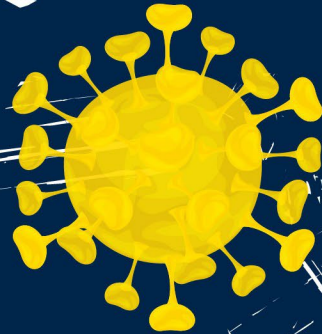


CROI ICAR

CROI Affiliated Event for
Italian Young Investigators

AWARDS 2024



CHAIRS:

F. Kirchhoff

(Ulm, Germany, EU)

D. Margolis

(Chapel Hill, North Carolina, USA)



Denver, Colorado

March 4th, 2024

h 06.00 - 07.30 pm

CROI 31st CONFERENCE ON
Retroviruses and Opportunistic Infections
MARCH 3-6 2024 DENVER, COLORADO

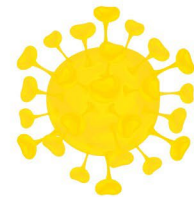
ICAR 16th NATIONAL CONGRESS
Italian Conference on AIDS and Antiviral Research
JUNE 19-21 ROME, ITALY



CROIICAR AWARDS 2024

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CHAIRS: **F. Kirchhoff** (Ulm, Germany, EU), **D. Margolis** (Chapel Hill, North Carolina, USA)



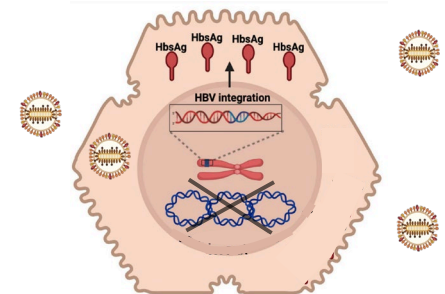
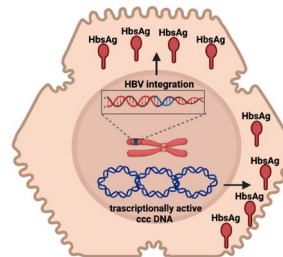
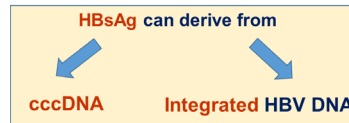
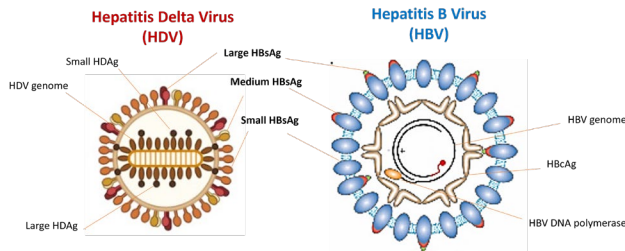
Intrahepatic HDV activity is fueled by integrated HBV DNA-derived HBs independently from cccDNA size

Stefano D'Anna
PhD Student

University of Rome Tor Vergata – Rome, Italy

Background

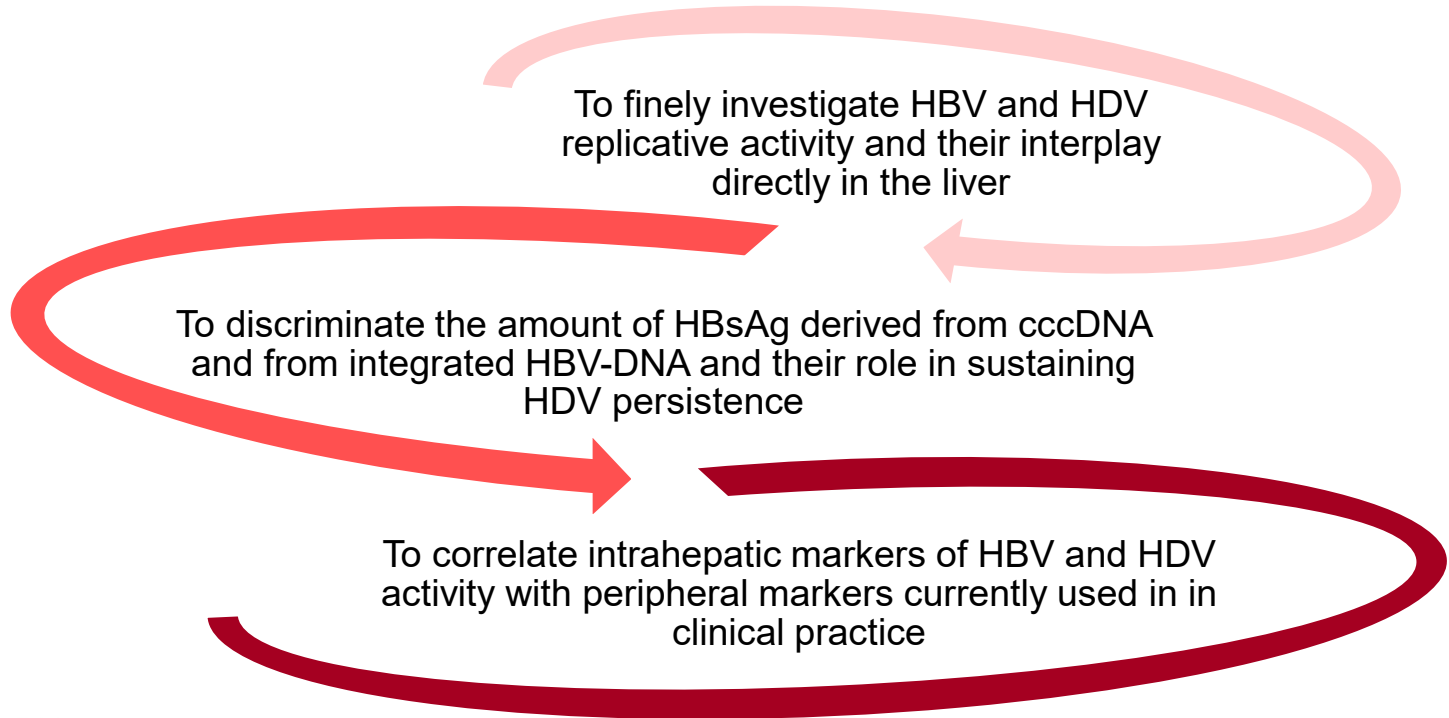
- HDV exploits **HBV surface glycoproteins (HBsAg)** for its morphogenesis and de novo entry into hepatocytes.
- In **HBV chronic infection**, it is known that **HBsAg can derive** not only from **cccDNA** but also from the **HBV-DNA integrated** into the genome of the hepatocytes.
- The contribution of **HBV integration** (as source of HBsAg) to **HDV persistence** has not been studied in vivo in the setting of **chronic HDV co-infection**.



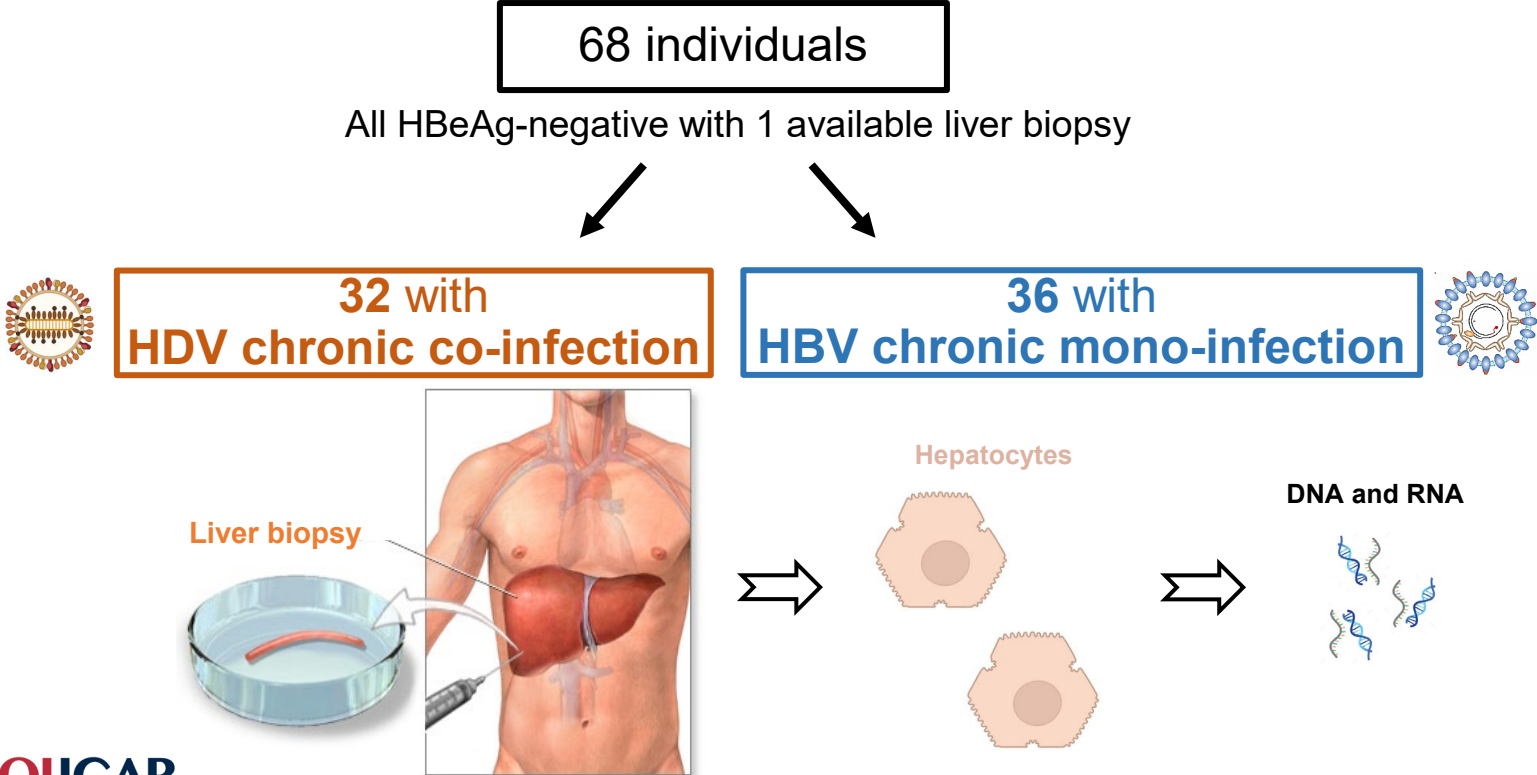
Mason, Gastroenterology, 2016
Budzinska et al., EMI, 2018
Podlaha, O; PlosOne, 2019
Rydell et al., JID, 2020
Svicher V and Salpini R., Gut, 2020
Meier MA, JHep, 2021



Aims of the study



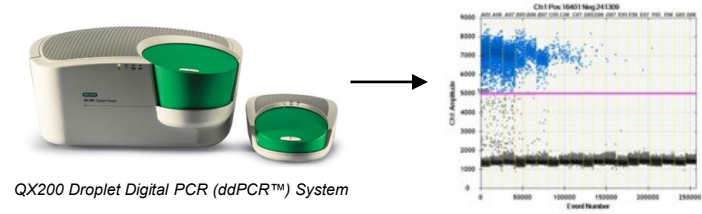
Methods



Methods

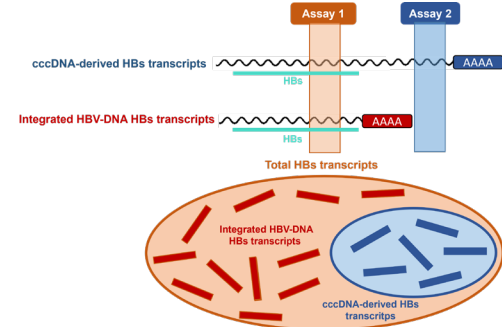
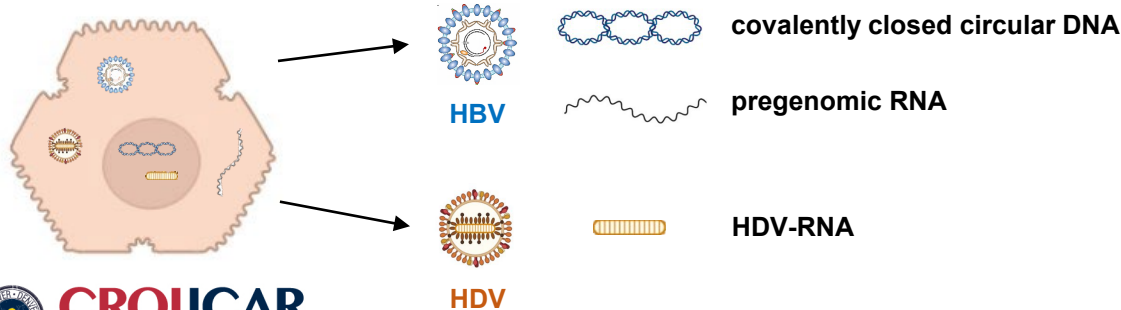
Highly-sensitive droplet digital PCR (**ddPCR**) used to quantify **intrahepatic levels** of:

- **Total HBV-DNA**
- **cccDNA** and **pgRNA**
- **HDV-RNA**



Two different **ddPCR assays** were set up to distinguish **HBs transcripts** deriving:

- from **cccDNA**
- from **integrated HBV-DNA**



ddPCR assays developed according to Gruda et al., 2022

$$\text{Integrated HBV-DNA HBs transcripts} = \text{Total HBs-transcripts} - \text{cccDNA derived transcripts}$$



RESULTS



Study population

Variables	HDV co-infection N=32	HBV mono-infection N=36	P-value
Age, median (IQR) years	49 (39 – 59)	42 (34 – 60)	0.6
Male, N (%)	21 (65.6%)	32 (88.9%)	0.04
Nationality ^a			
Italian, N (%)	17 (65.4%)	25 (73.5%)	0.6
East-European, N (%)	8 (30.8%)	6 (17.7%)	0.4
African, N (%)	1 (3.8%)	3 (8.8%)	0.6
NUC treatment, N (%)	27 (84.4%)	24 (66.7%)	0.1
NUC duration, median (IQR) years	6 (4 – 12)	6 (4 – 7)	0.5
Serum HBV-DNA, median (IQR) log IU/ml	1.3 (0 – 1.5)	3.6 (2.4 – 4.9)	<0.0001
Serum HBsAg, median (IQR) log IU/ml	4.0 (3.7 – 4.3)	3.8 (3.3 – 4.2)	0.3
Serum HDV-RNA, median (IQR) log IU/ml	6.0 (3.8 – 6.7)	-	-
Ishak fibrosis score ≥ 5, N (%)	16 (53.3%)^b	7 (19.4%)	0.005
ALT, median (IQR) U/l	68 (45 – 89)	28 (21 – 49)	0.001

^a Datum available for 26 individuals with HDV co-infection and for 34 individuals with HBV mono-infection.

^b Datum available for 30 individuals with HDV co-infection



HDV co-infection was characterized by a **high levels** of **HDV viraemia**, positively correlated with **intrahepatic levels** of HDV-RNA.

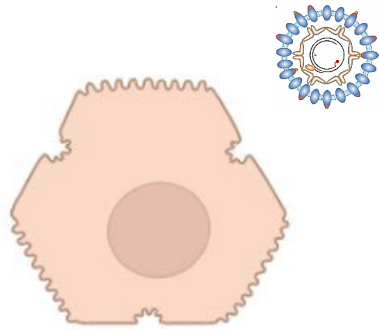
Variables	N=32
Serum HDV-RNA, median (IQR) log IU/ml	6.0 (3.8 – 6.7)
Intrahepatic HDV-RNA, median (IQR) copies/1000cells	784 (1 – 4266)



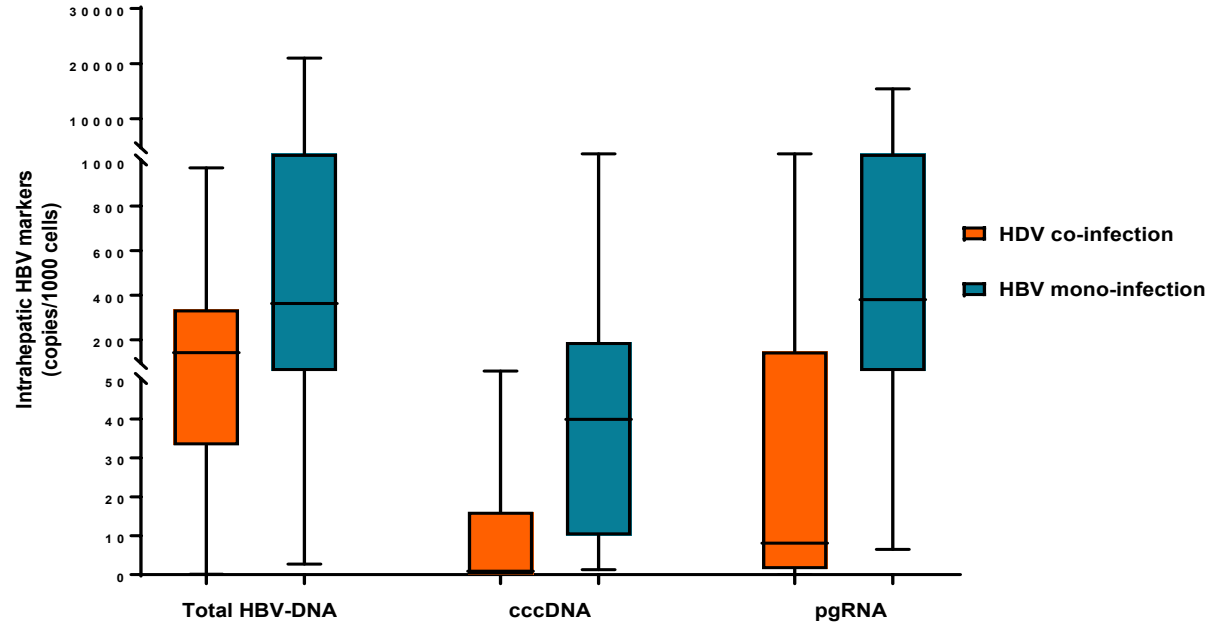
Rho=0.62
P=0.006



By comparing **intrahepatic HBV markers**, significant differences in **HBV reservoir size** were observed between HDV co-infection and HBV mono-infection.



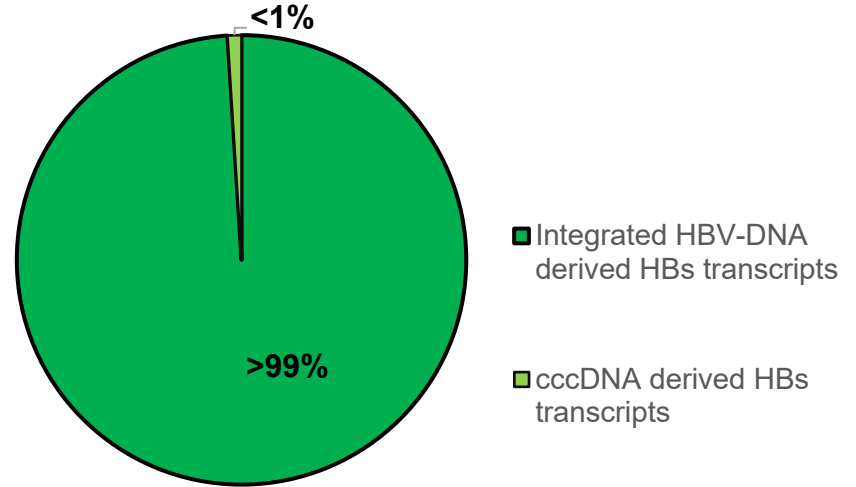
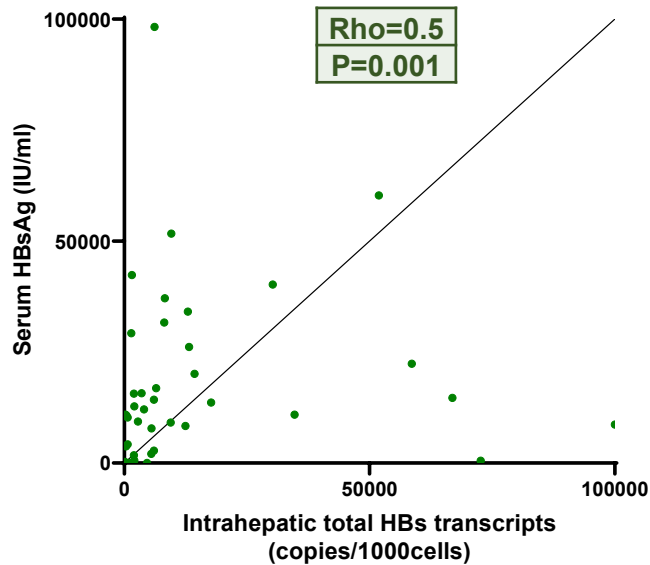
P<0.01 for all comparisons



The boxplots report the comparisons of the levels of intrahepatic HBV markers in copies/1000cells between HDV co-infection and HBV mono-infection. Mann-Whitney test was used to assess statistically significant differences.



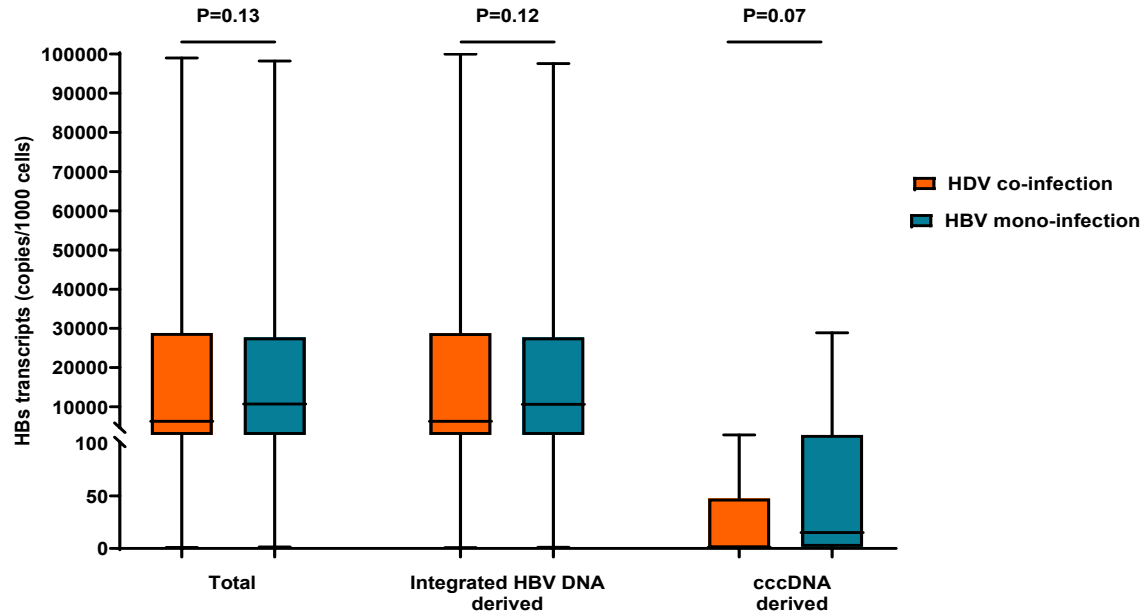
- In the overall population, intrahepatic levels of **total HBs transcripts positively correlated with serum HBsAg**.
- Notably, by analyzing the **source of HBs transcripts**, we found that **>99%** of them derived from **integrated HBV-DNA**, with a limited contribution of cccDNA transcriptional activity.



The graph reports the correlation between serum HBsAg and intrahepatic levels of total HBs transcripts in the overall population. Spearman test was used to assess statistically significant difference.

The pie-chart reports the percentages of HBsAg transcripts derived from integrated HBV-DNA and of those derived from cccDNA, calculated respect to the total levels of HBs transcripts in the overall population.

- Despite the **limited HBV reservoir**, HDV co-infection was characterized by a production of **total and integrated HBV DNA-derived HBs transcripts comparable** to HBV mono-infection.
- In line with HBV markers, the production of **cccDNA-derived HBs transcripts tends to be lower** in the setting of **HDV co-infection**.



The boxplots report the comparisons of the levels of intrahepatic HBs transcripts in copies/1000cells between HDV co-infection and HBV mono-infection. Mann-Whitney test was used to assess statistically significant differences.



68 individuals



**32 with
HDV chronic co-infection**

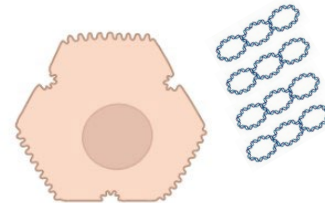
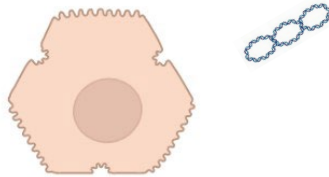
cccDNA levels, median (IQR) copies/1000cells 1 (0.03 – 14)



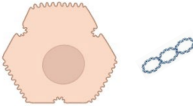
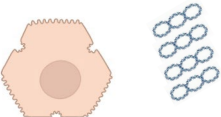








**17 with
cccDNA <1 copy/1000cells**



**15 with
cccDNA >1 copy/1000cells**



- **Intrahepatic HBV markers** were **different** according to the amount of cccDNA.
- Conversely, **HDV-RNA** levels were **comparable** independently from cccDNA...

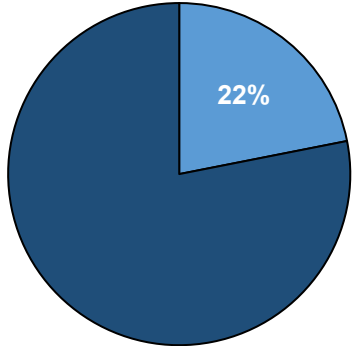
Intrahepatic markers, median (IQR) copies/1000cells		cccDNA <1 copy/1000cells N=17		cccDNA >1 copy/1000cells N=15	P-value
					
Total HBV-DNA		43 (1 – 128)		269 (174 – 414)	0.001
HBV pgRNA		1.4 (0.4 – 78)		108 (5 – 411)	0.01
cccDNA		0.02 (0 – 0.14)		17 (6 – 33)	<0.0001
HDV-RNA		782 (1 – 5,559)		844 (1 – 6,371)	0.6

Mann-Whitney test was used to assess statistically significant differences.

...suggesting the existence of pathways underlying HDV activity independent from HBV reservoir.



**32 with
HDV chronic co-infection**



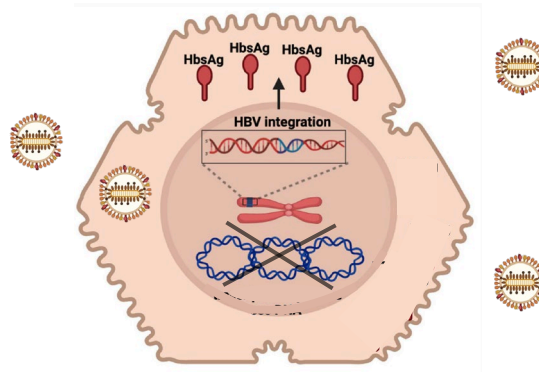
7 with:

- no cccDNA,
- no cccDNA-derived HBs transcripts
- undetectable HBV viraemia



Focusing on these 7 individuals, we observed **an intensive HDV activity** at both serum and intrahepatic levels, suggesting that HDV persistence can be sustained by **HBsAg derived from integrated HBV-DNA**.

Variables	N=7
Serum HDV-RNA, median (IQR) log IU/ml	6.0 (5.9 – 7.3)
Intrahepatic HDV-RNA, median (IQR) copies/1000cells	1,659 (660 – 12,261)
Integrated HBV DNA-derived HBs transcripts, median (IQR) copies/1000cells	3 (2 – 690)



Conclusions

- **HDV chronic co-infection** can be characterized by **high levels of intrahepatic HDV replication** in spite of the presence of a **limited HBV reservoir**.
- **Pathways sustaining HDV activity** are independent from the size of **HBV reservoir** and are **fueled by a considerable production of HBs transcripts**, mainly derived from **integrated HBV-DNA**.
- Overall, these issues are crucial for deciphering **mechanisms underlying HDV persistence**, that could **jeopardise the success of anti-HDV therapies** and should be carefully considered for the identification of **novel strategies aimed to finally achieve HDV cure**.



Acknowledgements



Unit of Virology

Valentina Svicher
Romina Salpini
Lorenzo Piermatteo
Giulia Torre
Francesca Ceccherini Silberstein

Unit of Infectious Diseases

Loredana Sarmati
Annamaria Geretti
Elisabetta Teti
Andrea Di Lorenzo
Vincenzo Malagnino
Marco Iannetta

Unit of Hepatology

Leonardo Baiocchi
Simona Francioso
Ilaria Lenci



Mario Rizzetto
Gian Paolo Caviglia
Antonella Olivero



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Giovanni B. Gaeta



Patrick T.F. Kennedy
Upkar S. Gill

