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P58
Poster for you

High diversity of the TCR *repertoire* in hepatitis delta virus patients with undetectable viral RNA.

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Introduction/Summary

- The hepatitis delta virus (HDV) causes a chronic infection and only a limited percentage of patients can achieve spontaneous or treatment-related control of HDV replication.
- The function of the T cells is regulated by the activation of the T-cell receptor (TCR), whose genetic diversity is essential for inducing the immune response.
- **AIM:** Given that the interrelation between HDV infection and the immune response is still little-known, this study aimed to analyze the TCR repertoire diversity in HDV patients in relation to the HDV RNA state.

Study Design

25 HDV adult patients (anti-HDAg positive) had been selected.

At sampling, the patients were grouped based on the HDV RNA titer and their main virological and clinical characteristics are reported in **Table 1**.

	HDV RNA	
	Undetectable (N=8)	Detectable (n=17)
Log_{10} HDV RNA median [Q1;Q3]	0 [0;0]	4.4 [3.5;5.1]
ALT median [Q1;Q3]†	27 [17;34]	71.5 [31.8;107.8]
N Treatment (%)	1 (12.5%)	6 (35%)
N Cirrhosis (%)	0	9 (53%)

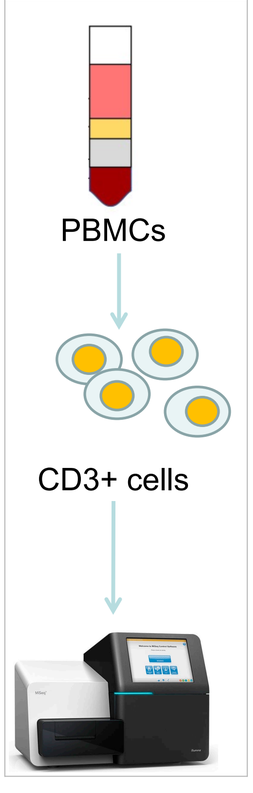
† for 3 patients this data was not available at sampling.

Control Group: 27 adult healthy donors (HD)

Methods

- PBMCs were collected from the blood of the 25 HDV patients.
- CD3+ cells were positively selected through the EasySep™ Human CD3 Positive Selection Kit (StemCell)
- Cellular DNA was isolated and the variable (TRBV), diversity (TRBD) and joining (TRBJ) gene rearrangements (forming the CDR3 sequence) of the beta chain of the TCR were studied by next-generation sequencing (Miseq, Illumina)

Figure 1 **Experimental procedure**



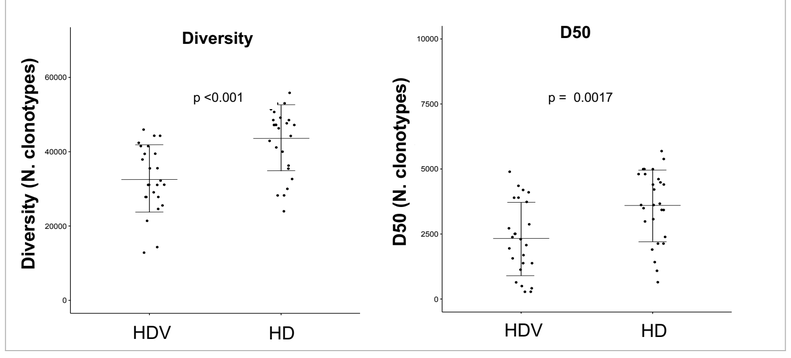
- **Bioinformatics analysis:**
  - Diversity: Number of unique clonotypes
  - D50: N clonotypes forming the 50% of the repertoire
  - TRBV-TRBJ rearrangements
  - TRBV and TRBJ usage frequency

Results

1. TCR repertoire diversity of HDV patients.

HDV patients exhibited a lower diversity of the TCRβ repertoire (both diversity and D50) than healthy donors.

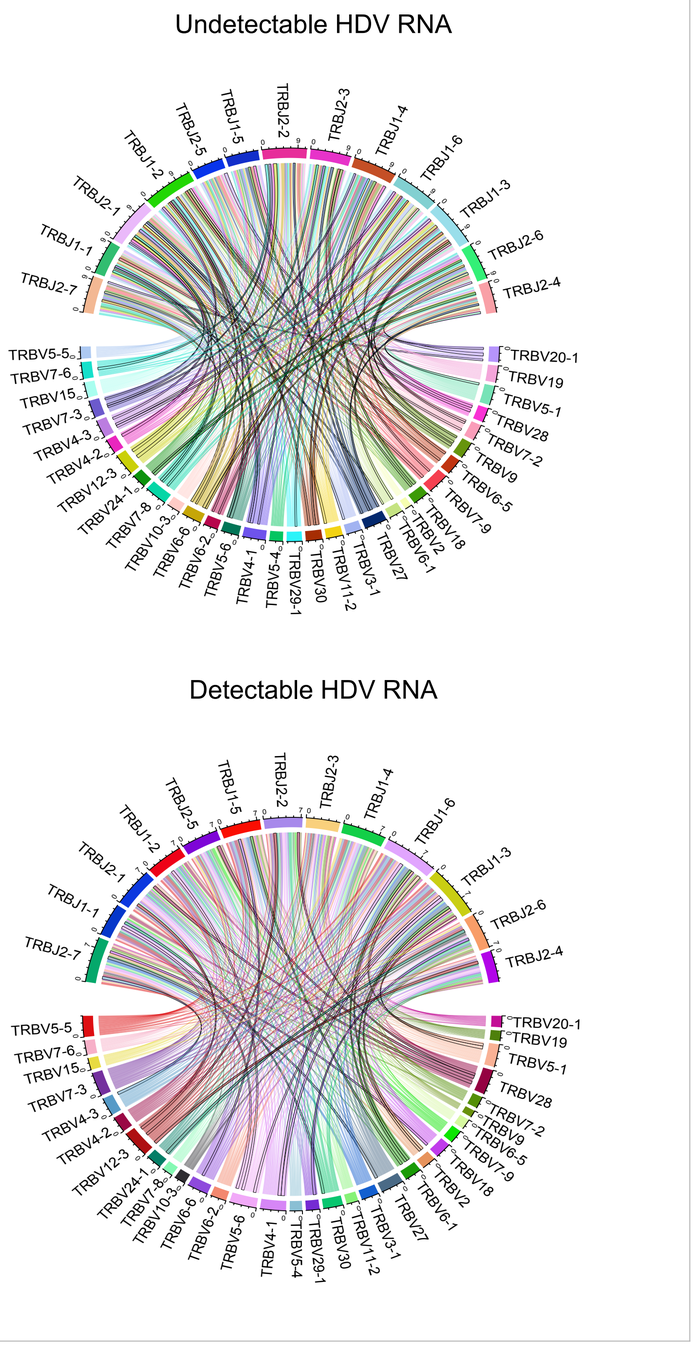
Figure 2 **TCRβ *repertoire* diversity in HDV patients related to HD control group.**



3. TRBV-TRBJ rearrangements between HDV-patients with undetectable and detectable HDV RNA.

Patients with undetectable HDV RNA presented a higher number of TRBV-TRBJ rearrangements (forming the CDR3 sequence) than patients with detectable HDV RNA (n = 56 versus 25, p = 0.0003), thus confirming this increased diversity.

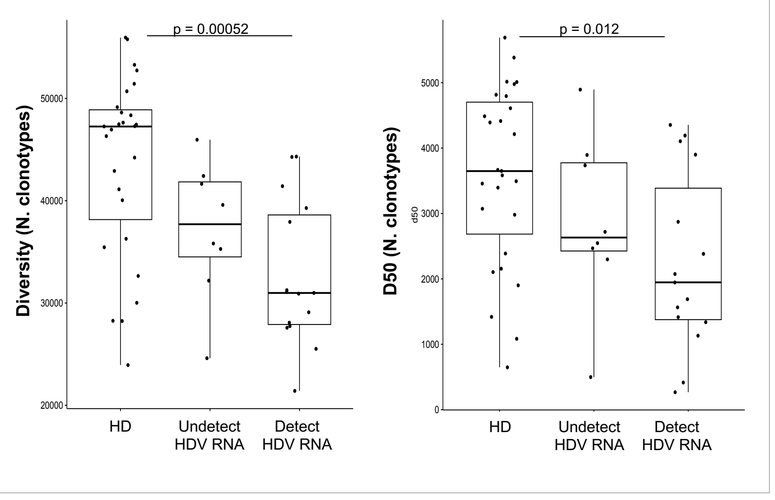
Figure 4 **Chord Diagram of the TRBV-TRBJ rearrangements in HDV patients with detectable or undetectable HDV RNA. The black lines show those rearrangements with a correlation of > | 0.5 | and p < 0.05**



2. TCR repertoire diversity between HDV patients with undetectable or detectable HDV RNA.

When considering the HDV RNA state, the patients with undetectable HDV RNA showed higher diversity than those with detectable HDV RNA. Nevertheless, the diversity was still lower than HD.

Figure 3 **TCRβ *repertoire* diversity related the HDV RNA state**

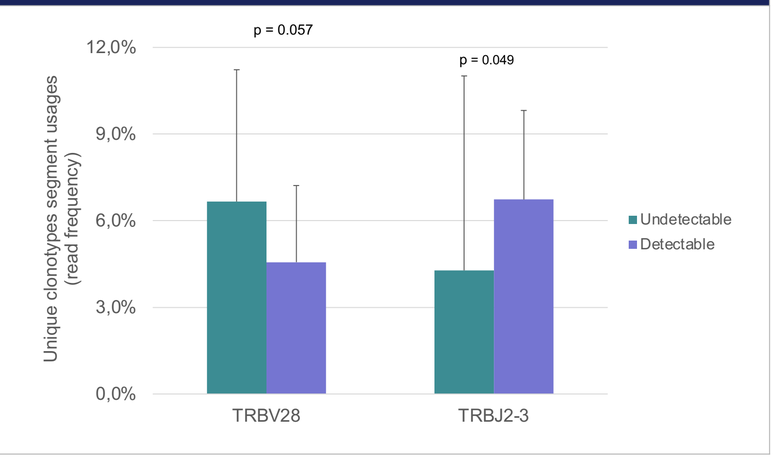


4. Usage frequency of TRBV and TRBJ segments in HDV patients with detectable and undetectable HDV RNA.

Patients with undetectable HDV RNA presented a higher frequency of the TRBV28 segment than patients with detectable HDV RNA.

Conversely, the usage of the TRBJ2-3 was higher in patients with detectable HDV RNA.

Figure 4 **Frequency of usage of TRBV and TRBJ segments related to the HDV RNA state**



Conclusion

- HDV-patients presented a lower diversity of the TCR *repertoire* than healthy donors, thus suggesting a kind of enrichment in some clonotypes due to the infection
- When comparing patients with detectable and undetectable HDV RNA, the latest showed a higher diversity, confirmed also when considering the TRBV-TRBJ rearrangements.
- This higher diversity of the HDV RNA undetectable group was closed but still lower to those of the HD, suggesting that a kind of enrichment is maintained also in absence of active viral replication.
- A more complete immune profile and a larger cohort of patients are needed to confirm these results.

Acknowledgements

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