Pitfalls of HBV DNA testing during NUC therapy

Planning for next generation antivirals

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Disclosures over the last 36 months

- Expert Scientist for Roche Pharma Research & Early Development (pRED)
- Consulted for BMS, Cepheid, Gilead, Janssen, Roche Diagnostics, ViiV Healthcare, WHO Global Hepatitis Programme
- Research funding from BMS, Gilead, Roche pRED, ViiV Healthcare, WHO GHP

Case study

- 34 year-old woman
- From Macau, moved to the UK aged 13
- Diagnosed HBsAg positive at the antenatal clinic in 2014

Case study

Profile at diagnosis

- HBeAg^{+}/anti-HBe^{-}
- HBV DNA 7.2 $\log_{10}$ IU/ml
- ALT 26 U/L and AST 18 U/L
- Preserved hepatic function
- Platelet count 301 x 10^{9}/L
- eGFR >90 ml/min
- HIV, HCV, HDV negative
- HAV immune

Case study

- 34 year-old woman
- From Macau, moved to the UK aged 13
- Diagnosed HBsAg positive at the antenatal clinic in 2014
- Started TDF in 2nd trimester for the prophylaxis of MTCT, stopped after delivery by own decision
- Child HBsAg negative

TDF = tenofovir disoproxil fumarate
MTCT = Mother to child transmission

Case study

Profile in first half of 2015

- $HBeAg^{+}$/anti-HBe^{-}
- HBV DNA 8.4 and 7.3 $\log_{10}$ IU/ml
- ALT ranging from 46 to 87 U/L
- Preserved hepatic function
- Platelet count stable
- eGFR >90 ml/min
- Fibroscan: 4.6 kPa [IQR 1.1]
- Abdominal US scan: Normal

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- Oct 2015: Restarted TDF and continued through a second pregnancy in 2016
- Mar 2017: HBV DNA load 3.2 $\log_{10}$ IU/ml; ALT 34 U/L; $HBeAg^{+}$

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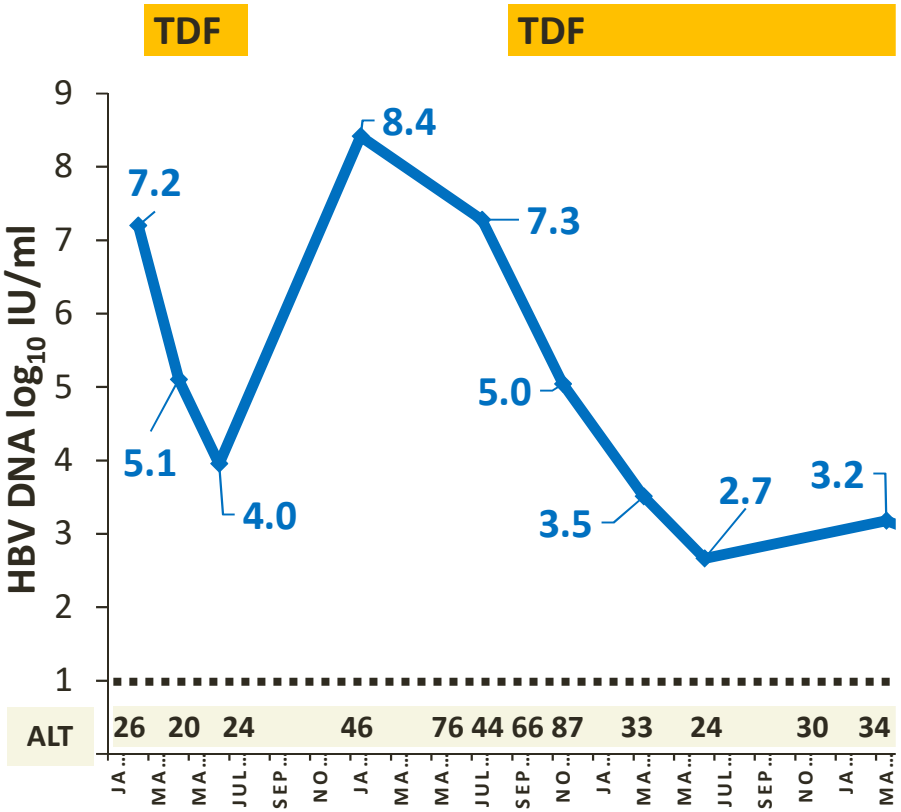
Case study

HBV DNA and ALT levels over time



Management options: Which is your choice?

1. Stop antiviral therapy
2. Do nothing, continue to monitor
3. Intensify adherence counselling, see again in 1 month
4. Add entecavir to TDF
5. Request a HBV drug resistance test

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- Adherence counselling and follow-up intensified, resistance test requested, entecavir added and then dose increased

Case study

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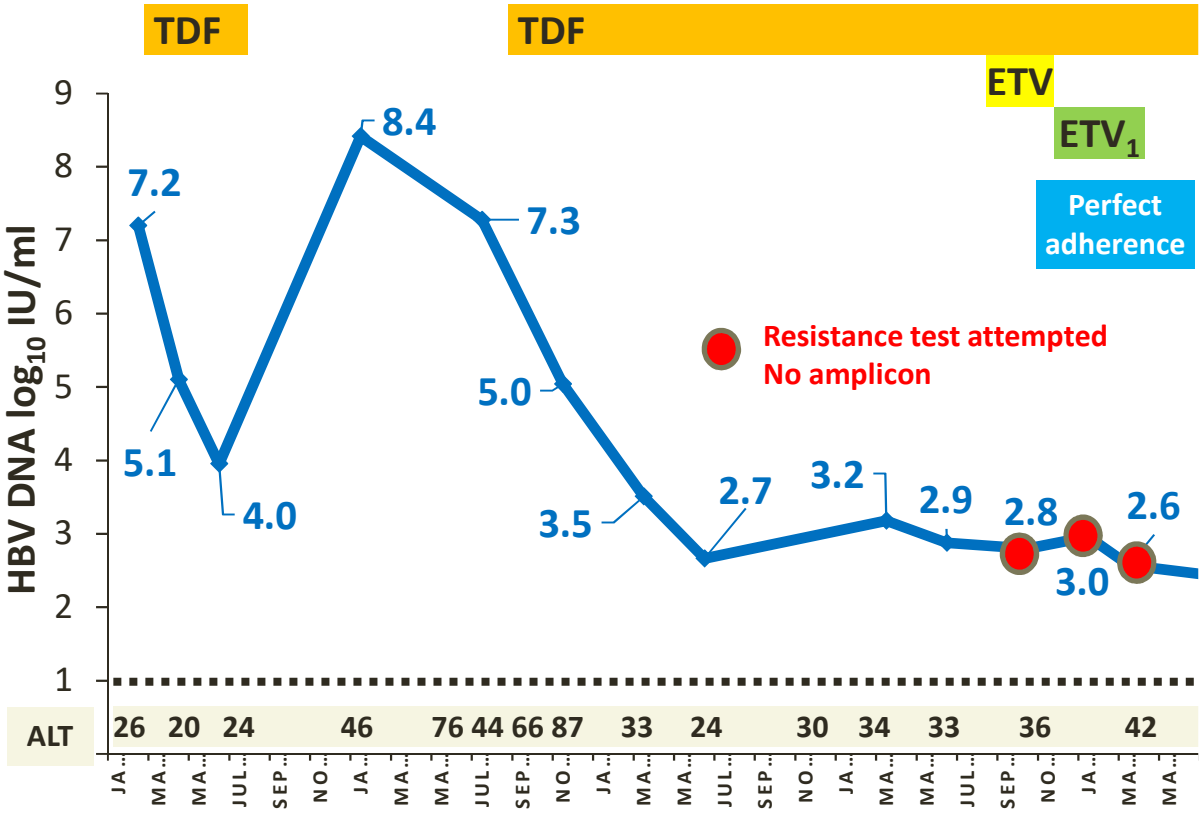


Management options: Which is your choice?

1. Stop all antiviral therapy
2. Stop entecavir
3. Do nothing, continue to monitor
4. Request a different HBV DNA load test

Case study

Solving the puzzle

- In discussion with the local lab, plasma samples are sent to a different laboratory for HBV DNA load retesting by a different assay

HBV DNA load:

- Jan 2018: 913 IU/ml at local lab vs. <10 IU/ml at referral lab
- Mar 2018: 372 IU/ml at local lab vs. 15 IU/ml at referral lab

Assay format:

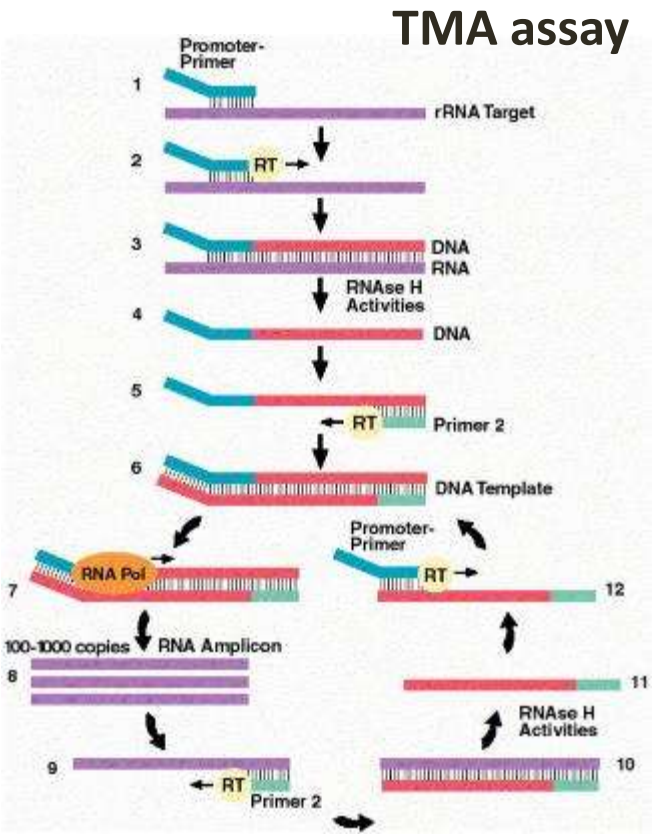
- Local lab = TMA-based commercial HBV DNA assay
- Referral lab = Real-time PCR-based commercial HBV DNA assay

➢ **Entecavir is discontinued**

TMA = Transcription-mediated amplification; PCR = Polymerase chain reaction

HBV DNA assays & their amplification enzymes

- Real-time PCR: 1 enzyme
  - DNA polymerase
- TMA: 2 enzymes
  - Moloney murine leukemia virus **reverse transcriptase**
  - T7 RNA polymerase
- The local lab adopted the TMA-based Aptima HBV Quant assay (Hologic) in Jan 2017



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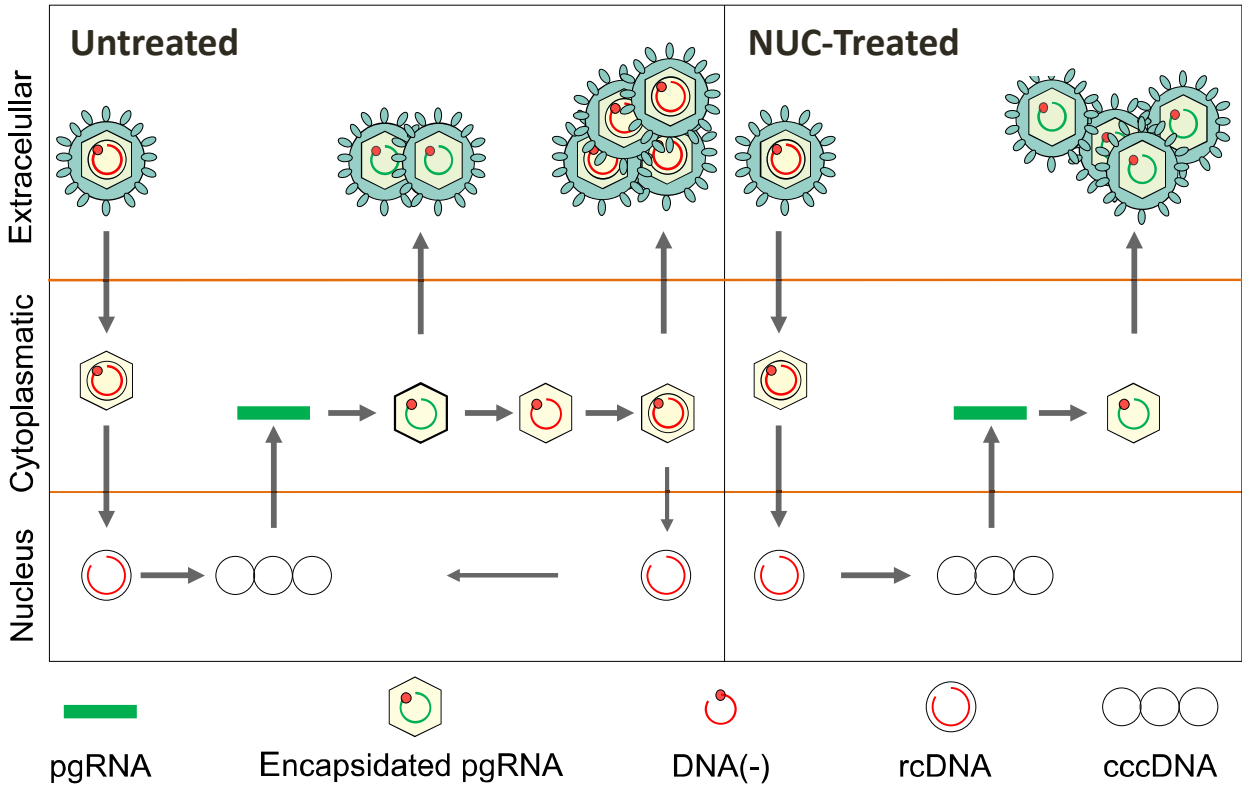
HBV as a DNA and RNA particle



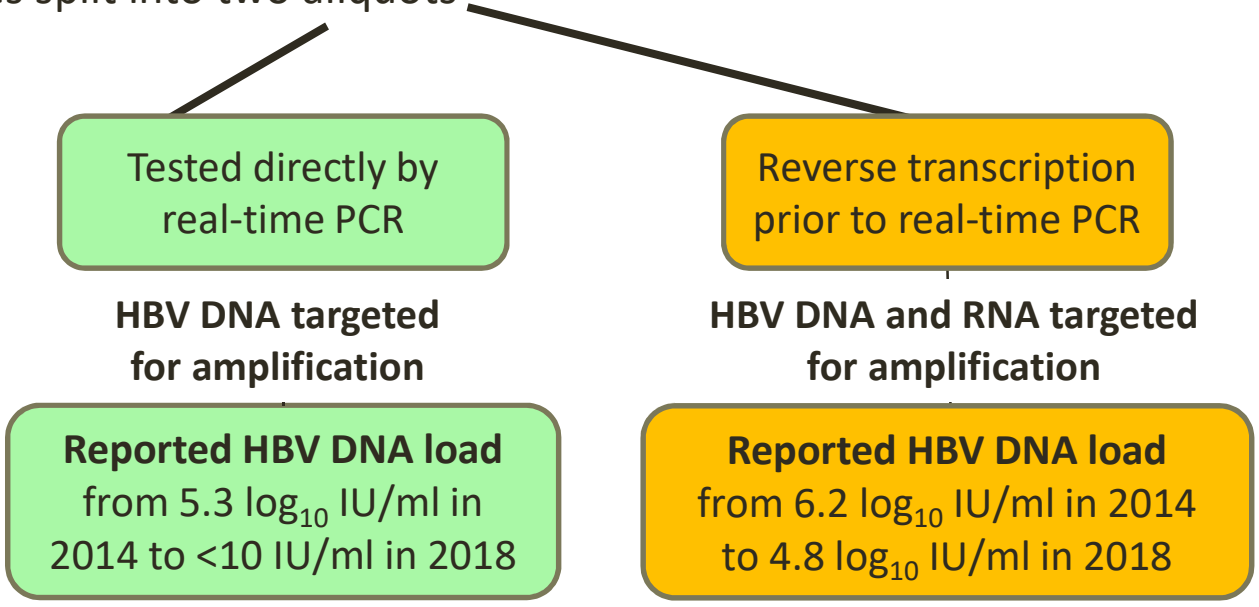
Wang et al. J Hepatology 2016

Hypothesis:

- In a NUC-treated patient with suppressed HBV DNA, high production of pgRNA from transcriptionally active cccDNA may result in the release of HBV RNA in circulation
- A TMA-based HBV DNA load assay could amplify HBV RNA and report this as a HBV DNA signal

Solving the puzzle

- Stored samples from regular intervals between 2014 and 2018 retrieved for retesting by a commercial real-time PCR assay in a referral lab
- Samples split into two aliquots



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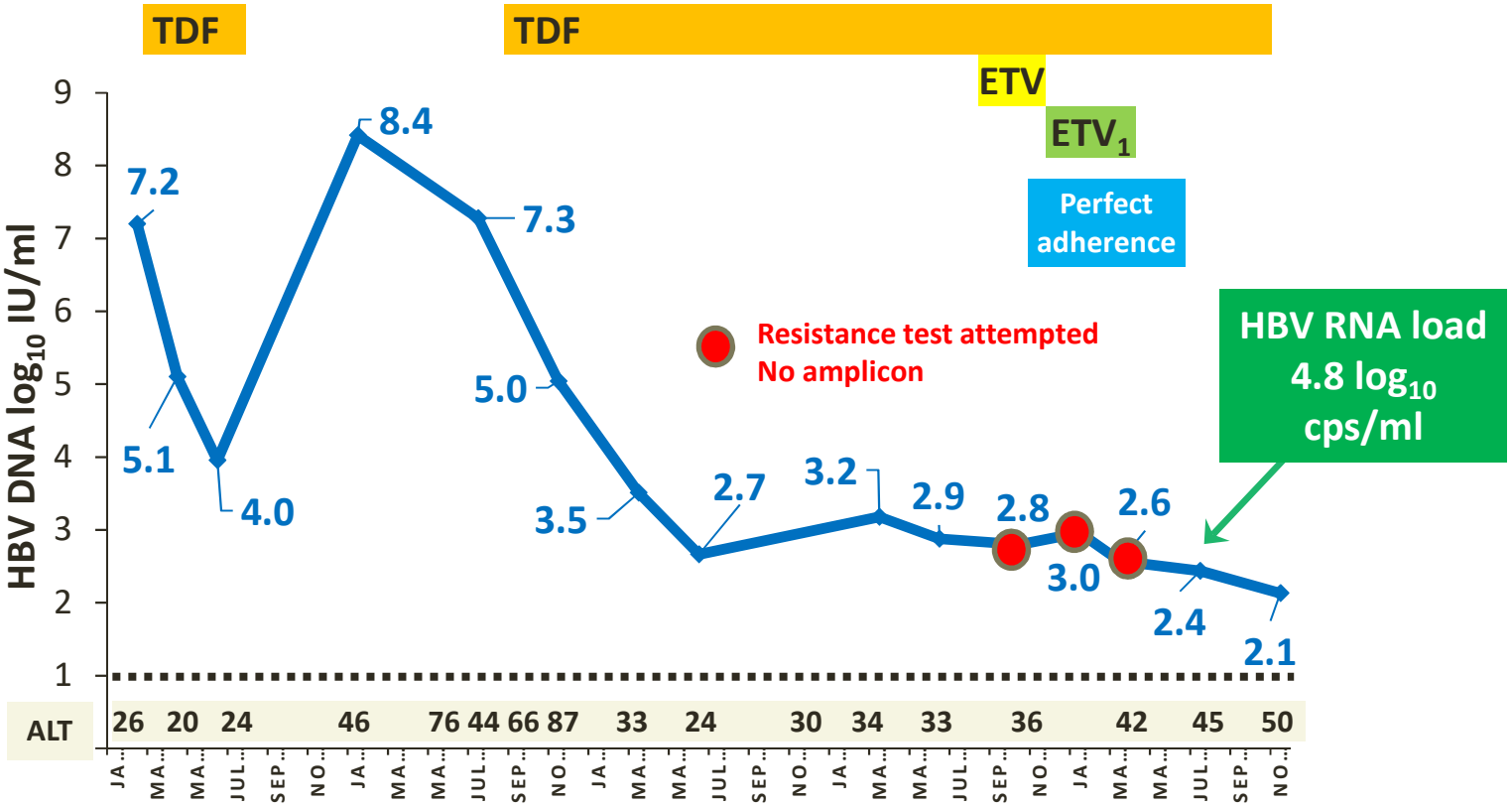
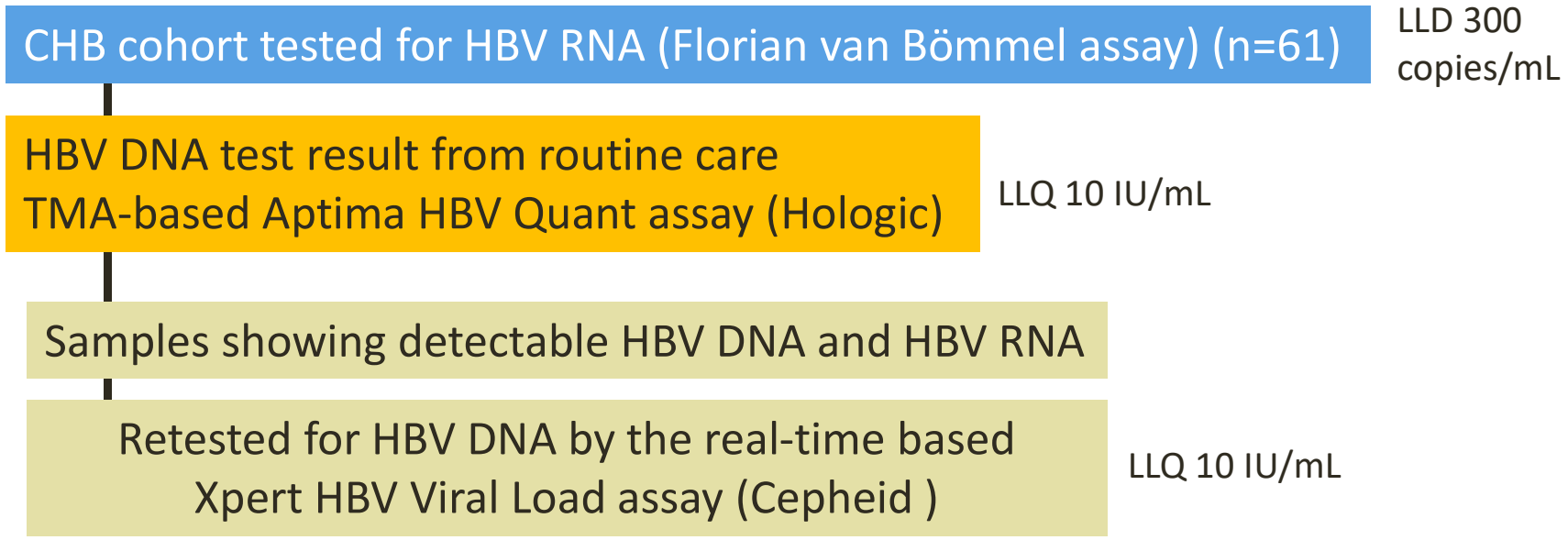


Next steps



LLD = Lower limit of detection; LLQ= Lower limit of quantification

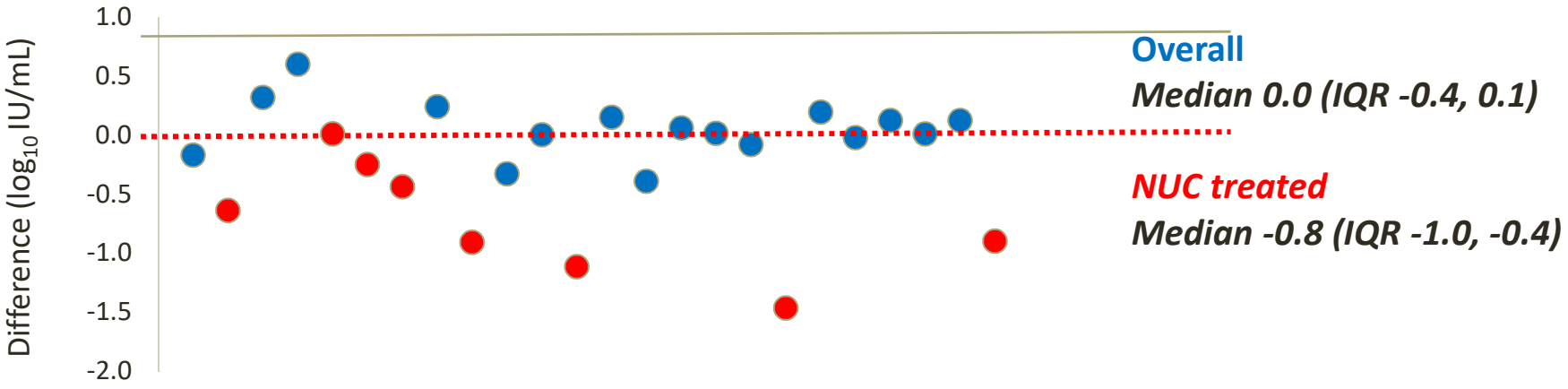
Study population

Characteristics		N=61
Age, median yrs (IQR)		37 (32, 45)
Female, n (%)		20 (33)
Ethnicity	Asian	35 (57)
	Black African	14 (23)
	White	12 (20)
HBeAg/anti-HBe	-/+	39 (64)
	-/-	5 (8)
	+/-	17 (28)
NUC	None	28 (47)
	TDF	24 (39)
	ETV	8 (13)
	TDF+ETV	1 (2)
NUC duration, median yrs (IQR)		2.2 (1.1-4.9)
HBV RNA detected, n (%)		**31 (51)**
HBV RNA, median $\log_{10}$ cps/ml		**3.8 (2.7, 7.0)**

HBV RNA detection by patient group

Characteristics		Total N	HBV RNA + N (%)	HBV RNA – N (%)
Female		20	15 (75)	5 (25)
Male		41	16 (39)	25 (61)
Ethnicity	Asian	35	23 (66)	12 (34)
	Black African	14	3 (21)	11 (79)
	White	12	4 (33)	8 (67)
HBeAg/anti-HBe	-/+	39	13 (33)	26 (67)
	-/-	5	2 (40)	3 (60)
	+/-	17	16 (94)	1 (6)
NUC	None	28	16 (57)	12 (43)
	Any	33	15 (45)	18 (55)
	TDF	24	12 (50)	12 (50)
	ETV	8	3 (38)	5 (62)
	TDF+ETV	1	0 (0)	1 (100)

Difference in HBV DNA quantification between real-time PCR and TMA in patients with detectable HBV RNA



- 15 of 33 patients on NUCs had detectable HBV RNA [median 4.3 log_{10} cps/mL (IQR 2.7, 6.4)]
- Of the 15 with detectable HBV RNA, 8 had detectable HBV DNA by Hologic
- In these 8 patients, HBV DNA was median 3.7 log_{10} IU/ml (IQR 3.2, 4.5) by Hologic and 3.1 log_{10} IU/ml (IQR 2.6, 3.4) by real-time PCR

Implications

- In chronic hepatitis B, circulating HBV DNA levels provide a direct measure of the efficacy of antiviral therapy
- In NUC treated patients, high transcriptional activity of cccDNA leads to excess production and export of HBV RNA
- HBV DNA load assays that incorporate a reverse transcription step can amplify HBV RNA and report it as a HBV DNA signal
- The HBV DNA overestimation is median 0.8 $\log_{10}$ IU/mL
- Results may mistakenly suggest lack of HBV DNA suppression in a treated patient, leading to unnecessary clinic visits, tests and treatment changes, and causing distress for the patient

HBV RNA in HBV cure trials

- **Eligibility criteria** commonly target NUC-treated patients with evidence of stably suppressed HBV DNA based on local testing (confirmed at screening at central lab)
  - *False positive HBV DNA detection may lead to unnecessary exclusion*

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- About **40-50%** of NUC treated patients have quantifiable HBV RNA
  - *In house tests suffer from limited sensitivity, although this is likely to improve*
- Lack of commercially available test, but evolving landscape
  - *Development also influenced by likely use in routine practice (e.g., decisions on NUC discontinuation)*

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- HBV RNA included as exploratory pharmacodynamic (PD) end-point in trials
  - *Endorsed by the FDA as promising marker of cccDNA silencing*
- About 40-50% of NUC treated patients have quantifiable HBV RNA at baseline
  - *In house tests suffer from limited sensitivity, although this is likely to improve*
- Lack of commercially available test, but evolving landscape
  - *Development also influenced by likely use in routine practice (e.g., decisions on NUC discontinuation)*
- Need for improved characterisation of **factors associated with HBV RNA circulation**


Thank you