia, S Cretella (Bologna); EM Erne, A Pieri (Bolzano); E Quiros Roldan, E Focà, C Minardi (Brescia); B Menzaghi, C Abeli (Busto Arsizio); L Chessa, F Pes (Cagliari); P Maggi, L Alessio (Caserta); B Cacopardo, B Celesia (Catania); J Vecchiet, K Falasca (Chieti); A Pan, S Dal Zoppo (Cremona); D Segala (Ferrara); MA Di Pietro, C Costa (Firenze); S Lo Caputo, S Ferrara (Foggia); M Bassetti, E Pontali, S Blanchi, N Bobbio, G Mazzarello (Genova); M Lichtner, L Fondaco (Latina); S Piconi, C Molteni (Lecco); S Rusconi, G Canavesi (Legnano); G Nunnari, G Pellicanò (Messina); G Marchetti, S Antinori, G Rizzardini, M Puoti, A Castagna, A Bandera, V Bono, MV Cossu, A Giacomelli, R Lolatto, MC Moioli, L Pezzati, S

Diotallevi, C Tincati (Milano); C Mussini, C Puzzolante (Modena); P Bonfanti, G Lapadula (Monza); V Sangiovanni, I Gentile, V Esposito, N Coppola, FM Fusco, G Di Filippo, V Rizzo, N Sangiovanni, S Martini (Napoli); AM Cattelan, D Leoni (Padova); A Cascio, C Colomba (Palermo); D Francisci, E Schiaroli (Perugia); G Parruti, F Sozio (Pescara); P Blanc, SI Bonelli (Pistoia); C Lazzaretti, R Corsini (Reggio Emilia); A Antinori, R Cauda, C Mastroianni, L Sarmati, A Latini, A Cingolani, V Mazzotta, S Lamonica, M Capozzi, A Mondi, M Rivano Capparuccia, G Iaiani, C Stingone, L Gianserra, J Paulicelli, MM Plazzi, G d'Ettore, M Fusto (Roma); I Coledan (Rovigo); G Madeddu, A De Vito (Sassari); M Fabbiani, F Montagnani (Siena); A Franco, R Fontana Del Vecchio (Siracusa); BM Pasticci, C Di Giuli (Terni); GC Orofino, G Calleri, G Di Perri, S Bonora, G Accardo (Torino); C Tascini, A Londero (Udine); V Manfrin, G Battagin (Vicenza); G Starnini, D Farinacci (Viterbo)