

Low expression of circulating liver-enriched miRNAs in anti-HDAg patients in absence of active viral replication.

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Background and Aims

- Only a limited percentage of patients with hepatitis delta virus (HDV) infection can achieve spontaneous or treatment-related control of viral replication (undetectable viral RNA).
- The mechanism behind this virological control is still unclear.
- The microRNAs (miRNAs) are small RNAs that participate in several biological processes, including the host response to viral infections.
- AIM: To analyze the miRNA profiles of HDV-infected patients to identify potential biomarkers that differentiate virological controllers from non-controllers.

Cohorts of the study

- Explorative cohort:** 30 HDV patients (anti-HDAg positive, 30 samples in total, **Table 1**) in presence or absence of detectable HDV RNA (at least two consecutive samples).

	HDV RNA	
	Undetectable (n=15)	Detectable (n=15)
N male (%)	9 (60)	9 (60)
Age at sample (median[Q1;Q3])	54 [47.5; 57]	45 [33.5; 52]
N detectable HBV DNA (%)	4 (26.7%)	2 (13.3%)
N treatment at sampling (%)	2 (13.3%)	2 (13.3%)
Previous treatment (%)	5 (30%)	5 (30%)
Log10 HBsAg (median[Q1;Q3])	3.0† [0.1; 3.4]	4.0 [3.6; 4.2]
ALT (median[Q1;Q3])	31 [21.5; 39.5]	64 [50; 118.7]††

Table 1. Main clinical and demographic characteristics of the patients included in the explorative cohort. † Three patients showed undetectable HBsAg; †† 1 missing data.

- Validation cohort:** 18 untreated HDV patients (**Table 2**) grouped based on their HDV RNA or ALT levels in:

- A. Undetectable HDV RNA
- B. Detectable HDV RNA and normal ALT (<50 U/L)
- C. Chronic hepatitis delta

For each patient, three samples were collected at intervals of 0.5-1yr and then analyzed.

For group A, the T0 was the last available sample with detectable HDV RNA.

A group of 8 healthy donors (HD) was used as control.

	A (n=3)	B (n=6)	C (n=9)
Log ₁₀ HDV RNA (median[Q1;Q3])			
T0	2.78 [2.8; 3.3]	3.9 [3.7; 4.4]	4.9 [4.3; 5.2]
T1	0 [0; 0]	3.9 [3.3; 4.5]	4.7 [3.8; 5.3]
T2	0 [0; 0]	3.5 [3.2; 3.7]	4.7 [3.5; 5.2]
Log ₁₀ HBsAg (median[Q1;Q3])			
T0	2.9 [2.6; 3.2]	4.0 [3.7; 4.0]	4.0 [3.9; 4.3]
T1	3.5 [2.9; 3.8]	4.0 [3.7; 4.1]	3.6 [3.4; 4.0]
T2	3.4 [2.9; 3.7]	4.0 [3.6; 4.1]	3.8 [3.4; 4.2]
ALT (median[Q1;Q3])			
T0	58 [56; 191]	40 [34.5; 46.2]	144 [75.2; 188.5]
T1	26 [22.5; 34.5]	40.5 [35; 43.7]	164 [96.5; 204.2]
T2	25 [24.5; 26.5]	40.5 [37.7; 42.5]	128.5 [76; 165.8]
Previously treated (%)	0 (0)	1 (16.7)	4 (44.4)

Table 2. Clinical and virological characteristics of the patients included in the validation cohort. The T0 in A group (undetectable HDV RNA) corresponds to the last available sample with detectable HDV RNA.

Methods

- Circulating miRNAs were isolated from the plasma.

Explorative study:

- Analysis of the isolated miRNAs by microArray (Afflymetrix).
- Identification of the most differently expressed miRNAs by adjusting a linear model with empirical Bayes moderation of the variance.

Validation study:

- Quantification of the most differently expressed miRNAs by digital PCR (Quiacuity, Qiagen).

Results of the Explorative Study

- Several miRNAs were differently expressed between HDV patients with undetectable or detectable HDV RNA (**Figure 1**).
- Of them, 6 miRNAs were particularly down-regulated in the undetectable group (log2FC >1 and raw p-value < 0.05; **Figure 2**) and formed two different patterns of expression (the liver-enriched miR122-5p + miR-194-5p and miR-192-5p; miR26a-5p+ miR-23b-3p; **Figure 3**).

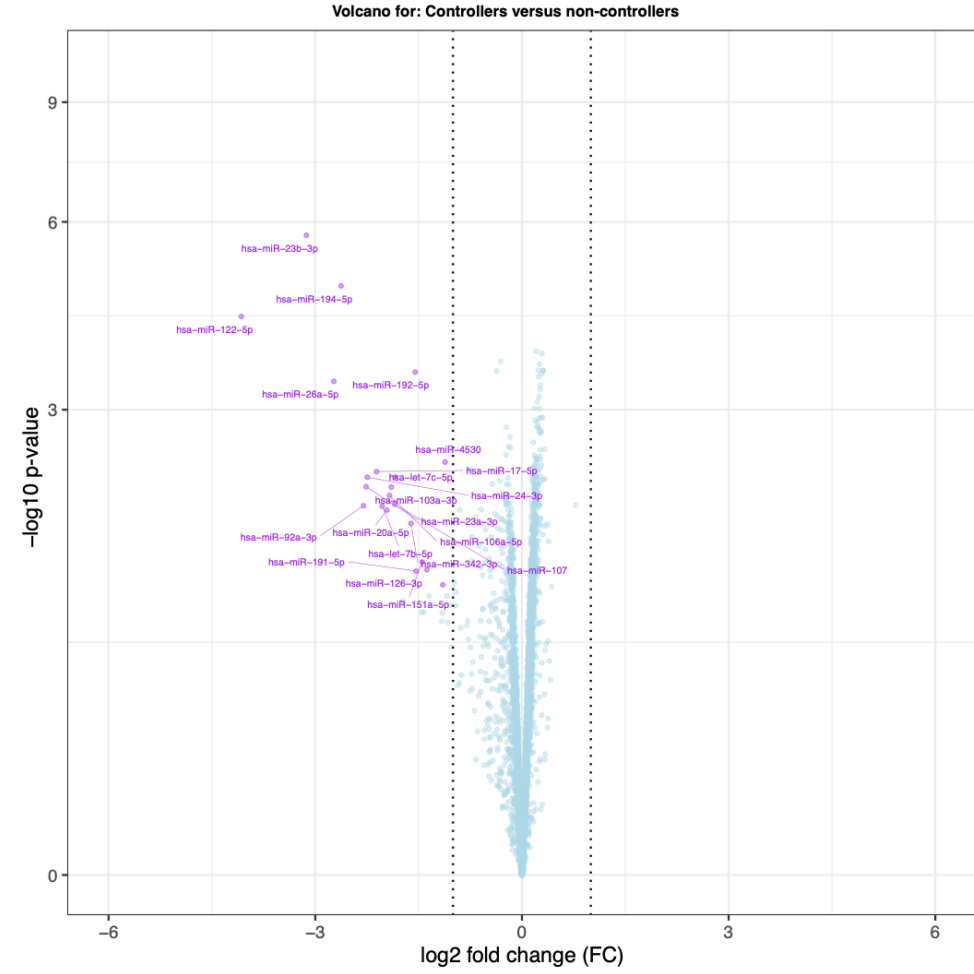


Figure 1. Vulcan Plot showing the first 21 miRNAs differently expressed between the explorative cohort.

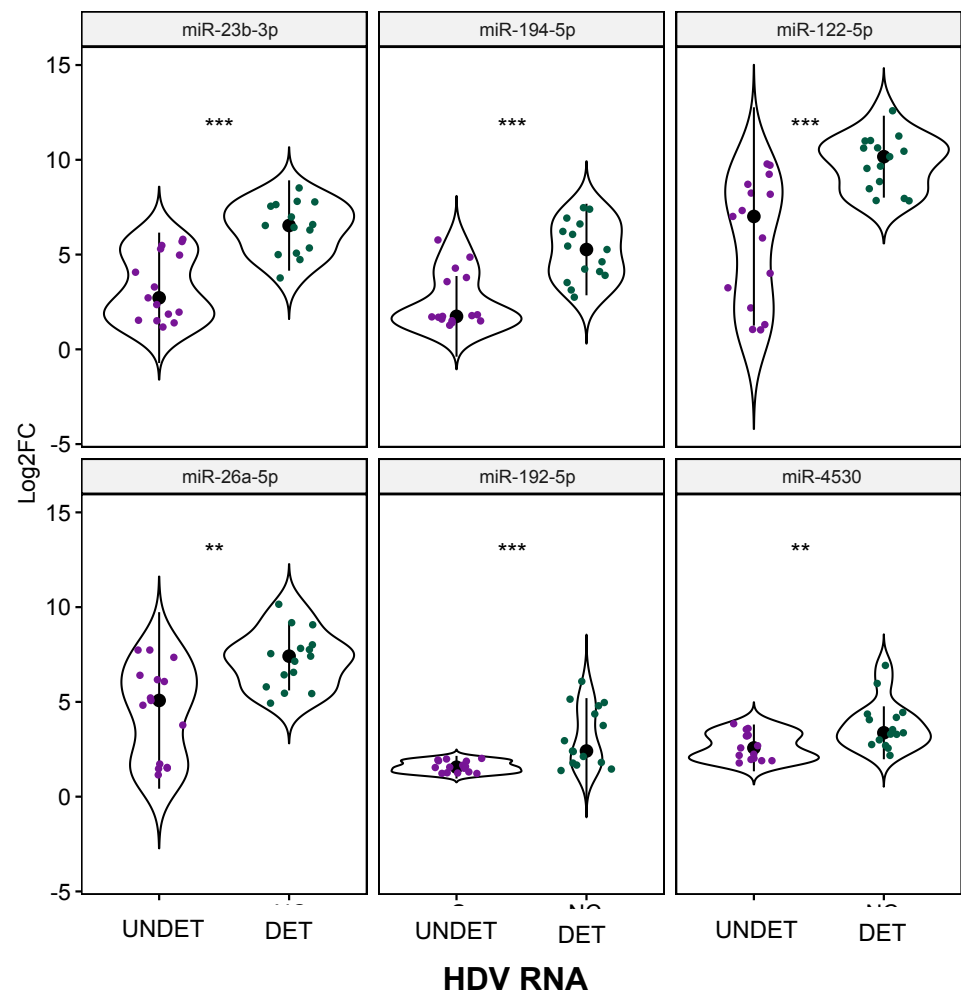


Figure 2. Expression of the 6 most miRNAs between HDV patients with detectable (DET) and undetectable (UNDET) HDV RNA.

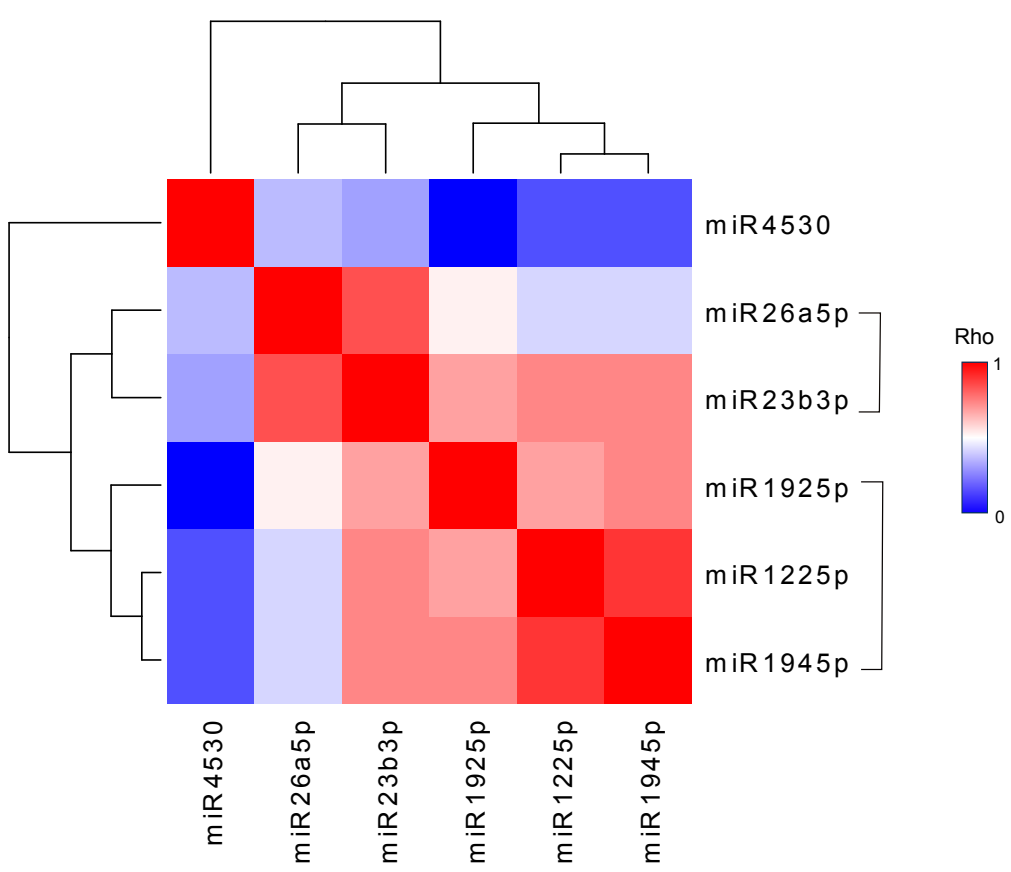


Figure 3. Correlation between the 6 miRNAs and their hierarchical relationship. The gradient color shows the spearman rho value.

Results of the validation study

- The 3 liver-enriched miRNAs tested in the validation cohort were down-regulated in group A (undetectable HDV RNA; **Figure 4**) even when HDV RNA was still detectable (T0), especially for the miR-194-5p (with a mean of 38.3-fold when comparing A versus C).
- Patients with detectable HDV RNA and normal ALT (group B) presented an intermediate behavior in all the timepoints.
- The miRNAs positively correlated with the ALT levels (**Figure 5**), especially for the group C (chronic hepatitis delta), that, when considered alone, showed a rho correlation of respectively 0.55, 0.5 and 0.67 (p-values < 0.05).

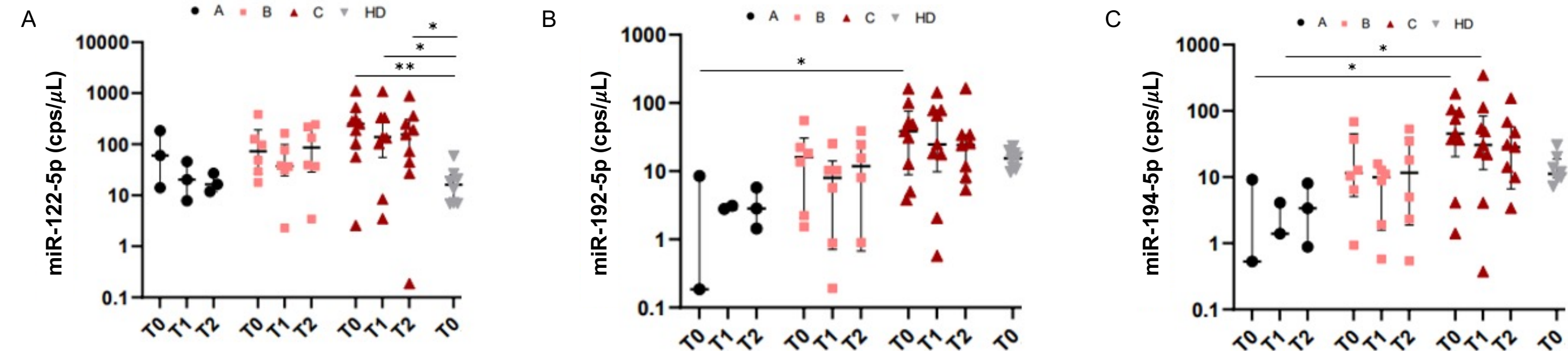


Figure 4. Concentration of the liver-enriched miRNAs in all the samples from the validation cohort: (A) miR-122-5p, (B) miR-192-5p and (C) miR-194-5p. Bonferroni-adjusted p-values (Pairwise Test for Multiple Comparisons of Mean Rank Sums; Dunn's-Test) are reported as asterisks (* for ≤ 0.05 and ** for ≤ 0.01).

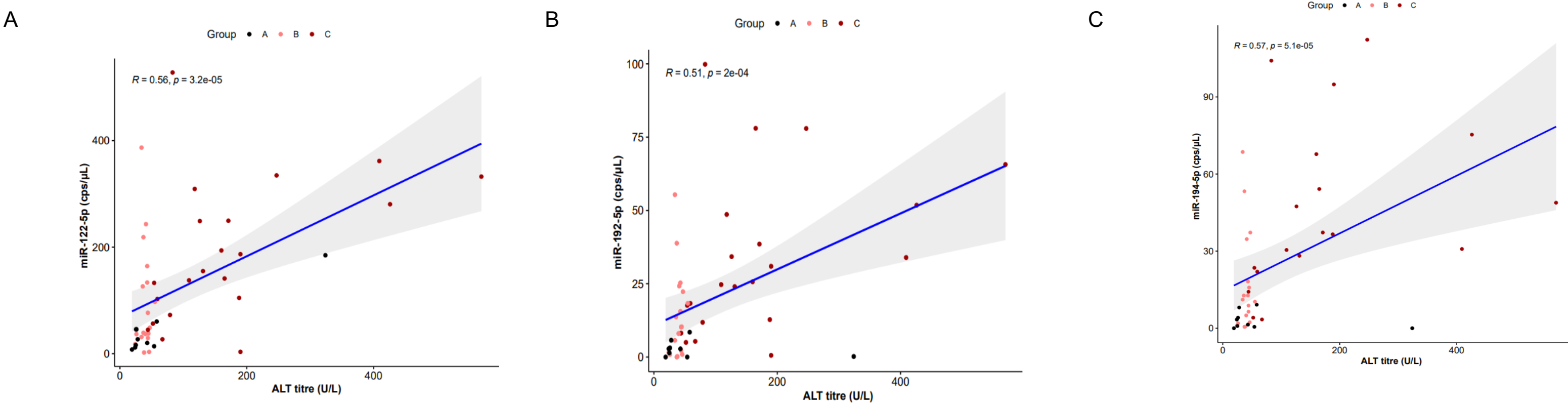


Figure 5. Correlations between miR-122-5p (A), miR-192-5p (B) and miR-194-5p (C) and ALT (U/L) considering all the samples. The blue line represents the trend line considering all the data, whereas the grey shadow represents the confidence interval. The Spearman rho and the p-value are also reported.

Conclusion

- HDV patients with undetectable HDV RNA showed a down-regulation of several miRNAs (including three liver-enriched miRNAs), which persisted even when viral RNA was still detectable.
- The mechanism associated with miRNAs expression and their role in HDV replication needs further studies.

Acknowledgements

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