Biomarkers of HBV transcriptional activity - HBcrAg and pre-genomic HBV RNA during antiviral therapy with nucleos(t)ide analogue help to predict optimal timing of therapy withdrawal

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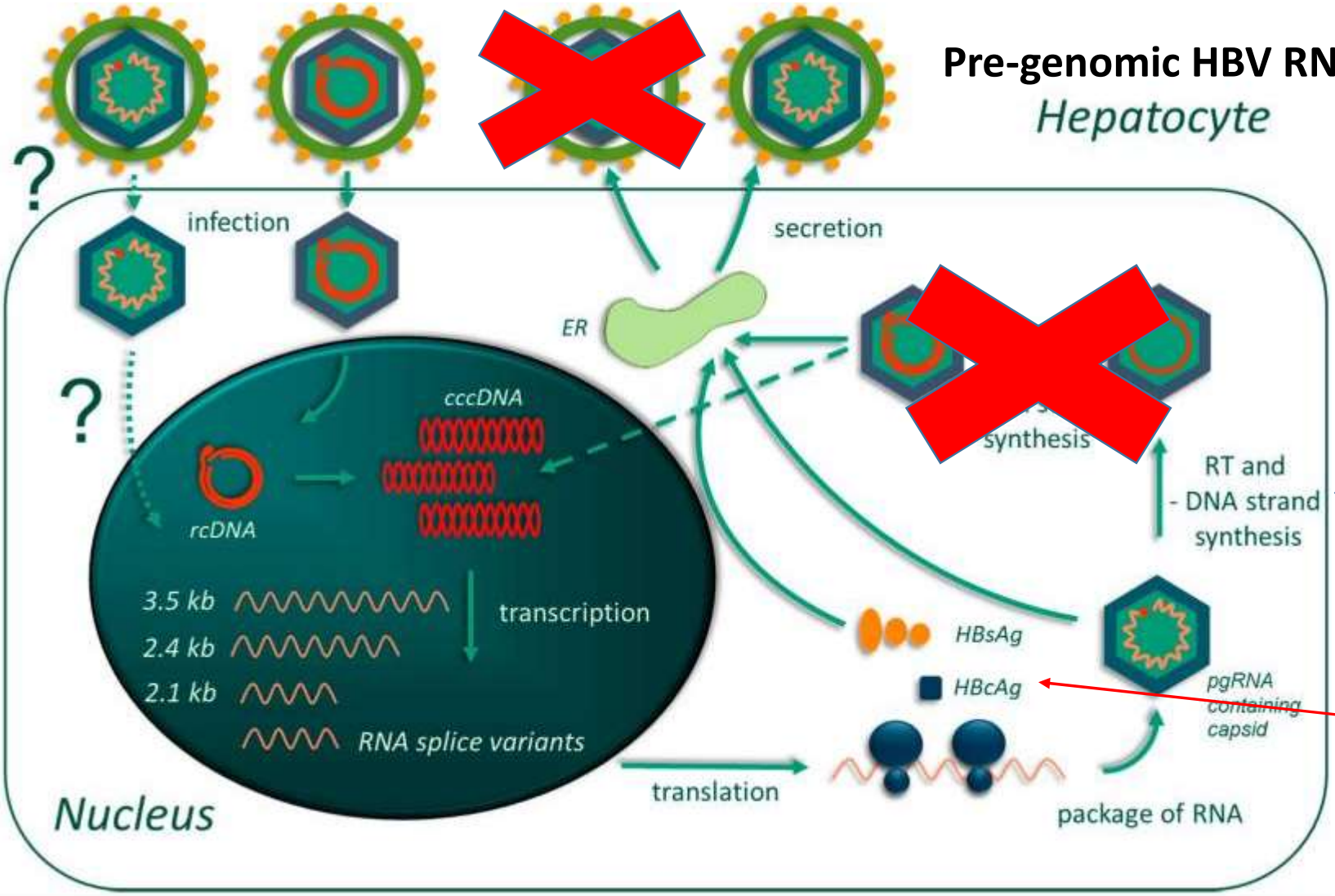
Non-invasive biomarkers of cccDNA transcription



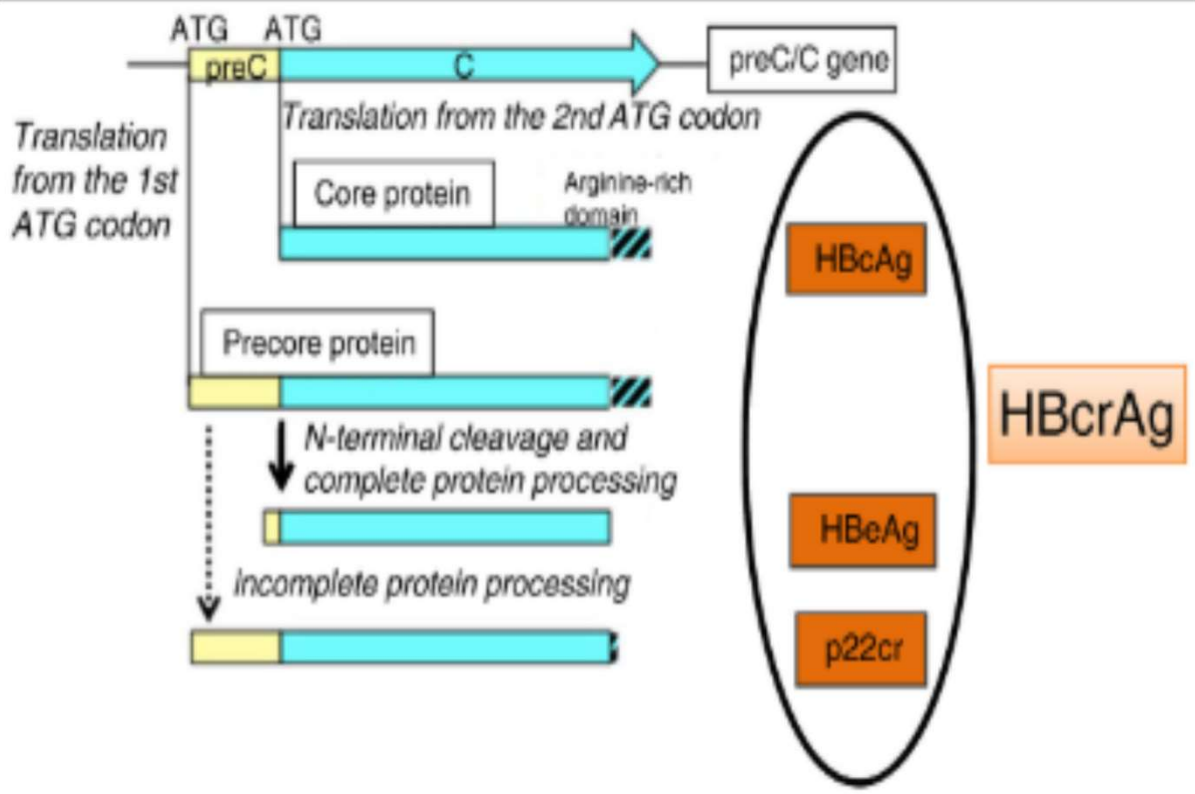
Pre-genomic HBV RNA is still produced

Nucleos(t)ide analogue

<u>Viral proteins are still produced:</u>
HBcAg, HBeAg, HBsAg

Kostyusheva A et al Genes 2018

Hepatitis B core related antigen
Non-invasive biomarker of cccDNA transcription



Hepatitis B core-related antigen (HBcrAg)

3 related HBV proteins sharing 149 amino acids:

- **HBcAg**
- **HBeAg**
- **p22Cr - a truncated 22kDa precore protein**

Mak L-Yet al Aliment Pharmacol Ter. 2018

3 different cohorts aims

To evaluate:

- The concentrations of **pg HBV RNA** and **HBcrAg** in 3 selected cohorts of patients with chronic hepatitis B
- the utility of these markers to predict clinical outcomes (ALT flares and HBV DNA re-activation) after NA withdrawal

Cohort A

What predicts high ALT flares after stopping NA?

Nucleos(t)ide analogue therapy withdrawal

- Nucleos(t)ide analogue (NA) suppresses HBV DNA replication, but does not provide complete cure due to minimal impact on cccDNA transcriptional activity
- Withdrawal of long-term NA therapy is possible in non-cirrhotic patients, but it is not clear who would be **a good candidate** for this approach at time of stopping NA
- Traditional markers, HBeAg negative status, low baseline HBV DNA and HBsAg decline >1 $\log_{10}$IU/ml during NA therapy were associated with successful NA withdrawal
- New biomarkers of cccDNA transcriptional activity (**HBcrAg** and **pre-genomic HBV RNA**) are still detected in patients with fully suppressed HBV DNA and might be helpful in identifying **good candidates** for NA withdrawal approach

Aims

To compare:

- the concentrations of **HBV DNA**, **HBsAg**, **HBcrAg** and **pre-genomic (pg) HBV RNA** during therapy in patients with chronic hepatitis B treated long-term with nucleos(t)ide analogue before stopping NA therapy

To evaluate:

- whether on-treatment markers of cccDNA transcriptional activity can help with selecting good candidates and timing of successful NA withdrawal

Patients

25 patients with long-term suppressed HBV DNA (for at least 3 years) and stopped NA (median follow up 52 weeks)

Baseline characteristics at initiation of NA therapy

	All cohort (n=25)
Male patients (#, %)	n= **16** (64%)
HBeAg positive patients (#, %)	n= **2** (8%)
Median fibrosis stage by Ishak (range)	**3** (2-3)
Median age (range)	**48** years (24 - 66)
Median baseline HBV DNA (IQR; range)	**4.6** $\log_{10}$ IU/ml (1.2; 2.5 - 8.8)
Median baseline HBsAg (IQR; range)	**3.81** $\log_{10}$ IU/ml (0.8; 2.4 – 4.9)
Median baseline ALT activity (IQR; range)	**32** IU/L (26; 17 – 256)
HBV genotypes distribution • **A** • **B** • **C** • **D** • **E**	# of patients (%) n= **5** (20%) n= **3** (12%) n= **1** (4%) n= **5** (20%) n= **11** (44%)
Duration of therapy at time of withdrawal (IQR; range)	**84** months (52; 38-118)
Type of therapy - TDF vs. ETV (# of patients)	**21: 4**

Serum concentrations of the following markers were analysed:

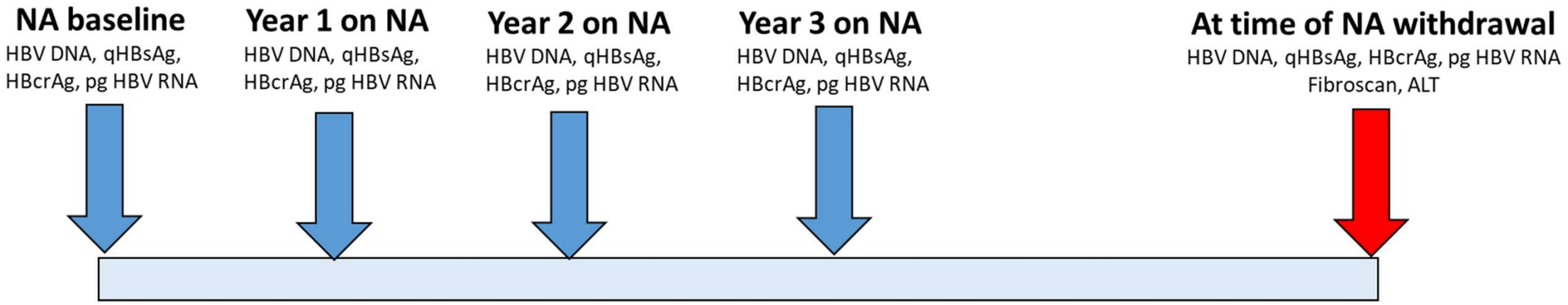
During therapy on-treatment assessments

- **HBV DNA** (IU/mL) using COBAS AmpliPrep/TaqMan real-time PCR assay (Roche);
- **Quantitative HBsAg** (IU/mL) using Abbott ARCHITECT chemiluminescent microparticle immunoassay (Abbott);

Retrospective analysis

- **HBcrAg** (U/mL) using Lumipulse G HBcrAg chemiluminescent enzyme immunoassay (Fujirebio);
- **Pre-genomic (pg) HBV RNA** (U/mL) by a novel dual-target real-time PCR research assay (Abbott Diagnostics) as reported by Butler et al

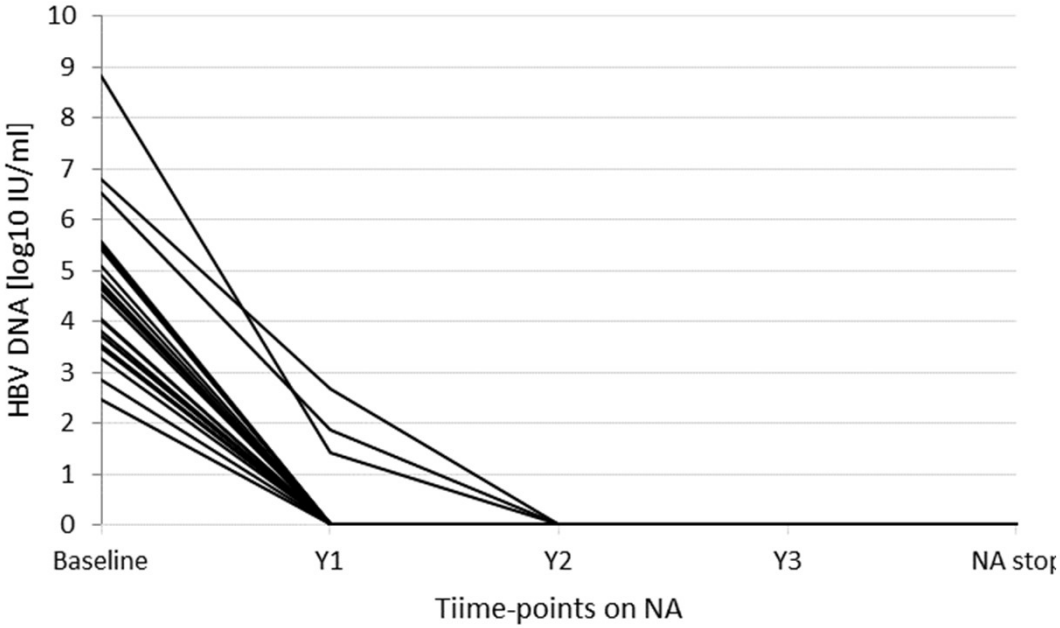
Serum concentrations of HBV biomarkers were analysed at the following time-points:



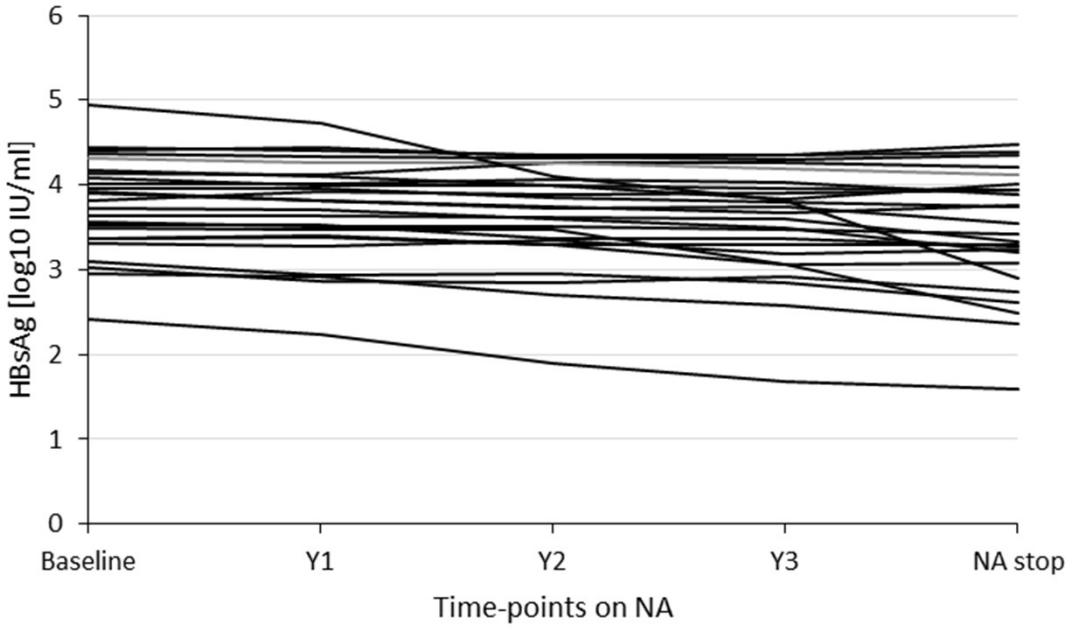
Median duration of therapy: 84 months (range 38 -118)

Changes in traditional on-treatment markers

HBV DNA

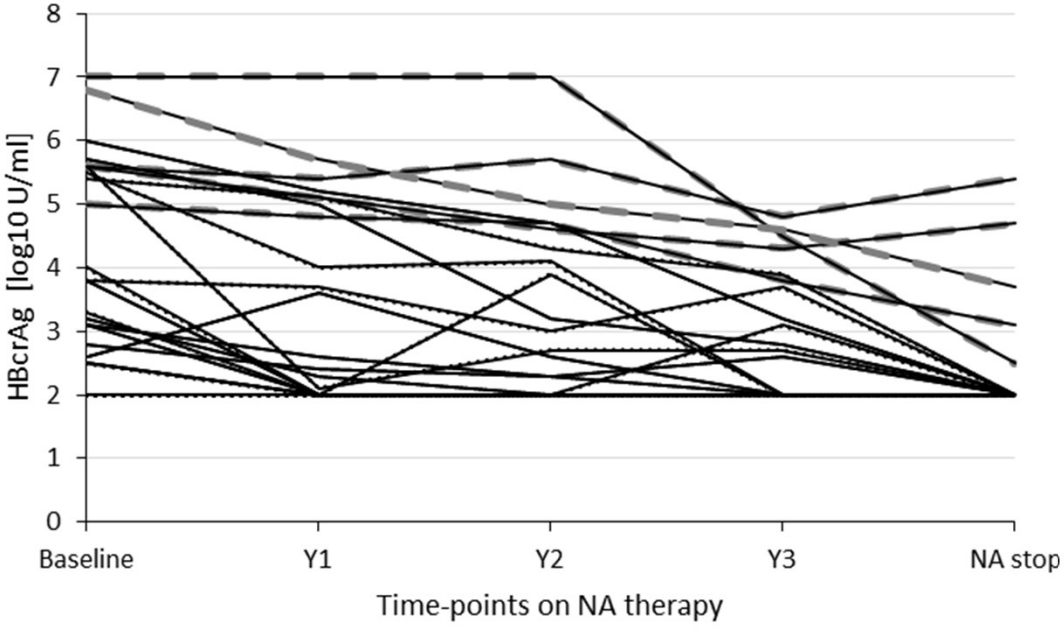


HBsAg

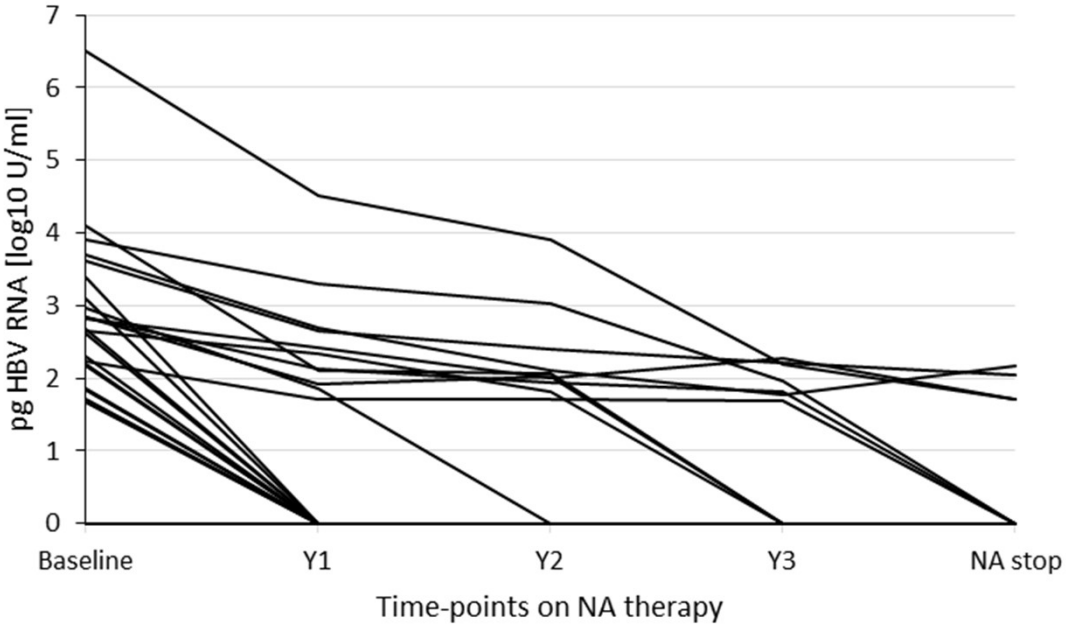


Changes in on-treatment cccDNA transcription markers

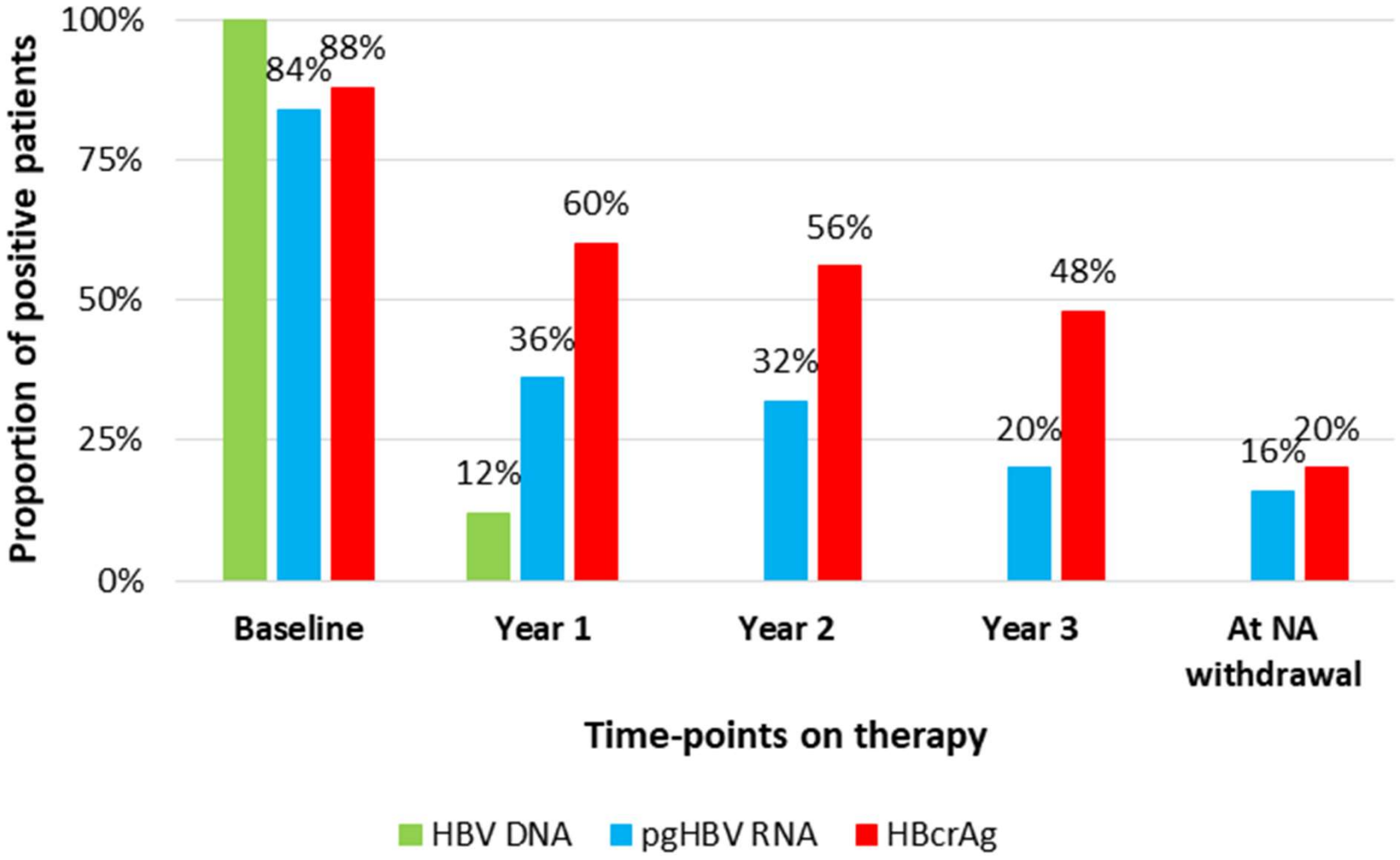
HBcrAg



Pre-genomic HBV RNA



Proportion of positive patients on therapy



Patients

25 patients with long-term suppressed HBV DNA (for at least 3 years) and stopped NA (median follow up 52 weeks)

ALT and HBV DNA concentrations after NA withdrawal

ALT flares after NA withdrawal:

- **No/minimal flare (< 2 UNL ALT)** – 9 patients (36%)
- **Mild flare (>2 < 5 UNL ALT)** – 11 patients (44%)
- **Severe flare (> 10 UNL ALT)** – 5 patients (20%)

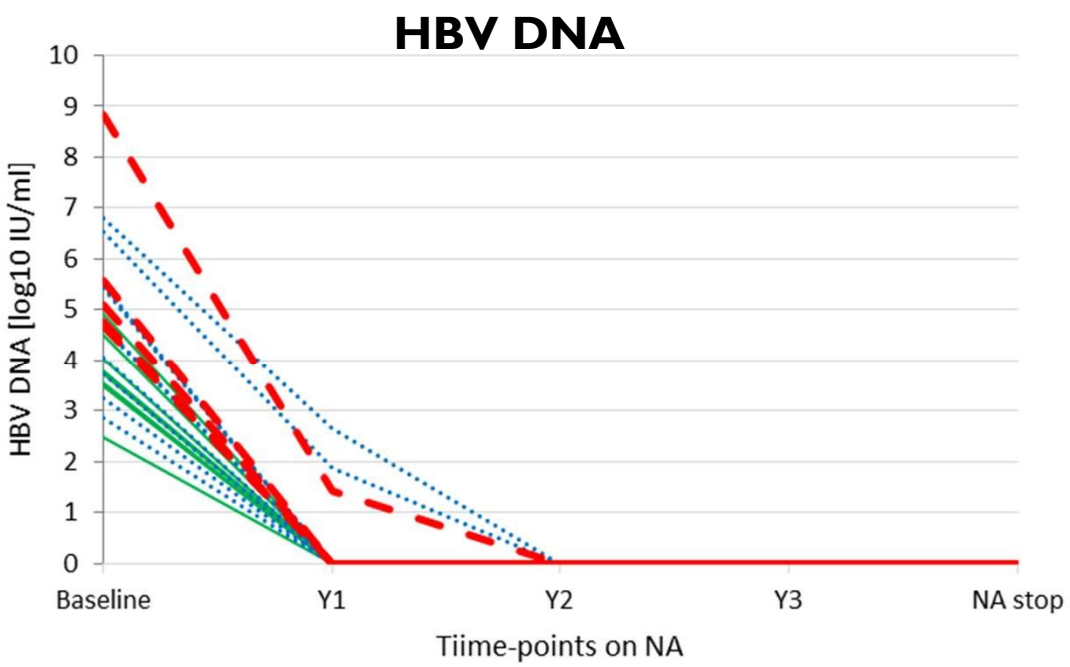
Patients

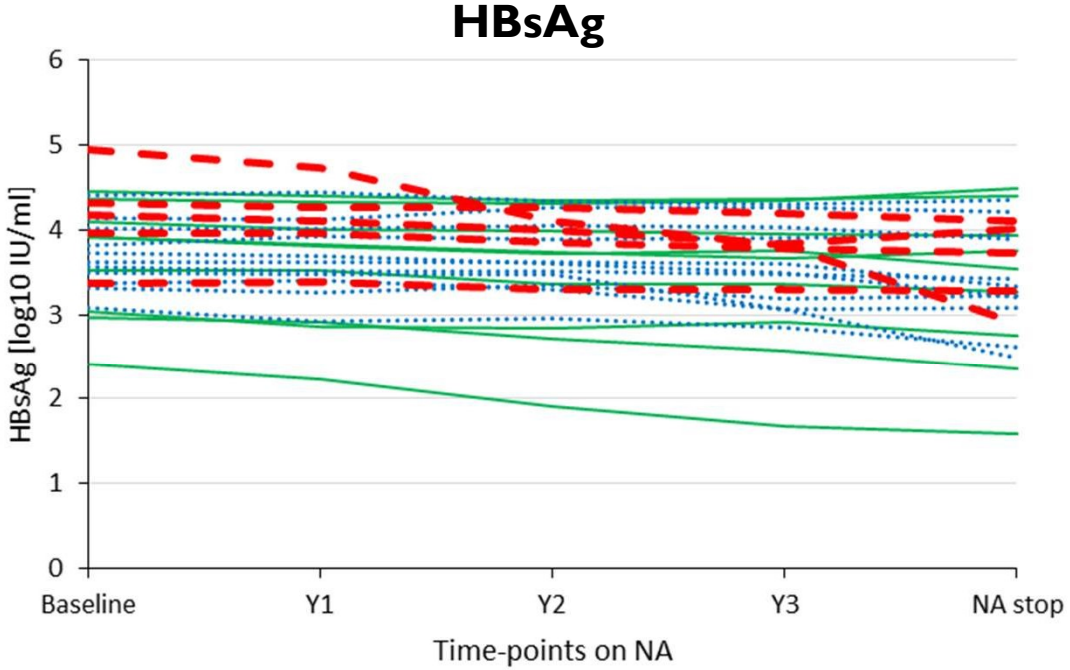
25 patients with long-term suppressed HBV DNA (for at least 3 years) and stopped NA (median follow up 52 weeks)

NA treatment baseline characteristics

	No flare (n=9)	Mild flare (n=11)	Severe flare (n=5)	
Male patients (#, %)	n= **4** (44%)	n= **7** (64%)	n= **5** (100%)	**p<0.01**
Median fibrosis stage by Ishak (range)	**3** (2-3)	**3** (2-3)	**3** (2-3)	
Median age (range)	**49** years (34 - 64)	**47** years (31 - 57)	**51** years (24 - 66)	
Median baseline HBV DNA (IQR; range)	**3.73** $\log_{10}$ IU/ml (0.8; 2.5 - 4.9)	**4.67** $\log_{10}$ IU/ml (1.8; 2.9 – 6.8)	**5.1** $\log_{10}$ IU/ml (2.5; 4.7 – 8.8)	**p<0.01**
Median baseline HBsAg (IQR; range)	**3.91** $\log_{10}$ IU/ml (1.2; 2.4 - 4.4)	**3.63** $\log_{10}$ IU/ml (0.7; 3.1 - 4.4)	**4.17** $\log_{10}$ IU/ml (1.0; 3.4 - 4.9)	
Median baseline HBcrAg (IQR; range)	**2.8** $\log_{10}$ U/ml (1.3; 2.0 – 6.0)	**3.8** $\log_{10}$ U/ml (3.0; 2.0- 5.7)	**5.6** $\log_{10}$ U/ml (1.6; 5.1 – 7.0)	**p<0.01**
Median baseline pg HBV RNA (IQR; range)	**2.19** $\log_{10}$ U/ml (0.9; 0 - 3.9)	**2.67** $\log_{10}$ U/ml (1.44; 0 - 4.1)	**3.6** $\log_{10}$ U/ml (2.6; 2.2 – 6.5)	**p<0.01**
Median baseline ALT activity (IQR; range)	**21** IU/L (20; 17 - 62)	**44** IU/L (30; 20 - 256)	**61** IU/L (44; 25 - 83)	**p<0.05**
Duration of therapy at time of withdrawal (IQR; range)	**55** months (42; 38-114)	**100** months (46; 58-118)	**72** months (49; 54-110)	

On-treatment HBV DNA and HBsAg according to ALT flares post NA withdrawal

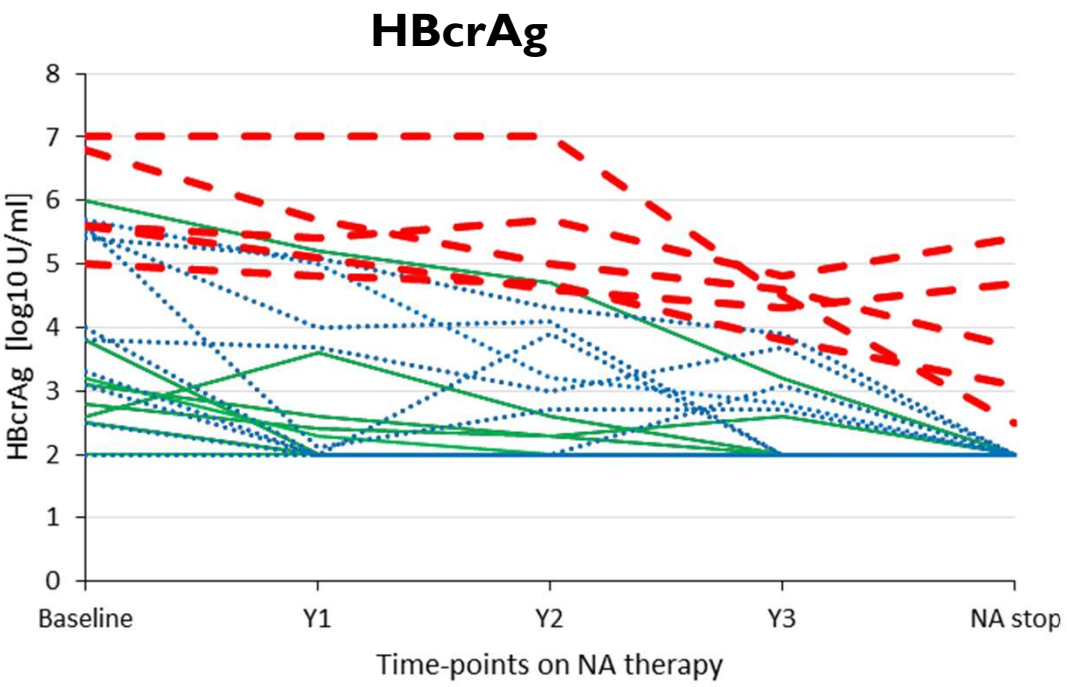




	Baseline	Year1	Year 2	Year 3	NA stop
No flare (n=9)	100%	0%	0%	0%	0%
Mild flare (n=11)	100%	18%	0%	0%	0%
Severe flare (n=5)	100%	20%	0%	0%	0%

	Baseline	Year1	Year 2	Year 3	NA stop
No flare (n=9)	100%	100%	100%	100%	100%
Mild flare (n=11)	100%	100%	100%	100%	100%
Severe flare (n=5)	100%	100%	100%	100%	100%

On-therapy HBcrAg and pg HBV RNA

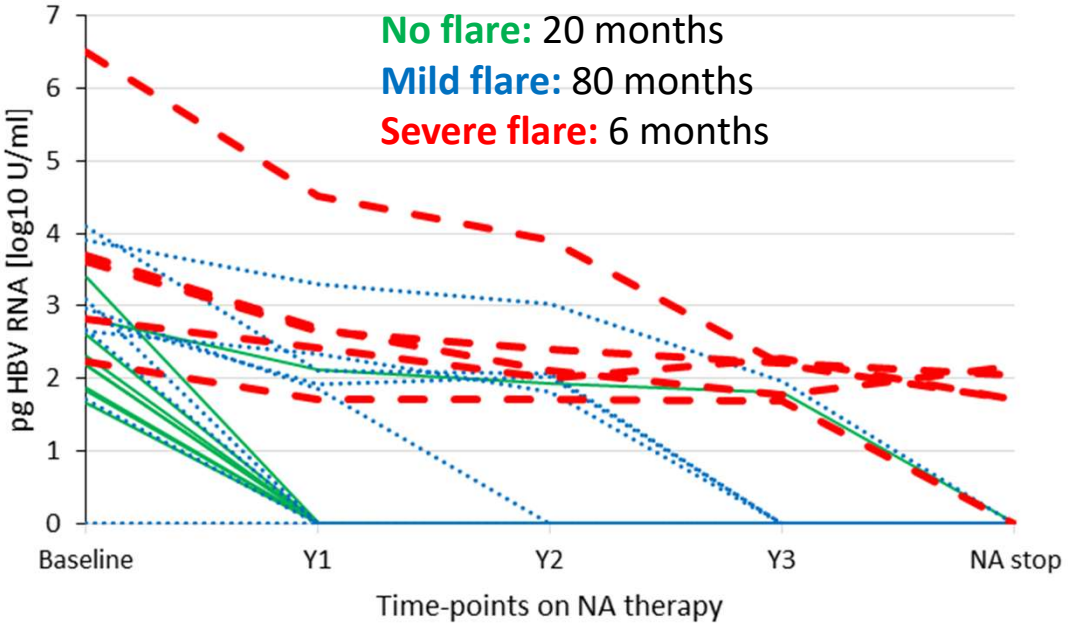


Time between last detected pg RNA and NA withdrawal

No flare: 20 months

Mild flare: 80 months

Severe flare: 6 months

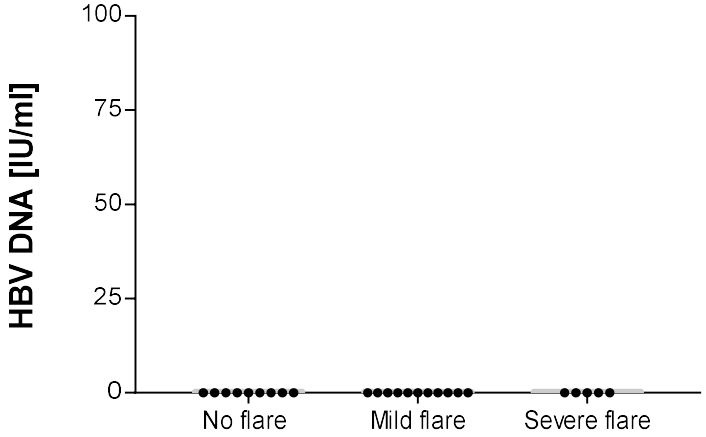


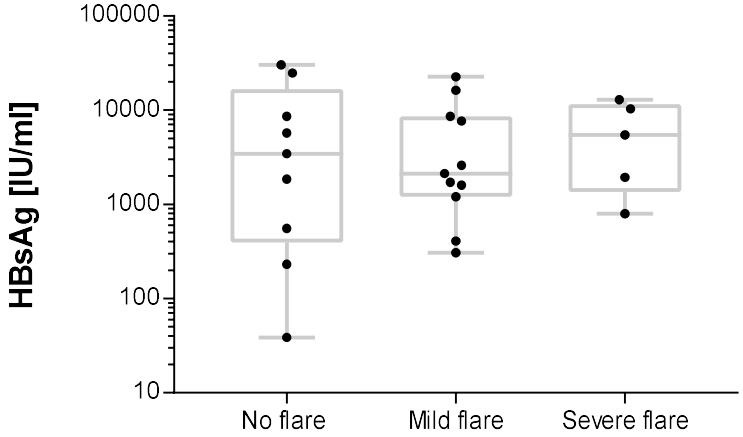
	Baseline	Year1	Year 2	Year 3	NA stop
No flare (n=9)	78%	56%	44%	22%	0%
Mild flare (n=11)	82%	45%	45%	36%	0%
Severe flare (n=5)	100%	100%	100%	100%	**100%**

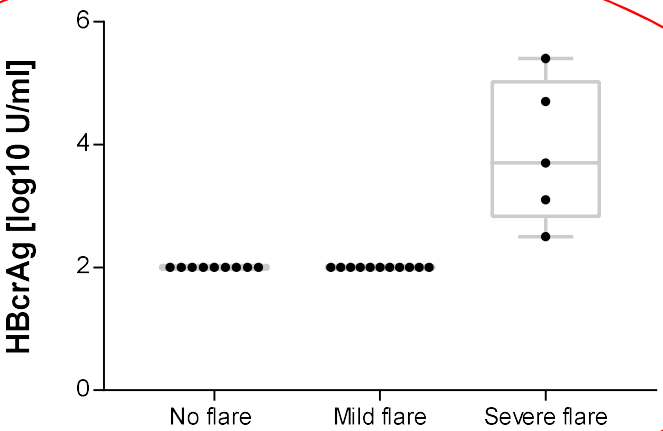
	Baseline	Year1	Year 2	Year 3	NA stop
No flare (n=9)	100%	11%	11%	11%	0%
Mild flare (n=11)	82%	45%	36%	9%	0%
Severe flare (n=5)	100%	100%	100%	100%	**80%**

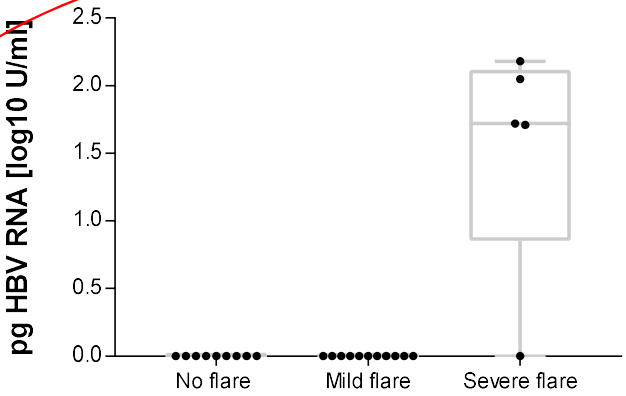
At time of NA withdrawal

Difference in HBV biomarkers at time of NA withdrawal according to flare









Conclusions – Cohort A

- Serum **HBcrAg** and **pg HBV RNA** appear to be sensitive biomarkers of continued cccDNA transcription in CHB patients despite inhibition of DNA synthesis during NA therapy.
- These markers, at time of NA withdrawal were strong predictors for severe ALT flares and might help with a timing of NA withdrawal.

Cohort B

Proportions of patients with still detected pg HBV RNA during NA therapy?

Aims for cohort B

To evaluate:

- the proportion of patients with still **detected** **HBcrAg** and **pre-genomic (pg) HBV RNA** during therapy in patients with chronic hepatitis B treated long-term with nucleos(t)ide analogue (NA)

Serum concentrations of the following markers were analysed:

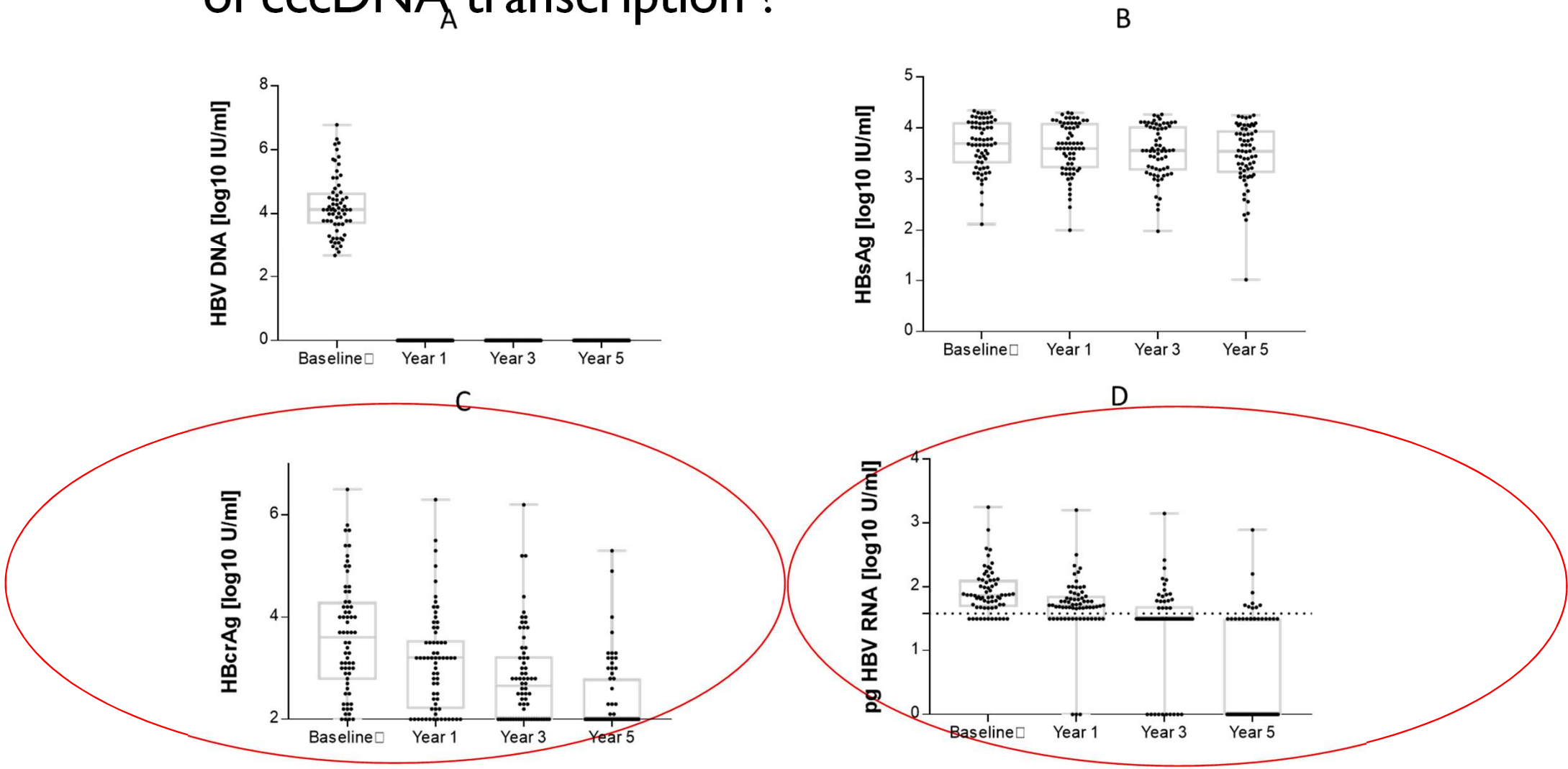
- **HBV DNA** (IU/mL) using COBAS AmpliPrep/TaqMan real-time PCR assay (Roche);
- **Quantitative HBsAg** (IU/mL) using Abbott ARCHITECT chemiluminescent microparticle immunoassay (Abbott);
- **HBcrAg** (U/mL) using Lumipulse G HBcrAg chemiluminescent enzyme immunoassay (Fujirebio);
- **Pre-genomic HBV RNA** (U/mL) by a novel dual-target real-time PCR research assay (Abbott Diagnostics) as reported by Butler et al

Cohort B

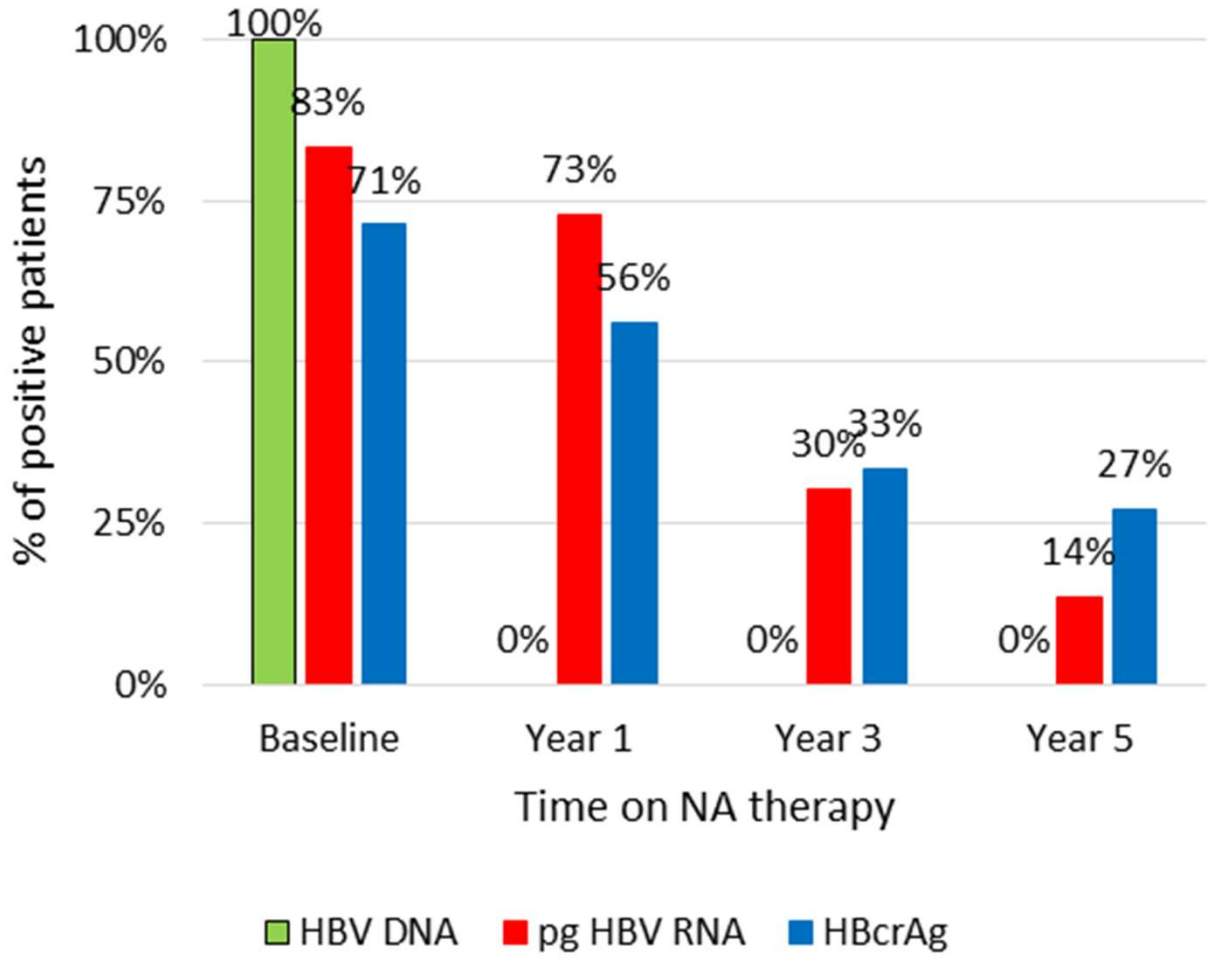
66 HBV DNA negative patients on long-term suppressive therapy with nucleoside analogue (NA), all HBeAg negative

Male patients (#, %)	n= **48** (73%)
Median fibrosis stage by Ishak (range)	**3** (2-3)
Median age (range)	**45** years (19 - 67)
Median baseline HBV DNA (IQR; range)	**4.12** $\log_{10}$ IU/ml (1.0; 2.67 - 6.78)
Median baseline HBsAg (IQR; range)	**3.69** $\log_{10}$ IU/ml (0.81; 2.12 - 4.34)
Median baseline HBcrAg (IQR; range)	**3.6** $\log_{10}$ U/ml (1.53; 2.0 – 6.5)
Median baseline pg HBV RNA (IQR; range)	**1.87** $\log_{10}$ U/ml (0.43; 1.65 – 3.25)
Median baseline ALT activity (IQR; range)	**45** IU/L (20; 33 – 212)
HBV genotypes distribution • A • B • C • D • E	**# of patients (%)** n= **12** (18%) n= **2** (3%) n= **10** (15%) n= **10** (15%) n= **32** (49%)

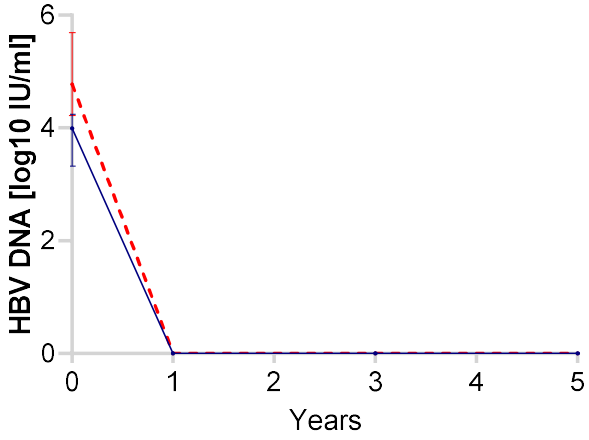
Cohort B: Dichotomy between HBV DNA suppression and markers of cccDNA transcription ?

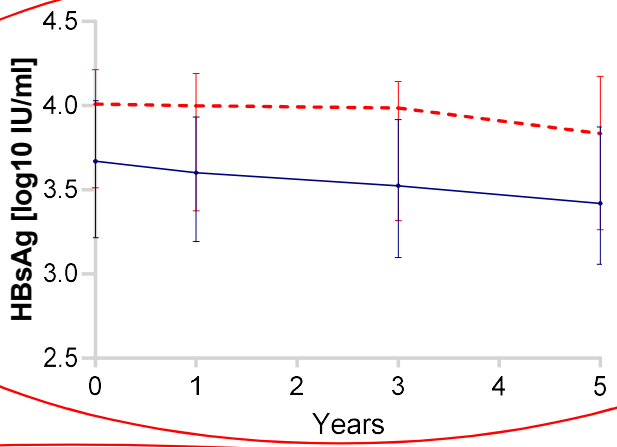


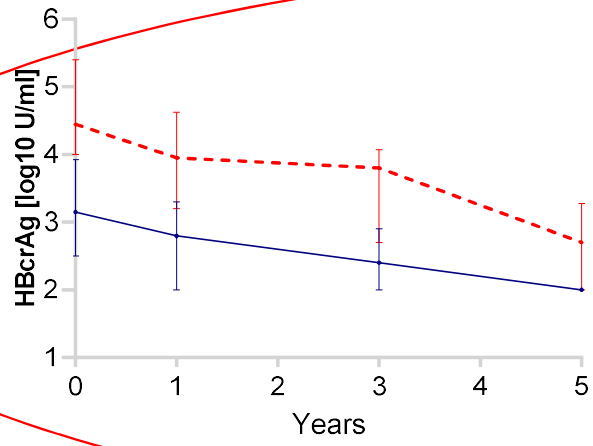
Cohort B: Dichotomy between HBV DNA suppression and markers of cccDNA transcription ?

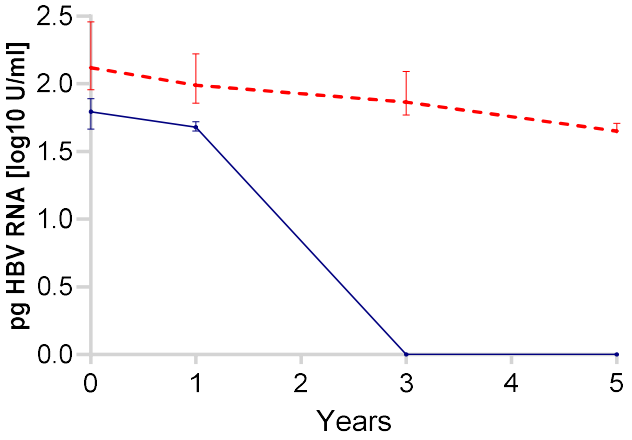


Cohort B: Baseline differences between patients pg HBV RNA detected vs. not detected after 3 years on NA?









pg HBV RNA not detected

pg HBV RNA detected

Conclusions – Cohort B

Despite long-term full suppression of HBV DNA during NA therapy about third patients had still **detectable** pre-genomic HBV RNA after 3 years of therapy and 14% patients after 5 years of therapy reflecting still active transcriptional activity of cccDNA suggesting that longer duration of therapy is required in some patients prior to considering NA withdrawal

Cohort C

The risk of HBV re-activation in chronic HBV patients treated with NA who achieved **HBsAg loss** - when we can stop the therapy?

Aims for cohort C

To assess:

- the role of ultra-sensitive **HBsAg** assay and new cccDNA transcriptional activity markers (**HBcrAg** and **pre-genomic HBV RNA**) in predicting HBV re-activation in chronic HBV patients who achieved HBsAg loss during antiviral and stopped antiviral therapy

Serum concentrations of the following markers were analysed:

- **HBV DNA** (IU/mL) using COBAS AmpliPrep/TaqMan real-time PCR assay (Roche);
- **Quantitative HBsAg** (IU/mL) using Abbott ARCHITECT chemiluminescent microparticle immunoassay (Abbott);
- **HBcrAg** (U/mL) using Lumipulse G HBcrAg chemiluminescent enzyme immunoassay (Fujirebio);
- **Pre-genomic HBV RNA** (U/mL) by a novel dual-target real-time PCR research assay (Abbott Diagnostics) as reported by Butler et al;
- **Ultrasensitive HBsAg** (mIU/ml) using Lumipulse G HBsAg HQ chemiluminescent enzyme immunoassay (Fujirebio) with the low quantification limit 0.5 mIU/ml

Cohort C

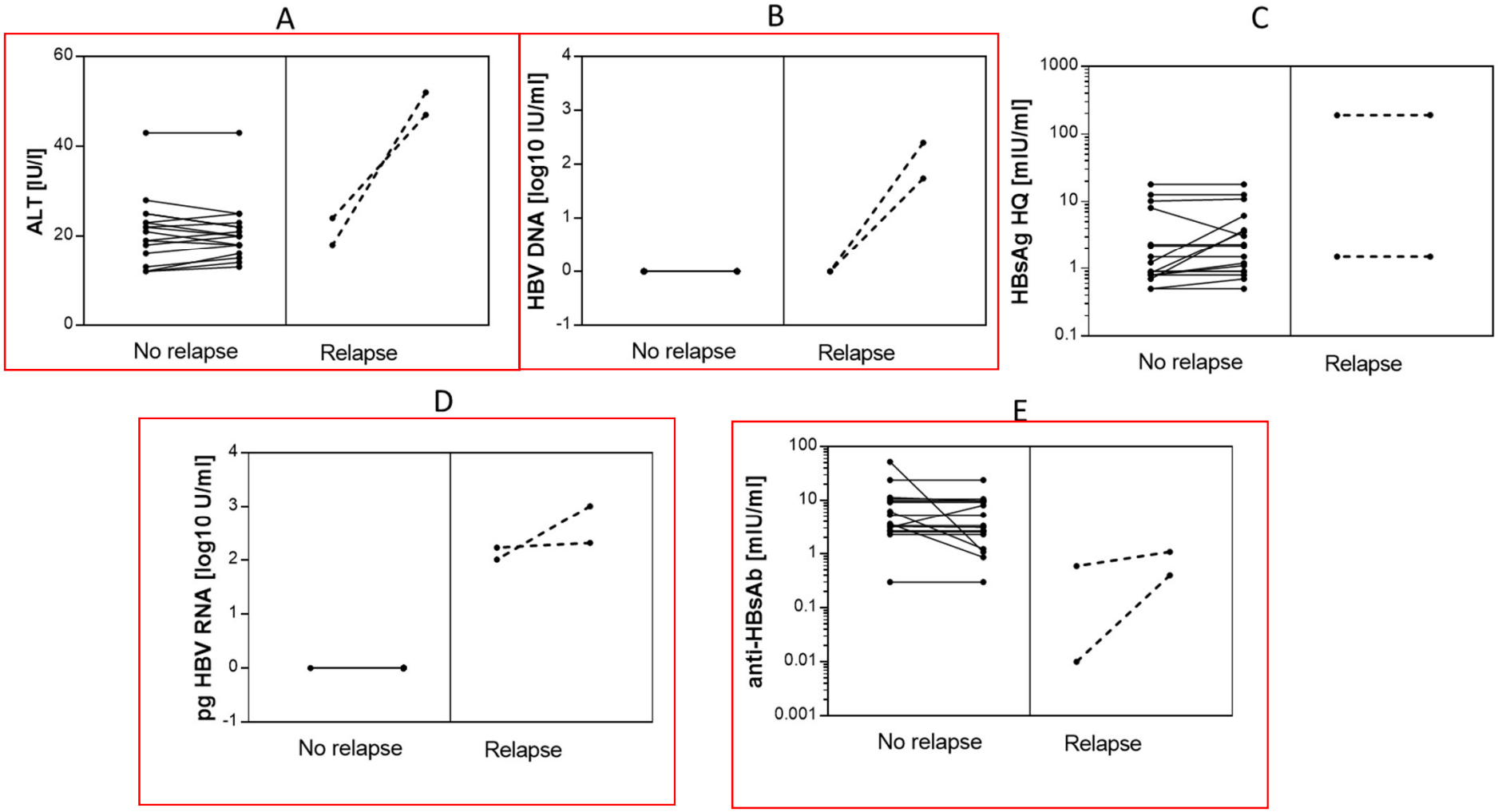
At time of withdrawal

	All cohort (n=19)
Median age (IQR; range)	**36** years (10; 21 - 57)
Median fibrosis stage by Ishak (range)	**2** (2-4)
Duration of therapy at time of withdrawal (IQR; range)	**8.58** years (3.5; 1.6 – 12.2)
Median duration on therapy since HBsAg loss (IQR; range)	**1.76** years (0.6; 0.5 – 3.8)
Median HBV DNA at time of withdrawal (IQR; range)	**0** $\log_{10}$ IU/ml (0; 0)
Median HBsAg HQ at time of withdrawal (IQR; range)	**1.23** mIU/ml (7.3; 0.5 - 190)
Median HBcrAg at time of withdrawal (IQR; range)	**0** $\log_{10}$ U/ml (0; 0)
Median pg HBV RNA at withdrawal (IQR; range	**0** $\log_{10}$ U/ml (0; 0 – 2.2)
Median anti-HBsAb at time of withdrawal (IQR; range)	**3.32** mIU/ml (8; 0 - 53)
Median ALT activity at time of withdrawal (IQR; range)	**21** IU/L (8; 12 - 43)

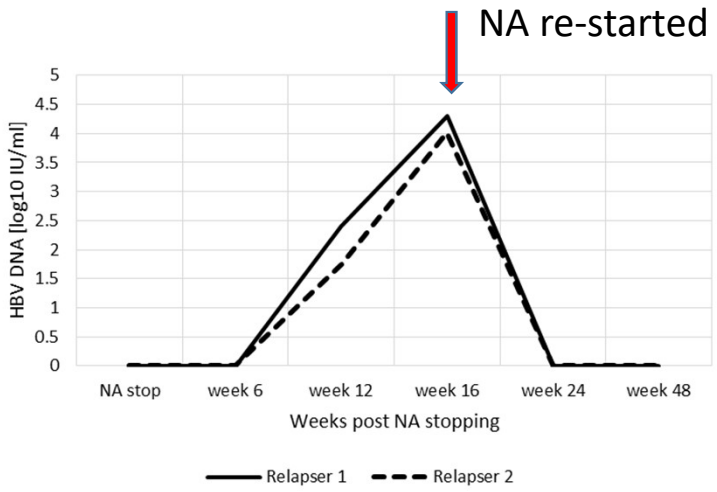
HBV DNA reactivation after NA withdrawal

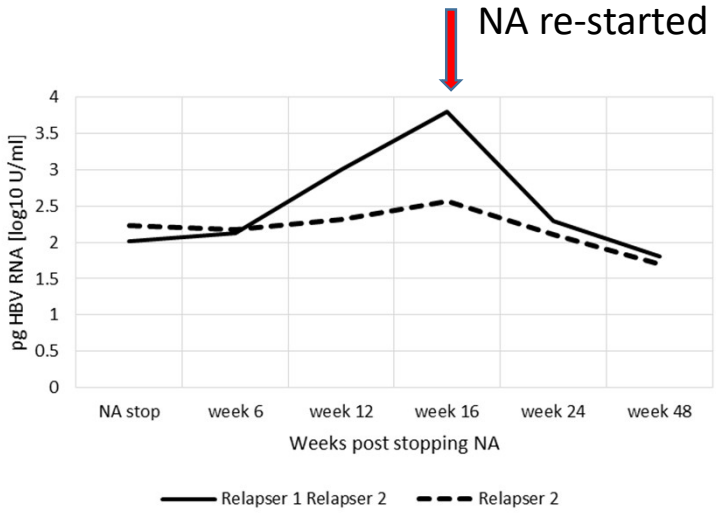
- **No relapse**– 17 patients
- **HBV DNA reactivation** – 2 patients

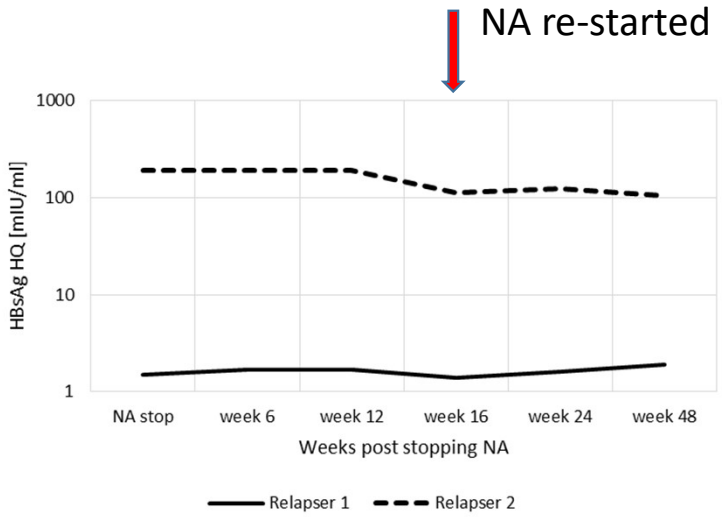
Cohort C: Difference in markers after withdrawal (no relapse vs. relapse)

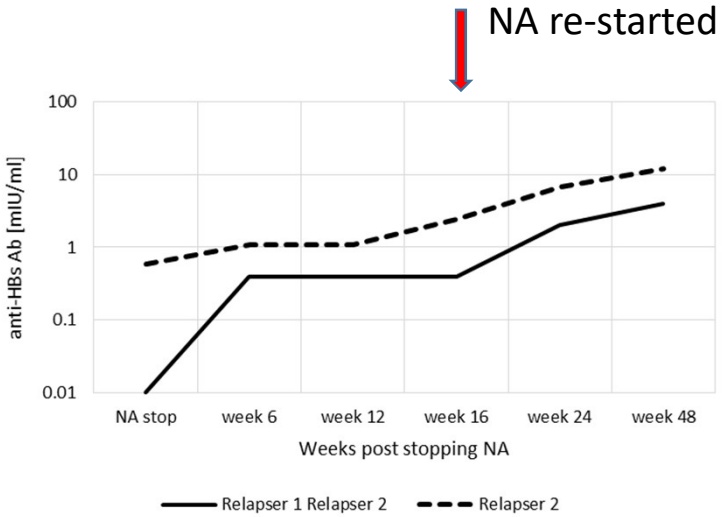


Cohort C: Therapy response after re-activation – changes in markers









Conclusions – Cohort C

Pre-genomic HBV RNA was exclusively detected only in the patients with HBV re-activation after achieving HBsAg loss

Ultra-sensitive HBsAg was not helpful into predicting HBV re-activation

Summary

- Serum HBcrAg and pg HBV RNA appear to be sensitive biomarkers of continued cccDNA transcription in CHB patients despite inhibition of DNA synthesis during NA therapy.
- These markers, at time of NA withdrawal were strong predictors for severe ALT flares and HBV DNA reactivation (post HBsAg loss).
- These biomarkers may reflect differential intra-cellular pathways between HBV replication and transcription.

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