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P55
Poster for you

HDV persistence can be independent from the extent of HBV reservoir and can be sustained by HBsAg production mainly derived from HBV-DNA integration

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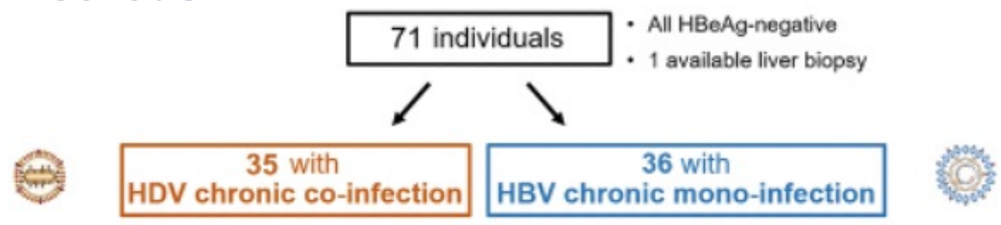
Introduction & Aims

- **HDV exploits** HBV surface glycoproteins (**HBsAg**) for its morphogenesis and de novo entry into hepatocytes.
- HBsAg consists of **3 different proteins**: small (**S-HBs**), middle (**M-HBs**) and large (**L-HBs**) HBsAg (present only in virions and responsible for binding to NTCP receptor).
- 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**.

In this light, this study aims at:

- finely investigating the **size** and the **activity** of **intrahepatic HBV reservoir** in the setting of HBeAg-negative chronic HDV coinfection and HBV monoinfection;
- at correlating the **levels of HBV intrahepatic markers with the degree of HDV activity**;
- at elucidating **the role of integrated HBV-DNA** in sustaining **HDV replicative activity** and **viral persistence**.

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**
- **Serum levels** of **HBV-DNA**, **total HBsAg** and **HDV-RNA** were quantified by commercial assays.
- Ad-hoc ELISA assays were used to quantify **serum levels of HBsAg isoforms**.

Results

Table 1. Patients' characteristics

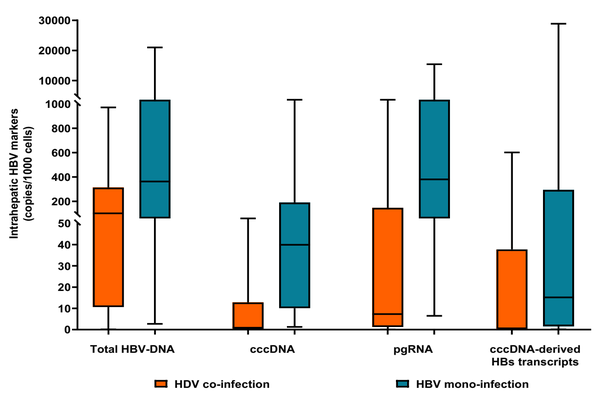
Variables	HDV co-infection N=35	HBV mono-infection N=36	P-value
Age, median (IQR) years	50 (38 – 60)	42 (34 – 60)	0.6
Male, N (%)	22 (62.9%)	32 (88.9%)	0.01
Nationality [a]			
Italian, N (%)	17 (48.6%)	25 (73.5%)	0.1
East-European, N (%)	13 (37.1%)	6 (17.7%)	0.06
African, N (%)	5 (14.3%)	4 (11.4%)	0.7
NUC treatment, N (%)	30 (85.7%)	23 (63.9%)	0.06
NUC duration, median (IQR) years	6 (4 – 12)	6 (4 – 7)	0.5
Serum HBV-DNA, median (IQR) log IU/ml	**1.1 (0 – 1.5)**	**3.6 (2.4 – 4.9)**	**<0.0001**
Serum HBsAg, median (IQR) log IU/ml	4.0 (3.6 – 4.2)	3.8 (3.3 – 4.2)	0.6
Serum HDV-RNA, median (IQR) log IU/ml	6.0 (4.0 – 6.9)	-	-
Ishak fibrosis score ≥5, N (%)	**17 (53.1%) [b]**	**7 (19.4%)**	**0.02**
ALT, median (IQR) U/l	**72 (48 – 119)**	**28 (21 – 49)**	**0.0004**

[a] Datum available for all individuals with HDV co-infection and for 35 individuals with HBV mono-infection.
[b] Datum available for 32 individuals with HDV co-infection
Mann-Whitney test and Chi-Squared test were used to assess statistically significant diferences.

- HDV co-infection was characterized by high levels of HDV viraemia, positively correlated with intrahepatic levels of HDV-RNA (Rho=0.62; P=0.006).

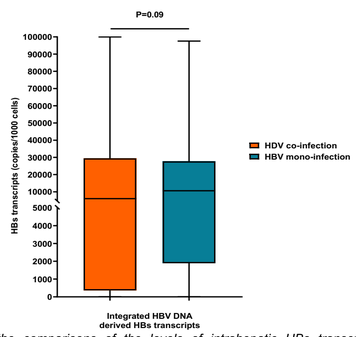
Variables	N=32
Serum HDV-RNA, median (IQR) log IU/ml	6.0 (4.0 – 6.9)
Intrahepatic HDV-RNA, median (IQR) copies/1000cells	787 (1 – 7596)

- By comparing intrahepatic HBV markers, HDV co-infection was associated with significantly lower HBV reservoir size and with a more limited transcriptional activity of HBV reservoir.



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.

- Superimposable results were observed by focusing on individuals under NUC treatment.
- By analyzing the source of HBs transcripts in both groups, we found that >99% of them derived from integrated HBV-DNA, with comparable levels between HDV co-infection and HBV mono-infection.



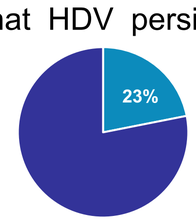
The boxplots report the comparisons of the levels of intrahepatic HBs transcripts in copies/1000cells between HDV co-infection and HBV mono-infection. Mann-Whitney test was used to assess statistically significant differences.

- In order to verify if intrahepatic levels of HDV-RNA were dependent on the size of cccDNA, we divided the CHD population on the basis of the median levels of cccDNA (1 copy/1000cells).
- A lower cccDNA pool correlated with lower levels of all intrahepatic HBV markers.
- Conversely, HDV-RNA levels were comparable independently from cccDNA size.

Intrahepatic markers, median (IQR) copies/1000cells	cccDNA <1 copy/1000cells N=19	cccDNA >1 copy/1000cells N=16	P-value
Total HBV-DNA	**30 (1 – 73)**	**247 (158 – 406)**	**<0.001**
HBV pgRNA	**1.4 (0.4 – 25)**	**89 (6 – 238)**	**0.005**
cccDNA	**0.02 (0 – 0.1)**	**15 (5 – 32)**	**<0.0001**
cccDNA-derived HBs transcripts	**0.3 (0.1 – 0.9)**	**41 (7 – 179)**	**0.001**
Integrated-derived HBs transcripts	**432 (3 – 7748)**	**19,312 (5476 – 42,448)**	**0.003**
HDV-RNA	782 (1 – 5559)	1026 (40 – 6984)	0.5

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

- These results were confirmed also focusing on 8 out of 35 individuals with CHD that showed no signs of cccDNA, cccDNA-derived HBs transcripts and with undetectable HBV viremia.
- Despite no evidence of HBV reservoir, an intensive HDV activity was revealed at both serum and intrahepatic levels in these individuals, coupled to the presence of integrated HBV DNA-derived HBs transcripts, suggesting that HDV persistence can be sustained by HBV integration.



8 with:
- **no cccDNA**,
- **no cccDNA-derived HBs transcripts**
- **undetectable HBV viraemia**

Variables	N=8
Serum HDV-RNA, median (IQR) log IU/ml	6.0 (4.8 – 6.9)
Intrahepatic HDV-RNA, median (IQR) copies/1000cells	5495 (976 – 14,946)
Integrated-derived HBs transcripts, median (IQR) copies/1000cells	3 (1 – 497)

- In order to corroborate this hypothesis, we quantified HBs isoforms levels in matched plasma samples of these 8 individuals.
- All the three HBs isoforms showed no statistically significant differences between the 8 individuals without cccDNA and cccDNA-derived HBs transcripts and the other individuals with signs of cccDNA, supporting the role of HBV integration in sustaining HDV morphogenesis.

Variables, median (IQR)	cccDNA –	cccDNA +	P-value
Serum S-HBs, median (IQR) ng/ml	1116 (123 – 3987)	6183 (2043 – 9735)	0.2
Serum M-HBs , median (IQR) ng/ml	368 (8 – 1894)	1637 (345 – 2533)	0.2
Serum L-HBs, median (IQR) ng/ml	1.4 (0.2 – 5.6)	3.2 (1 – 11.1)	0.5
Serum HDV-RNA, median (IQR) log IU/ml	6.0 (4.8 – 6.9)	6.4 (4.9 – 7.2)	0.3

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

Conclusion

- HDV chronic co-infection can be characterized by high levels of 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, capable to support the production of all three HBs isoforms.
- 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.

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