Torque Teno Virus: A Predictive Biomarker for SARS-CoV-2 Vaccine Response in Immunosuppressed People

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BACKGROUND

- Solid organ transplant recipients are highly susceptible to severe COVID-19 due to chronic immunosuppressive treatment aimed at preventing organ rejection.
- Their vaccine responses are often diminished compared to the general population. Identifying a biomarker that reflects immune status and predicts vaccination response is crucial for optimizing vaccination strategies.
- Torque Teno Virus (TTV), a component of the human virome, has previously been identified as a marker of immune competence.
- Our objective was to develop an in-house qPCR assay to measure TTV viral load and evaluate its potential as a predictor of humoral response to SARS-CoV-2 vaccination.

METHODS

- A cohort of liver transplant recipients (LTR) and non-transplanted individuals (NT) was recruited from multiple centers. Vaccination schemes included initial doses (1st and 2nd) and a booster dose (3rd), with combinations determined by the national vaccination plan.
- Blood samples were collected to evaluate CD4+ T cells count and CD4/CD8 ratio by flow cytometry, and plasma was separated to measure anti-Spike IgG antibodies by ELISA and extract DNA.
- TTV viral load was determined using an in-house qPCR assay with Sybr Green and specific primers targeting the TTV genome.
- Mann-Whitney U test was used for group comparisons. P-values less than 0.05 were considered significant.

RESULTS

Tables 1 & 2. STUDY POPULATION CHARACTERISTICS

Baseline characteristics of the LTR and NT cohorts after receiving the initial vaccination schedule (Table 1) or the booster dose (Table 2) for SARS-CoV-2.

BL characteristics between LTR and NT after receiving the initial vaccination scheme

Characteristics	LTR (n=118)	NT (n=25)	p-value
Demographic variables			
Age (years), median (IQR)	64 (55-71)	67 (56.5-73.5)	0,227
Sex, n(%)			0,186
Male	70 (59.3%)	11 (44%)	
Female	48 (40.7%)	14 (56%)	
Body mass index (BMI), median (IQR)	27.6 (24.3-31.2)	27.2 (23.8-31.1)	0,711
Comorbidities			
Hypertension, n (%)	69 (58.5%)	11 (44%)	0,193
Diabetes, n (%)	43 (36.4%)	5 (20%)	0,162
COPD/Asthma, n (%)	9 (7.6%)	2 (8%)	1
Time intervals			
Days between the second dose and sample collection, median (IQR)	55.5 (40-71)	64 (45.5-72.5)	0,394
Days between the first and second doses, median (IQR)	87.5 (60-107)	96 (62.5-119.5)	0,368
Vaccination schedule			
ChAdOx1 (AstraZeneca), n (%)	37 (31.4%)	8 (32%)	1
BBIBP-CorV (Sinopharm), n (%)	20 (16.9%)	3 (12%)	0,766
rAd26-rAd5 (Sputnik V), n (%)	39 (33.1%)	6 (24%)	0,48
rAd26/mRNA-1273 (Sputnik V/Moderna), n (%)	22 (18.6%)	8 (32%)	0,175

For categorical variables, Fisher's exact test was used.
For continuous variables, the Mann-Whitney test was used.

BL characteristics between LTR and NT after the booster dose

Characteristics	LTR (n=66)	NT (n=16)	p-value
Demographic variables			
Age (years), median (IQR)	65 (55-71)	60.5 (43-73.5)	0,395
Sex, n(%)			**0,025**
Male	42 (63.6%)	5 (31.3%)	
Female	24 (36.4%)	11 (68.8%)	
Body mass index (BMI), median (IQR)	27.5 (24.6-31.3)	27.2 (23.8-31.6)	0,600
Comorbidities			
Hypertension, n (%)	37 (56.1%)	6(37.5%)	0,265
Diabetes, n (%)	26 (39.4%)	5(31.3%)	0,775
COPD/Asthma, n (%)	4(6,1%)	0(0.00%)	0,581
Time intervals			
Days between the third dose and sample collection, median (IQR)	59.5 (44-74)	49.5 (36-57.5)	**0,033**
Days between the second and third doses, median (IQR)	130.5 (86-156)	237 (143.5-316.5)	**<0.001**
Vaccination schedule			
IN/IN/IN, n (%)	2 (3%)	0 (0%)	1
VV/VV/VV, n (%)	30 (45.5%)	10 (62.5%)	0,271
VV/VV/RNA, n (%)	12 (18.2%)	3 (18.8%)	1
IN/IN/VV, n (%)	6 (9.1%)	0 (0%)	0,592
IN/IN/RNA, n (%)	0 (0.0%)	1 (6.3%)	0,195
VV/RNA/VV, n (%)	10 (15.2%)	0 (0.0%)	0,197
VV/RNA/RNA, n (%)	6 (9.1%)	2 (12.5%)	0,65

IN: Inactivated, VV: Viral Vector, RNA: Messenger RNA

High TTV viral load is a negative predictor of humoral response to SARS-CoV-2 vaccination in liver transplant recipients.

Figure 1. DETECTION OF ANTI-SPIKE IgG ANTIBODIES

Anti-Spike antibody levels were lower in LTR than in NT after both the initial schedule and booster (Fig. 1A). While all NT had detectable antibodies after the booster, 25% of LTR lacked a humoral response (Fig. 1B).

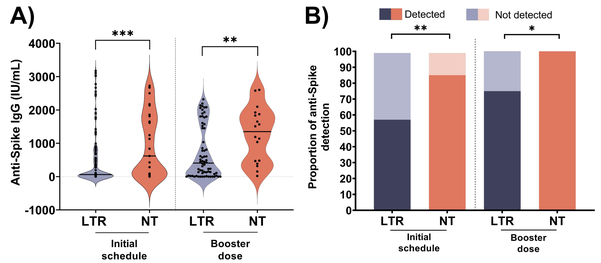


Figure 2. DEVELOPMENT OF A QUANTITATIVE PCR (qPCR) ASSAY FOR TTV MEASUREMENT

We developed a quantitative PCR assay using Sybr Green to determine TTV viral load. A TTV standard was generated and confirmed by sequencing to contain a single viral copy of TTV. The detection limit is 3.26 copies/mL or 0.51 $\log_{10}$(copies/mL).

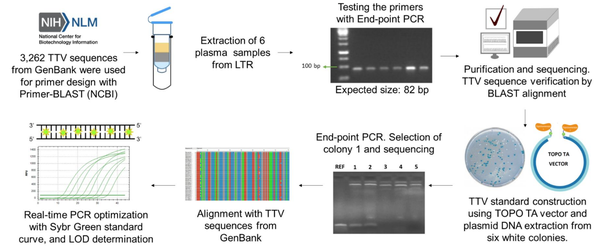


Figure 3. PREVALENCE AND TTV VIRAL LOAD

TTV prevalence was similar in both groups after the initial schedule (LTR: 89.04%, NT: 92.9%) and after the booster dose (LTR: 87.23%, NT: 88.88%). LTR had a higher TTV viral load than NT after the initial schedule with a median of 4.09 vs. 2.71 $\log_{10}$(copies/mL) and after the booster dose (4.38 vs. 2.99 $\log_{10}$ copies/mL).

Within LTR, non-responders (NR) had higher viral loads than responders (R) after the initial schedule (4.61 vs. 3.79 $\log_{10}$ copies/mL and booster 4.58 vs. 4.32 $\log_{10}$ copies/mL).

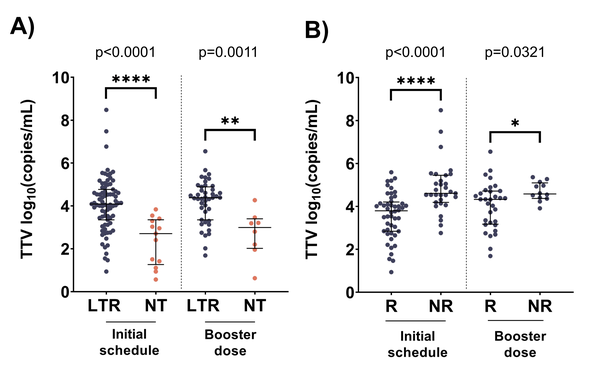


Figure 4. IMMUNE PROFILE EVALUATION OF LTR

Within the LTR group, NR had lower CD4⁺ counts than R after both the initial schedule (1131 vs. 836.2) and the booster dose (993.7 vs. 748) (Fig. A). No differences were observed in the CD4/CD8 ratio (Fig. B).

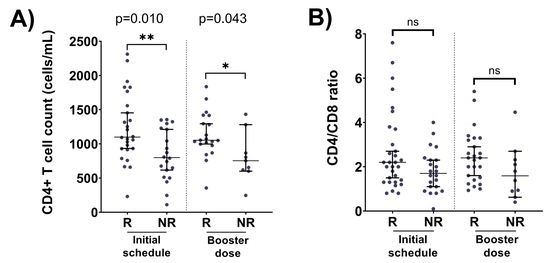
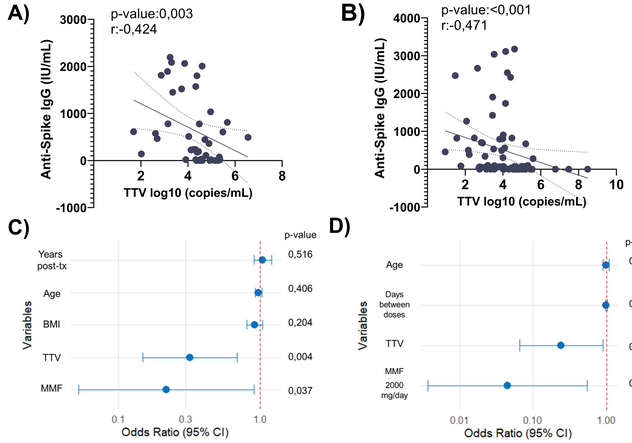


Figure 5. PREDICTIVE FACTORS FOR HUMORAL RESPONSE

Non-parametric Spearman correlation between anti-SARS-CoV-2 antibodies and TTV viral load after the initial schedule (Fig. A) and booster dose (Fig. B). Multivariate logistic regression shows that high TTV viral load and MMF use negatively predict humoral response in both vaccination phases (Fig. C: initial schedule, Fig. D: booster dose). Odds ratios are shown for each factor.



CONCLUSIONS

A high TTV viral load predicts a poor humoral response to SARS-CoV-2 vaccination and may serve as a biomarker for optimizing vaccination strategies in immunocompromised patients.

ADDITIONAL KEY INFORMATION

Reference:

- Ducasa N et al. Vaccine. DOI: 10.1016/j.vaccine.2023.11.039
- Mendizabal M et al. Hepatology Communications. DOI: 10.1002/hep4.2034.

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