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Metabolic determinants of torque teno virus load across the diabetes spectrum: insights from a cross-sectional analysis

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Torquetenovirus (TTV) is a ubiquitous non-pathogenic DNA virus whose replication mirrors immune competence. We profiled TTV viremia (TTVv) across type 1 diabetes (T1D), type 2 diabetes (T2D), and non-diabetes (ND) controls and assessed clinical associations. Cross-sectional, multicentre analysis of 485 individuals (T2D n. 277; T1D n. 61; ND n. 147). Plasma TTV-DNA was quantified by real-time PCR. Clinical/metabolic variables were harmonized across cohorts. Associations with TTVv were tested with uni- and multivariable models adjusting for age, sex, body mass index (BMI), glycated haemoglobin (HbA1c), and antidiabetic therapies. TTVv was detectable in 74.4%, with higher prevalence in T2D (79.0%) and T1D (73.8%) versus ND (69.0%). Age was an independent predictor. In T2D, unlike T1D, TTVv correlated negatively with BMI and HbA1c; those with poor control (HbA1c ≥ 8%) had significantly lower TTVv. Dipeptidyl peptidase-4 inhibitors (DPP-IVi) were independently associated with both TTVv presence and higher titers. Among T2D, individuals with TTVv < 4.0 log copies/ml were more often obese, female, and less frequently treated with DPP-IVi. TTVv is more prevalent and higher in diabetes, yet lower with poor glycaemic control and obesity, suggesting a directionality paradox. TTVv may index immunometabolic balance in diabetes; longitudinal and mechanistic studies are warranted.

Keywords Clinical immunology, Clinical science, Clinical diabetes

Torquetenovirus (TTV) is a small, single-stranded, non-pathogenic DNA virus belonging to the Anelloviridae family[1]. It is a ubiquitous component of the human virome present in up to 98% of the population[2] and detected in many sample types other than plasma, suggesting a broad tropism.

While TTV is not associated with overt pathogenicity in immunocompetent individuals, its replication is tightly regulated by host immune function, rendering circulating TTV DNA levels a dynamic and informative biomarker of immune competence. Given its quantitative relationship with immune activity, the measure of TTV DNA load, rather than its mere detection, provides a more accurate and clinically meaningful indicator of fluctuations in host immune competence. This property has been particularly leveraged in immunocompromised populations, such as solid organ and hematopoietic transplant recipients[3], where TTV viremia correlates with the degree of immunosuppression and can serve as a predictor of opportunistic infections or graft rejection[4].

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Beyond the transplant setting, TTV has attracted increasing attention in a range of immune-mediated and chronic inflammatory diseases, such as asthma[5] and rheumatoid arthritis[6].

Diabetes is a heterogeneous condition that is associated with an increased risk of infection and hospitalization for infections, both for type 1 (T1D) and type 2 (T2D) diabetes[7].

The coronavirus disease 2019 (COVID-19) pandemic highlighted the vulnerability of patients with diabetes, identifying it as an independent predictor of severe illness and mortality[8]. In fact, mechanistically, hyperglycemia impairs multiple arms of the innate and adaptive immune responses[9], and glycemic control is a strong predictor of infection risk in diabetes[10], while insulin signaling pathways intersect with key modulators of immune cell activation and survival[11].

In this context, T1D offers a unique pathophysiological model to study the isolated effects of chronic hyperglycemia (glucotoxicity) on the immune system, independent of confounding factors such as obesity, hypertension, and dyslipidemia that commonly coexist in T2D and contribute to a more complex cardiometabolic profile. Conversely, T2D represents a multifactorial metabolic syndrome in which insulin resistance, adipose tissue inflammation, and altered cytokine signaling create an immunometabolic environment distinct from that of T1D.In this study, we conceptualize TTV viremia as a marker of immune imbalance in diabetes, reflecting the net interplay between immune suppression and immune activation, rather than a unidirectional indicator of either state alone.Given these differences, the present cross-sectional study was designed to assess TTV viremia across a large, well-characterized cohort comprising individuals with T1D, T2D, and non-diabetes (ND) controls. Our primary aim was to investigate the distribution of TTV load in relation to diabetes status and, secondarily, to explore the association between TTV viremia and specific metabolic parameters. By comparing T1D and T2D patients, we sought to gain mechanistic insights into the interplay between glycemic control and viral dynamics in humans.

Materials and methods

Subjects

In this study, we retrospectively included a cohort of healthy subjects with normal glucose tolerance (ND), T1D and T2D patients attending the Diabetes outpatients clinic of Azienda Ospedaliero-Universitaria Pisana in Pisa and Fondazione Policlinico Universitario A. Gemelli IRCCS, Rome, Italy from May 2022 to December 2024, for their usual screening for diabetic complications or participation in a multicenter observational study aiming to explore novel biomarkers of early beta-cell dysfunction (NCT02175459). An additional cohort of patients with obesity with or without T2D who were candidates for metabolic bariatric surgery and age-matched lean controlsubjects who underwent studies employing metabolic imaging at Turku PET Centre (Turku, Finland) was assessed[12]. All contributing studies were approved by the appropriate local ethics committees (GR-2018-12365577 approved on November 19th 2019 for the University of Pisa and 30051/19 ID 2616 approved on July 4th 2019, for Fondazione Policlinico Universitario A. Gemelli IRCCS, Rome, Italy), and written informed consent was obtained from all participants in accordance with the Declaration of Helsinki.Subjects were included if they met predefined diagnostic criteria for T1D, T2D, or were classified as non-diabetes controlsubjects based on fasting glucose, glycated haemoglobin (HbA1c), and clinical history. Exclusion criteria comprised medical conditions expected to substantially influence immune competence (e.g., active/chronic infections; autoimmune diseases other than type 1 diabetes), advanced malignancy or organ failure, and treatment with immunosuppressive or immunomodulatory agents.

Despite the different designs and recruitment strategies of the contributing studies, all cohorts applied consistent inclusion and exclusion criteria relevant to diabetes diagnosis and metabolic assessment.To ensure comparability across datasets, we implemented a standardized protocol for the harmonization of clinical and laboratory data, including glycemic indices, anthropometric measures, and comorbid conditions.To further mitigate inter-cohort variability, all analyses were adjusted for key demographic and metabolic differences across cohorts, and plasma samples were processed under uniform laboratory conditions. All plasma samples were processed and stored under uniform conditions at −80 °C until analysis.A cross-sectional analytical approach was employed using the most recent available samples for each participant. For all individuals, demographic, clinical, and biochemical data were retrieved from the corresponding study databases and integrated into a unified dataset for statistical analysis.

Quantification of TTV viremia (TTVv)

TTV quantification was performed using a TaqMan®-based real-time PCR assay targeting a highly conserved region within the untranslated region (UTR) of the viral genome, common to *Alphatorquevirus* species. The method was adapted and analytically validated on the fully automated Panther Fusion® platform (Hologic, Inc) using the Open Access channel, as previously described[13]. Briefly, 250 µL of plasma was mixed 1:1 with specimen diluent and loaded into lysis tubes containing 710 µL of lysis buffer. Nucleic acids were automatically extracted and eluted in 50 µL. PCR amplification was carried out using 5 µL of eluate in a final 25 µL reaction volume, including 1 µM each of forward primer AMT-S (5′-GTGCCGIAGGTGAGTTTA-3′) and reverse primer AMT-AS (5′-AGCCCGGCCAGTCC-3′), and 0.3 µM of probe AMT-P (5′-FAM- TCAAGGGGCAATTCGGG CT-BHQ1-3′), targeting a 63-nt amplicon. The reaction mix also contained 4 mM $MgCl_2$, 50 mM KCl, and 10 mM Tris-HCl (pH 8.0). The thermal cycling conditions were: 95 °C for 2 min, followed by 45 cycles of 95 °C for 8 s and 55 °C for 28 s. Absolute quantification was performed using standard curves obtained from serial dilutions of a plasmid containing the target region. The analytical limit of quantification, which defines the assay's sensitivity threshold, was determined at 42 copies/ml (1.6 log copies/ml). Internal controls were included to ensure specificity and amplification efficiency.To mitigate batch effects, all PCR assays were conducted in a centralized laboratory utilizing a standardized amplification protocol and the same instrumental platform. Identical controls were used throughout, and all values were normalized to the designated calibrator.

Mixed meal tolerance test and related calculations

A subgroup of participants ($n = 223$) from the cohort of patients recruited in Pisa and Rome underwent a mixed meal tolerance test (MMTT) as previously described[14], while the cohort from the subgroup of patients recruited in Turku ($n = 71$) underwent a standard (75 g) oral glucose tolerance test (OGTT). In both metabolic tests, frequent blood sampling was performed (every 30 min) for determination of plasma glucose, insulin and C-peptide levels to assess beta-cell function and insulin sensitivity. Plasma glucose concentrations were measured by glucose oxidase. Plasma insulin and C-peptide levels were determined by radioimmunoassay (Beckman Coulter, Brea, CA); the detection limits of the assay were 2 units/mL and 0.1 ng/mL, respectively, with intra- and interassay coefficients of variation < 4%. Beta-cell function and insulin sensitivity parameters were calculated from the MMTT and OGTT data as previously described[15]. Estimated glomerular filtration rate (eGFR) was calculated by the Chronic Kidney Disease Epidemiology Collaboration (CKD-EPI) equation[16].

Statistical analysis

Summary statistics for continuous data are presented as mean ± standard deviation or median (25th–75th percentile, interquartile range, IQR), as appropriate. Normality of variables distribution was tested using the Shapiro-Wilk test. For comparisons between groups, one-way ANOVA was used for normally distributed variables, and Mann–Whitney U test or Kruskal–Wallis test for skewed variables. A comparison of the distribution of categorical variables was performed using chi-square test. Associations between two continuous parameters were analyzed by Spearman's rank correlation coefficient (rho). In linear and logistic regression models, the association between clinical/biochemical parameters and TTVv was adjusted for potential confounders that were selected upon background knowledge of their involvement in metabolic and clinical determinants of diabetes. To address inter-cohort variability, we additionally adjusted the main models for recruiting center/cohort (Pisa/Rome/Turku) and performed cohort-restricted and group- by-center interaction sensitivity analyses. Specifically, we repeated logistic regression models for TTV positivity and linear regression models for quantitative viral load (log10 copies/mL) among TTV-positive participants including center as a fixed effect and tested for effect modification by adding group x center interaction terms. In T2D participants with detectable TTVv, we evaluated HbA1c both as a continuous predictor (Spearman correlation and linear models) and as clinically meaningful thresholds (7.0%, 7.5%, 8.0%, 9.0%) using non-parametric group comparisons.To assess robustness to age/sex imbalance and group-size asymmetry, we performed two complementary sensitivity analyses. First, we conducted 1:1 nearest-neighbor matching without replacement with exact matching on sex and an age caliper (± 10 years for pooled diabetes vs. controls and for T2D vs. controls; ±5 years for T1D vs. controls). Within matched pairs, TTVpositivity was compared using McNemar's test; among TTV-positive participants, quantitative TTV load (log10 copies/mL) was compared using linear regression with age adjustment and robust standard errors. Second, we performed weighted regression using propensity-score overlap weights estimated from age and sex (weights trimmed at the 1st–99th percentile), followed by weighted logistic regression for TTV positivity and weighted linear regression for log10 viral load among TTV-positive participants, using robust (sandwich) standard errors. Covariate balance after weighting was evaluated using standardized mean differences (absolute SMD < 0.1 considered adequate).

Statistical analyses were performed using JMP version 17.0 (SAS Institute, Cary, NC, USA). A two-sided p value < 0.05 was considered statistically significant.

Results

Overall, we included 277 patients with T2D (Male: Female = 162:115, median age: 65.3 years, IQR: 56.3–71.0), 61 patients with T1D (M: F = 31:30, median age: 42.0 years, IQR: 30.0–51.0) and 147 subjects with normal glucose tolerance (ND) (M: F = 53:94, median age: 46.0 years, IQR: 34.0–54.0) (Table 1). TTVv was detectable in 361/485 (74.4%) subjects and showed a non-normal distribution in the overall cohort (Fig. 1) with a median value of 3.67 (IQR: 2.84–4.37) log copies/ml. Overall, the prevalence of TTVv was 218/277 (79.0%) for T2D, 45/61 (73.8%) for T1D and 97/147 (69.0%) for ND ($p = 0.07$). In post-hoc analysis, however, the prevalence of TTVv was higher in patients with diabetes (78.0%) compared to ND subjects (69.0%) ($p = 0.03$). In a linear regression model, after adjusting for age, patients with diabetes showed higher TTVv (diabetes: median 3.8 Log copies/ml [IQR: 3.0-4.5] vs. non-diabetes: median 3.2 Log copies/ml [IQR: 2.6–3.9], standardized beta-coefficients for age: 0.341, diabetes: 0.112, p value for the regression model = 0.043). Similarly, this association was also confirmed after the exclusion of T1D patients (standardized beta-coefficients for age: 0.262, diabetes: 0.192, p-value for the regression model < 0.001).

Overall, TTV-positive subjects were older (median age: 56.6 vs. 51.3 years, $p < 0.001$), more frequently male ($p = 0.003$) and with diabetes ($p = 0.031$), had higher systolic blood pressure (median:131 mmHg vs. 126 mmHg, $p = 0.040$), lower High-density lipoproteincholesterol (median: 49 mg/dL vs. 55 mg/dL, $p = 0.016$), and higher gamma-glutamil transferase (median: 20 UI/L vs. 18 UI/L, $p = 0.044$) (Table 2).

In the overall cohort, in univariable analysis TTVv was associated with age (rho = 0.39, $p < 0.001$) and HbA1c (rho = 0.15, $p < 0.001$). In multivariable analysis, including age, sex and HbA1c, only age was a significant predictor of TTVv (rho = 0.388, $p < 0.001$). In patients with T2D, TTVv was associated with age (rho = 0.249, $p < 0.001$) and body mass index (BMI), (rho = − 0.177, $p = 0.009$), with also a tendency for a negative association with HbA1c (rho=-0.117, $p = 0.08$). At multivariate analysis, after adjusting for age, the association of HbA1c was lost, with a tendency for an inverse association of BMI with TTVv (standardized beta-coefficients for age: 0.181, $p = 0.011$, BMI: − 0.132, $p = 0.061$ HbA1c: − 0.100, $p = 0.0142$, adjusted R^2 for the regression model = 0.064, $p = 0.083$).

In T2D patients treated with dipeptidyl peptidase-4 inhibitors (DPP-IVi) we found a higher prevalence of TTVv (93.1% vs. 75.0%; $p < 0.05$),which was confirmed in a binary logistic model after adjusting for age, sex, BMI and (adjusted odds ratio, OR = 3.6, Confidence Intervals, CI, at 95% 1.3–9.6, $p = 0.003$). Also, among TTV

	T1D ($n=61$)	T2D ($n=277$)	Non-diabetes ($n=147$)	*P* value
Age (years)	42.0 (30.0–51.0)	65.3 (56.3–71.0)	46 (34–54)	< 0.001
Sex (Male: Female, n.)	31:30(51.7:48.3)	162:115(58.3:41.7)	53:94(64.4:35.6)	< 0.001
Diabetes duration (years)	19 (10–26)	7 (3–14)		
TTV-DNA positive: negative (n.)	45:16(73.8:26.2)	218:57(79.3:20.7)	98:44 (69:31)	0.077
TTV-DNA (logcopies/ml)	2.87 (2.52–3.74)	3.97 (3.30–4.60)	3.18 (2.56–3.89)	< 0.001
BMI (kg/m^2)	28 (25.2–32.1)	29.1 (25.9–32.9)	29.1 (25.9–32.9)	< 0.001
Obese(n.)	4 (6.6)	119 (43.9)	55 (37.4)	< 0.001
Waist circumference (cm)	79.5 (72.5–91.8)	104 (96–112)	90 (78–104)	< 0.001
Systolic blood pressure (mmHg)	122 (110–136)	136 (120–150)	120 (113–134)	< 0.001
Diastolic blood pressure (mmHg)	78 (70–81)	80 (75–89)	78 (70–85)	< 0.001
Hypertension treatment(n.)	8 (13.6)	113 (52.6)	12 (18.8)	< 0.001
Fasting plasma glucose(mg/dL)	150 (112–240)	137 (120–159)	97 (92–104)	< 0.001
Hba1c (%)	6.7 (6.4–7.3)	7 (6.5–7.5)	5.4 (5.2–5.6)	< 0.001
Hba1c (mmol/mol)	50 (46–56)	53 (48–58)	35 (33–38)	< 0.001
Hba1c > 6.5% (48 mmol/mol)(n.)	41 (67.2)	156 (56.3)	0 (0.0)	< 0.001
Hba1c > 7% (53 mmol/mol)(n.)	20 (32.8)	103 (37.2)	0 (0.0)	< 0.001
Total cholesterol (mg/dL)	166 (146–186)	164 (142–187)	169 (149–195)	0.113
LDL (mg/dL)	91 (74–109)	88 (69–109)	104 (81–120)	0.001
HDL (mg/dL)	63 (53–74)	47 (39–57)	54 (44–64)	< 0.001
Non-HDL (mg/dL)	105 (81–120)	113 (93–135)	116 (96–138)	0.027
Triglycerides (mg/dL)	63 (48–78)	121 (89–163)	89 (62–115)	< 0.001
Creatinine (mg/dL)	0.80 (0.71–0.91)	0.85 (0.71–0.99)	0.80 (0.70–0.93)	0.096
eGFR (ml/min/1.73 m^2)	105 (98–114)	91 (77–99)	101 (92–110)	< 0.001
CKD-STAGE(n.)	Stage II: 6 (9.8) Stage III: 2 (3.3) Stage IV: 1 (1.6) Stage V: 0	Stage II: 82 (29.6) Stage III: 19 (6.9) Stage IV: 0 Stage V: 0	Stage II: 0 (0) Stage III: 0 (0) Stage IV: 0 (0) Stage V: 0 (0)	< 0.001
AST (UI/L)	20 (17–26)	19 (15–23)	19 (16–23)	0.207
ALT (UI/L)	20 (14–30)	22 (14–30)	19 (15–28)	0.481
GGT (UI/L)	17 (12–23)	24 (16–41)	15 (12–24)	< 0.001
Metformin (n.)	4 (6.8)	233 (85.7) Monotreatment: 121 (48.6)	1 (1.3)	
Sulfonylureas (n.)		13 (4.8)		
DPP-IVi (n.)		59 (21.7)		
SGLT2-i (n.)	1 (1.7)	27 (9.9)	1 (1.3)	
GLP1-RA (n.)	0 (0.0)	21 (7.7)	0 (0.0)	
Basal insulin (n.)		11 (4.0)		
Basal-bolus insulin (n.)		16 (5.9)		
Anti-aggregation (n.)	4 (6.8)	59 (27.4)	0 (0)	< 0.001
Statin (n.)	15 (25.4)	117 (54.4)	1 (1.6)	< 0.001
Complications (n.)	No: 48 (81.4) Microvascular: 12 (20.3) Macrovascular: 1 (1.7) Micro- and Macrovascular: 0 (0.0)	No: 120 (60.3) Microvascular: 62 (31.6) Macrovascular: 34 (17.1) Micro- and Macrovascular: 19 (9.5)		

Table 1. Descriptive characteristics of the patients included divided as with type 1 diabetes (T1D), type 2 diabetes (T2D) and non-diabetes (ND). Continuous variables are expressed as median (interquartile range, IQR) and categorical variables as number (percentage).TTV: Torque Teno Virus. Abbreviations: T1D, type 1 diabetes; T2D, type 2 diabetes; ND, non-diabetes; TTV, Torquetenovirus; TTV-DNA, Torquetenovirus DNA; HbA1c, glycated haemoglobin; BMI, body mass index; BP, blood pressure; eGFR, estimated glomerular filtration rate; AST, aspartate aminotransferase; ALT, alanine aminotransferase; GGT, gamma-glutamyl transferase; HDL (HDL-C), high-density lipoprotein cholesterol; LDL (LDL-C), low-density lipoprotein cholesterol; non-HDL, non-high-density lipoprotein cholesterol; CKD, chronic kidney disease; DPP-IVi, dipeptidyl peptidase-IV inhibitors; GLP-1RA, glucagon-like peptide-1 receptor agonists; SGLT2i, sodium-glucose cotransporter-2 inhibitors; UI/L, international units per liter.

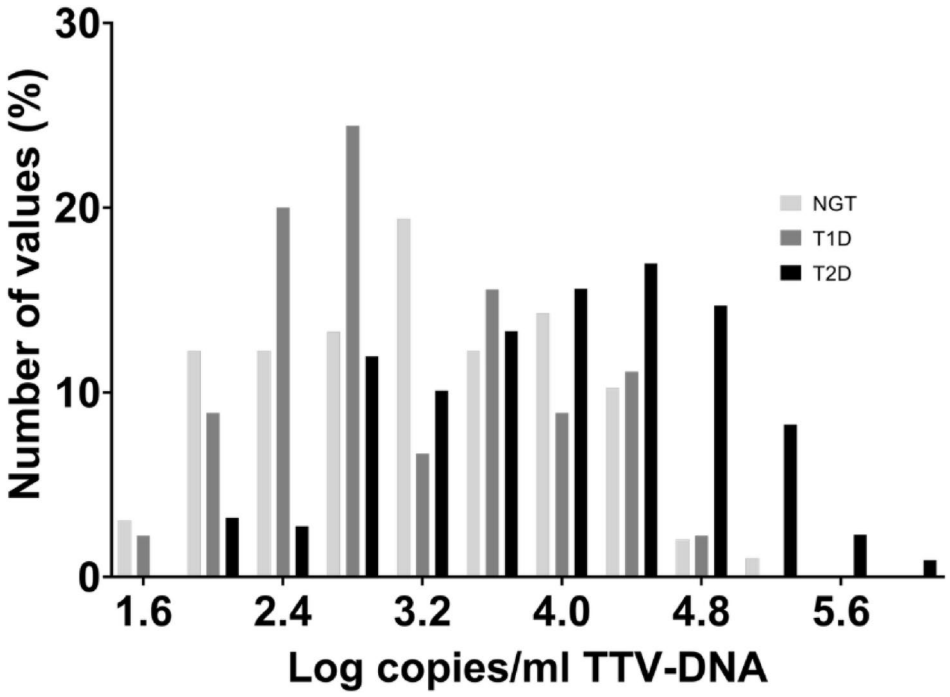

Fig. 1. Distribution of TTV-DNA (log copies/ml) in the overall cohort. TTV: Torquetenovirus.

	TTV positive	TTV negative	*P* value
Age (years)	56.6 (15.7)	51.3 (13.9)	**< 0.001**
Sex (Male/Female)	198/162	46/71	**0.003**
Non-diabetes/T2D/T1D	97/218/45	44/57/16	0.077
Non-Diabetes/Diabetes(T1D and T2D); Diabetes Prevalence (%)	98/263/72.8	44/73/62.3	**0.031**
Number of antidiabetic drugs in T2D only(more than 2 drugs / less than 2)	57/191	0/22	**0.010**
BMI (kg/m^2)	27.5 (24.6–32.5)	27.6 (23.3–31.3)	0.281
Waist circumference (cm)	102 (90–110)	99 (81–107)	0.113
Fasting plasma glucose (mg/dl)	121 (101–147)	118 (97–149)	0.658
Hba1c (%)	6.5 (5.7–7.3)	6.5 (5.5–7.4)	0.596
HbA1c (mmol/mol)	48 (39–56)	47 (37–57)	0.596
Systolic blood pressure (mmHg)	131 (18)	126 (17)	**0.040**
Diastolic blood pressure (mmHg)	80 (10)	79 (11)	0.884
Total cholesterol (mg/dl)	164 (143–187)	170 (149–192)	0.105
LDL (mg/dL)	91 (71–111)	96 (75–113)	0.409
HDL (mg/dL)	49 (41–59)	55 (43–65)	**0.016**
Triglycerides (mg/dL)	105 (71–142)	95 (66–142)	0.664
AST (UI/L)	19 (16–24)	20 (15–23)	0.956
ALT (UI/L)	22 (14–29)	18 (14–29)	0.588
GGT (UI/L)	20 (13–30)	18 (11–28)	**0.044**
eGFR (ml/min/1.73 m^2)	97 (14)	89 (19)	0.101

Table 2. Descriptive data and comparison of Torque Teno Virus (TTV) positive and negative subjects in the cohorts. Continuous variables are expressed as median (interquartile range, IQR) and categorical variables as number (percentage). T1D: type 1 diabetes; T2D: type 2 diabetes. Abbreviations: TTV, Torquetenovirus; T1D, type 1 diabetes; T2D, type 2 diabetes; HbA1c, glycated haemoglobin; BMI, body mass index; BP, blood pressure; eGFR, estimated glomerular filtration rate; AST, aspartate aminotransferase; ALT, alanine aminotransferase; GGT, gamma-glutamyl transferase; HDL (HDL-C), high-density lipoprotein cholesterol; LDL (LDL-C), low-density lipoprotein cholesterol; HOMA2-IR, homeostatic model assessment 2 of insulin resistance; OGIS, oral glucose insulin sensitivity index; UI/L, international units per liter. Significant values are in [bold].

positive T2D patients, those treated with DPP-IVi presented higher TTVv (DPP-IV treated/untreated: median 4.2 Log copies/ml [IQR: 3.6–4.7], vs. median 3.87 Log copies/ml [IQR: 3.1–4.5], $p = 0.015$), although this was lost after adjusting for age. No association was found between glucagon-like peptide-1-receptor agonists (GLP1-RA) and sodium-glucose cotransporter 2 inhibitors (SGLT2-i) in terms of TTV prevalence or viremia.

When stratifying the T2D cohort based on level of HbA1c, patients with HbA1c ≥ 8% (64 mmol/mol), after adjusting for age, sex, BMI and DPP-IVi therapy, presented lower TTVv (median values: 3.2 Logcopies/ml [IQR: 2.8–4.2] vs. 4.0 [IQR: 3.3–4.6], $p = 0.021$) (Fig. 2). This subgroup of 22 patients (Male: Female = 14:8, median age: 62.5 years, IQR: 51.6–69.2) was characterized by a high burden of macrovascular complications (27.8%, $p = 0.042$), higher diastolic blood pressure ($p = 0.029$) and lower DPP-IV inhibitor use (9.1%, $p = 0.064$) (Table 3).

T2D patients with TTVv < 4.0 Log copies/ml compared to those with ≥ 4 Log copies/ml were more frequently female (46.8% vs. 33.0%, $p = 0.038$) and obese (52.8% vs. 32.8%, $p = 0.001$), with higher BMI (30.5 kg/m^2 vs. 27.3 kg/m^2, $p = 0.035$) and alanine aminotransferase (ALT) (median: 24 UI/l vs. 16 UI/l, $p = 0.010$). They were less frequently treated with DPP-IVi (19.3% vs. 31.7%, $p = 0.037$) and anti-platelet therapy (21.3%, vs. 38.2%, $p = 0.019$), and more frequently with GLP1-RA (12.8% vs. 3.8%, $p = 0.018$) (Table 4).

No association was found between TTVv and clinical characteristics of ND subjects and T1D patients (data not shown).

Sensitivity analyses (age/sex matching and weighted regression) demonstrated thatin age- and sex-matched cohorts, TTV positivity did not differ between groups (pooled diabetes vs. controls: 80/115 [69.6%] vs. 76/115 [66.1%], McNemar $p = 0.683$; T2D vs. controls: 56/77 [72.7%] vs. 53/77 [68.8%], $p = 0.711$; T1D vs. controls: 43/59 [72.9%] vs. 36/59 [61.0%], $p = 0.230$). Among TTV-positive participants within matched samples, quantitative viral load showed a directionally higher signal for T2D vs. controls (median 3.65 vs. 3.31 log10 copies/mL; age-adjusted $\beta = 0.274$, 95% CI − 0.042 to 0.590; $p = 0.090$), while pooled diabetes vs. controls ($\beta = 0.095$, $p = 0.470$) and T1D vs. controls ($\beta = -0.171$, $p = 0.344$) were not significant.Using propensity-score overlap–weighted

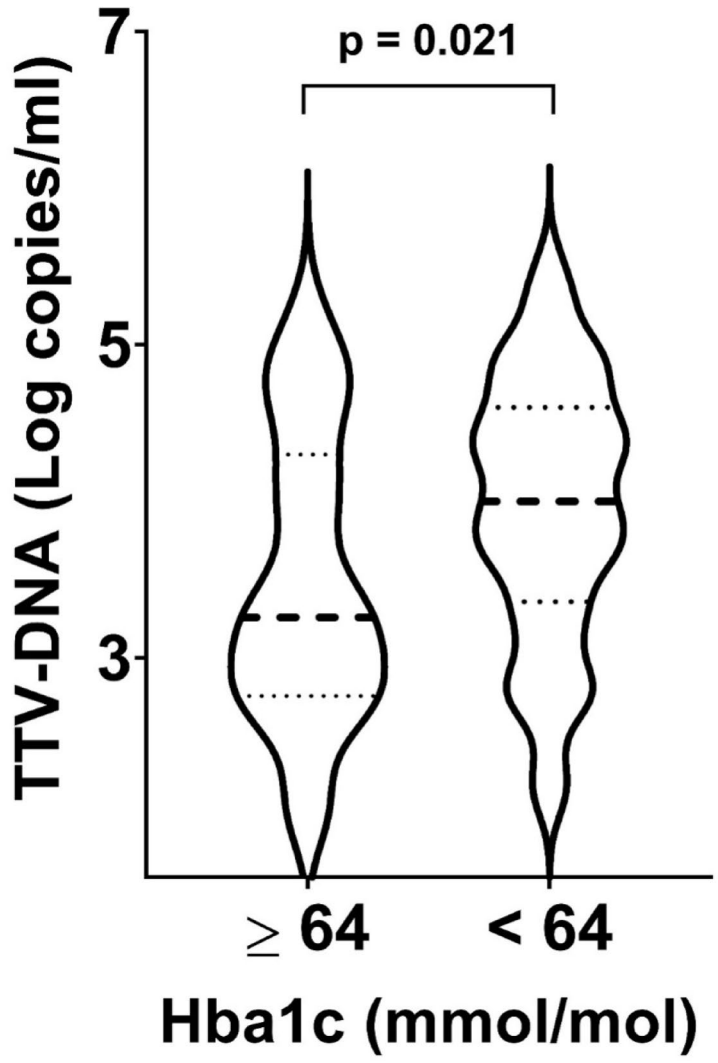

Fig. 2. Comparison of TTV-DNA (log copies/ml) between patients with type 2 diabetes (T2D) and Hba1c > 8% (64 mmol/mol) or < 8%(64 mmol/mol). TTV: Torquetenovirus.

	Hba1c < 8% (64 mmol/mol) (*n* = 196)	Hba1c ≥ 8% (64 mmol/mol) (*n* = 22)	*p* value
Age (yrs)	66.8 (57.6–72.9)	62.5 (51.6–69.2)	0.101
Sex (Male/Female)	115:81 (59.2:40.8)	14:8 (63.6:36.4)	0.685
Diabetes years	9 (3–14)	14 (2–19)	0.238
TTV-DNA(log copies/ml)	4.00 (3.36–4.6)	3.24 (2.77–4.20)	**0.021**
BMI (kg/mq)	28.4 (25.6–32.7)	28.6 (26.3–33.5)	0.573
Obese (n.)	82:114 (41.9:57.6)	9 (40.9)	0.914
Waist circumference (cm)	104 (96–112)	105 (101–112)	0.720
Systolic blood pressure (mmHg)	139 (122–150)	145 (128–160)	0.205
Diastolic blood pressure (mmHg)	80 (73–88)	85 (80–90)	**0.029**
Hypertension treatment (n.)	79 (54.9)	12 (60.0)	0.665
Total cholesterol (mg/dL)	163 (141–187)	141 (123–170)	**0.012**
LDL-c (mg/dL)	90 (70–108)	68 (64–83)	**0.005**
HDL-c (mg/dL)	48 (40–57)	42 (37–57)	0.223
Triglycerides (mg/dL)	120 (89–159)	115 (89–168)	0.961
eGFR (ml/min/1.73mq)	89 (75–98)	96 (78–101)	0.237
AST (UI/L)	18 (15–23)	18 (15–22)	0.864
ALT (UI/L)	21 (13–28)	23 (19–28)	0.310
G-GT (UI/L)	24 (15–39)	19 (12–35)	0.552
Metformin (n.)	165 (86.4)	17 (77.3)	0.278
Sulfonylureas (n.)	11 (5.8)	0 (0.0)	0.248
DPP-IVi (n.)	52 (27.2)	2 (9.1)	0.064
SGLT2i (n.)	23 (12.0)	1 (4.5)	0.243
GLP1-RA (n.)	17 (8.9)	1 (4.5)	0.453
Basal Insulin (n.)	10 (5.2)	1 (4.5)	0.576
Basal-Bolus Insulin (n.)	6 (3.1)	8 (36.4)	**< 0.001**
Anti-aggregation (n.)	43 (29.9)	7 (31.8)	0.640
Statin (n.)	77 (53.5)	15 (68.2)	0.069
Complications: (n.)	No: 74 (54.8) Microvascular:47 (35.3) Macrovascular: 23 (17) Micro- and Macrovascular: 11(8.1)	No: 9 (50.0) Microvascular: 7 (41.2) Macrovascular: 7 (38.9) Micro- and Macrovascular: 5 (27.8)	0.700 0.637 **0.042** **0.025**

Table 3. Characteristics of T2D patients with positive TTVv and Hba1c ≥ 8% (64 mmol/mol). Comparison with TTV-positive T2D patients with Hba1c < 8% (64 mmol/mol) is provided. Abbreviations: T2D, type 2 diabetes; TTV, Torquetenovirus; TTV-DNA, Torquetenovirus DNA; HbA1c, glycated haemoglobin; BMI, body mass index; BP, blood pressure; eGFR, estimated glomerular filtration rate; AST, aspartate aminotransferase; ALT, alanine aminotransferase; GGT, gamma-glutamyl transferase; HDL (HDL-C), high-density lipoprotein cholesterol; LDL (LDL-C), low-density lipoprotein cholesterol; DPP-IVi, dipeptidyl peptidase-IV inhibitors; GLP-1RA, glucagon-like peptide-1 receptor agonists; SGLT2i, sodium-glucose cotransporter-2 inhibitors; UI/L, international units per liter. Significant values are in [bold].

regression to balance age and sex while retaining larger samples, TTV positivity remained not significantly different (pooled diabetes vs. controls: OR = 1.10, 95% CI 0.53–2.28; $p = 0.789$; T2D vs. controls: OR = 0.98, 95% CI 0.41–2.36; $p = 0.964$; T1D vs. controls: OR = 1.12, 95% CI 0.42–2.99; $p = 0.817$). In contrast, among TTV-positive participants, quantitative TTV load was higher in pooled diabetes and in T2D vs. controls (pooled diabetes: $\beta = 0.235$, 95% CI 0.002–0.469; $p = 0.048$; T2D: $\beta = 0.348$, 95% CI 0.065–0.630; $p = 0.016$), with no significant difference for T1D ($\beta = -0.118$, $p = 0.437$).

In center-adjusted analyses (age- and sex-adjusted), TTV positivity did not differ between diabetes groups and controls (T2D vs. controls: OR = 1.04, 95% CI 0.55–1.95; $p = 0.910$; T1D vs. controls: OR = 1.46, 95% CI 0.70–3.02; $p = 0.310$), while a borderline higher positivity was observed in Rome vs. Pisa cohorts (OR = 2.15, 95% CI 1.00–4.65; $p = 0.051$). Among TTV-positive participants, quantitative viral load remained higher in T2D versus controls after center adjustment (beta = 0.450 log10 copies/mL, 95% CI 0.163–0.738; $p = 0.002$), with no significant difference for T1D (beta = − 0.114, 95% CI − 0.435 to 0.207; $p = 0.487$). The T2D-control viral-load contrast showed significant heterogeneity across centers in interaction models and center-restricted analyses (Pisa cohort beta = 0.842, $p < 0.001$; Rome cohort beta = − 0.804, $p < 0.001$; Turku cohort beta = 0.181, $p = 0.467$); importantly, the association remained robust when restricting to cohorts with larger internal control groups (Pisa+Turku: beta = 0.714, 95% CI 0.393–1.034; <0.0001). For a subgroup of T2D ($n = 148$) and ND ($n = 147$) subjects we analyzed the data of beta-cell function and insulin sensitivity derived from a MMTT. In this subgroup we found a tendency for an inverse correlation of oral glucose insulin sensitivity OGIS and TTVv (rho: − 0.142, $p = 0.076$). Similarly, in univariable analysis we found an inverse association between indexes of beta-cell function and TTVv (first phase secretion: rho = − 0.158, $p = 0.023$, and second phase secretion: rho

	TTV DNA < 4.0 log (*n* = 111)	TTV DNA ≥ 4.0 log (*n* = 107)	*p* value
Age (yrs)	65.0 (54.9–71.6)	68.2 (59.0-73.8)	0.056
Sex (Male/Female)	59:52 (53.2:46.8)	71:35 (67.0:33.0)	**0.038**
Diabetes years	9 (3–16)	7 (3–14)	0.769
TTV-DNA (log copies/ml)]	3.3 (2.8–3.7)	4.6 (4.3–4.9)	**< 0.001**
BMI (kg/mq)	30.5 (26.1–34.1)	27.3 (25.6–31.2)	**0.035**
Obese (n.)	57(52.8)	32 (30.8)	**0.001**
Waist circumference (cm)	106 (96–114)	102 (96–110)	0.375
Systolic BP (mmHg)	133 (120–148)	140 (130–154)	0.074
Diastolic BP (mmHg)	80 (72–88)	81 (76–89)	0.353
Hypertension treatment (n.)	40 (53.3)	51 (57.3)	0.610
Fasting plasma glucose (mg/dL)	136 (115–160)	132 (120–155)	0.869
Hba1c (%)	6.9 (6.4–7.5)	6.8 (6.5–7.4)	0.655
Hba1c (mmol/mol)	52 (46–58)	51 (48–57)	0.655
Hba1c > 6.5% (48 mmol/mol) (n.)	81 (74.3)	79 (76.0)	0.781
Hba1c > 7% (53 mmol/mol) (n.)	51 (46.8)	49 (47.1)	0.962
Total cholesterol (mg/dL)	160 (136–186)	160 (141–184)	0.678
LDL-c (mg/dL)	88 (68–111)	87 (69–102)	0.662
HDL-c (mg/dL)	45 (39–55)	48 (41–59)	0.141
Non-HDL-c (mg/dL)	113 (90–135)	110 (89–132)	0.738
Triglycerides (mg/dL)	121 (94–151)	116 (83–163)	0.838
Creatinine (mg/dL)	0.89 (0.73-1.00)	0.86 (0.73–1.02)	0.673
eGFR (ml/min/1.73mq)	91 (76–99)	89 (75–98)	0.418
CKD-STAGE (n.)	Stage II: 29 (26.1) Stage III: 6 (5.4) Stage IV: 0 Stage V: 0	Stage II: 38 (79.2) Stage III: 10 (20.8) Stage IV: 0 Stage V: 0	0.889
AST (UI/L)	19 (16–22)	17 (14–23)	0.153
ALT (UI/L)	24 (18–29)	16 (12–26)	**0.010**
G-GT (UI/L)	24 (15–40)	22 (14–34)	0.404
Metformin (n.)	92 (84.4)	90 (86.5)	0.659
Sulphanylureas (n.)	3 (2.8)	8 (7.7)	0.103
DPP-IVi(n.)	21 (19.3)	33 (31.7)	**0.037**
SGLT2i (n.)	16 (14.7)	8 (7.7)	0.107
GLP1-RA (n.)	14 (12.8)	4 (3.8)	**0.018**
Insulin therapy (n.)	14 (13.8)	10 (9.6)	0.099
Anti-aggregation (n.)	16 (21.3)	34 (38.2)	**0.019**
Statin (n.)	40 (53.3)	52 (58.4)	0.513
Complications	No: 38 (57.6) Microvascular: 22 (33.8) Macrovascular: 9 (13.6) Micro- and Macrovascular: 4 (6.1)	No: 45 (51.1) Microvascular: 33 (38.4) Macrovascular: 21 (23.9) Micro- and Macrovascular: 12 (13.6)	0.428 0.567 0.113 0.127

Table 4. Comparison of clinical characteristics of T2D patients with detectable TTV viremia and titer below or above 4.0log copies/mL. Abbreviations: T2D, type 2 diabetes; TTV, Torquetenovirus; TTV-DNA, Torquetenovirus DNA; HbA1c, glycated haemoglobin; BMI, body mass index; BP, blood pressure; eGFR, estimated glomerular filtration rate; AST, aspartate aminotransferase; ALT, alanine aminotransferase; GGT, gamma-glutamyl transferase; HDL (HDL-C), high-density lipoprotein cholesterol; LDL (LDL-C), low-density lipoprotein cholesterol; non-HDL, non-high-density lipoprotein cholesterol; CKD, chronic kidney disease; DPP-IVi, dipeptidyl peptidase-IV inhibitors; GLP-1RA, glucagon-like peptide-1 receptor agonists; SGLT2i, sodium-glucose cotransporter-2 inhibitors; HOMA2-IR, homeostatic model assessment 2 of insulin resistance; OGIS, oral glucose insulin sensitivity index; UI/L, international units per liter. Significant values are in [bold].

= − 0.45, $p = 0.037$, according to Stumvoll model). After accounting for age, however, these associations were no longer significant (standardized beta coefficient for first phase secretion: − 0.047, $p = 0.527$, for second phase secretion: − 0.033, $p = 0.651$, and for OGIS: − 0.007. $p = 0.938$). For both beta-cell function and insulin sensitivity parameters no association was found with TTVv when splitting the analysis for T2D and ND subjects (data not shown).

Discussion

This study investigated for the first time the prevalence of TTVv in individuals with T2D, T1D, and ND control subjects, and its association with relevant clinical parameters. Although, as reported in the literature, TTVv prevalence increased with age[17], it was more prevalent in patients with diabetes (78.0%) compared to ND subjects (69.0%), and its levels were higher in patients with diabetes after adjusting for age. This is at variance with data from a cohort of elderly Italian subjects, where diabetes was not a predictor of TTVv[18].

As highlighted in Table 2 in TTV positive patients, we found higher blood pressure, lower HDL and higher GGT, suggesting that other factors associated with metabolic syndrome may be determinants of TTVvbeyond diabetes. Also, no significant relationships were observed between TTVv and clinical characteristics in T1D or ND populations.

We compared T2D patients divided according to the TTVv cut-off of 4.0log copies/ml (Table 4). This cut-off was previously reported in the literature as the upper limit of normality in healthy subjects[17] and as clinically significant cut-off in patients with chronic obstructive pulmonary disease (COPD), ischaemic heart disease and in elderly subjects, where a TTVv ≥ 4.0log copies/ml was associated with negative clinical outcomes and increased mortality[18–20]. In our cohort, patients with TTVv < 4.0log copies/ml showed higher BMI and ALT, a higher prevalence of obesity and a lower prevalence of anti-platelet therapy. Similarly, in T2D patients after adjusting for age, there was a tendency for an inverse association of BMI with TTVv, which was not found in the overall cohort. A previous study evaluated TTVv in obese subjects from a bariatric surgery cohort compared to lean controlsubjects[21]. This study found higher TTVv in the obese group, although these patients were younger than the ones included in our study and none of them had diabetes. Also, there was no association between BMI and TTVv. On one hand, it is possible that such discrepancy in the results may be ascribed to the different characteristics of the cohort included in our study. On the other hand, an interesting hypothesis is the existence in T2D patients of an immune-mediated effect on TTVv on obesity and its comorbidities, such as metabolic-dysfunction associated steatosis liver disease (MASLD), which could be indicated by higher ALT in the patients with < 4.0log copies/ml. In chronic hepatitis B virus (HBV) infection, for example, both MASLD and higher BMI are known factors associated with earlier and more frequent HBsAg seroclearance[22,23]. Similarly, these conditions could promote more efficient control of TTV replication by the immune system and lower viremia. Also, a mechanistic explanation of the differences in the results of our study and those of the previous study[21] could be provided by the specific alteration of adipose tissue in T2D patients independent of BMI, where there is a higher prevalence of enlarged adipocytes[24]. These large adipocytes highly express major histocompatibility complex class II (MHC-II) and function as antigen presenting cells to stimulate interferon (IFN)-γ-expressing $CD4^+$ T cells[25], which could stimulate the immune control of TTV-replication. Lastly, anti-platelet therapy with low-dose aspirin was more prevalent in patients with TTVv ≥ 4.0log copies/ml, which could be explained, consistently with the previous findings, by its anti-inflammatory properties[26].

Both in the overall cohort and in T2D patients there was no association between HbA1c and viremia. However, we found that T2D patients with higher HbA1c (8%/≥64 mmol/mol) exhibited lower TTVv (Table 3). This degree of glycemic decompensation has deep implications on the immune system[27]. It could be argued that, since HbA1c is associated with high levels of circulating pro-inflammatory cytokines[28], as previously discussed above for the role of obesity, the *meta-inflammation* could promote the clearance of TTV infection. This hypothesis is challenged by the data of the MARK-AGE study, where both patients with and without ischemic heart disease and TTVv ≥ 4.0log copies/ml showed higher levels of pro-inflammatory cytokines (IFN-γ, interleukin-IL-1β, IL-6, IL-10, IL-12p70, and tumor necrosis factor-TNF-α)[19].Therefore, we may speculate the existence of a diabetes-specific mechanism, beyond what has already been discussed in relation to obesity, since the effect of HbA1c remained significant after adjusting for BMI. Ex vivo studies fromperipheral blood mononuclear cells derived from patients with diabetes and in vitro studies withhigh glucose medium or glycation products show reduced cytokine production in response to inflammatory stimuli[29–31]. These data suggest that reduced levels of cytokine production in T2D patients with high HbA1c may explain the different association between inflammation and TTVv found in the MARK-AGE study in respect of ourstudy. Intriguingly, in a cohort of newly diagnosed T2D with HbA1c > 11%,the nuclear factor-kappa B (NF-κB) signaling pathway, which is directly suppressed by the TTV protein open reading frame (ORF)-2[32], was found to be enriched and it was reduced after 1 week of insulin therapy[33]. Lastly, T lymphocytes, a suggested site of TTV replication[34], show reduced antigen-specific proliferation and compromised production of pro-inflammatory cytokines when lacking insulin receptor expression[35]. Taken together, these findings point to a U-shaped immunometabolic model. TTV viremia is generally higher in diabetes, thus suggesting weaker antiviral control in a state of metabolic stress, but on the contrary, it tends to decline with poor glycaemic control and obesity in T2D, possibly reflecting a stronger status of inflammation and/or of tissue immunomodulation that affects viral replication. This "directionality paradox" supports viewing TTV as a marker of immune imbalance, rather than of simple suppression or activation. In T2D the combination of poor glycaemic control and systemic inflammation appears to influence antiviral control, resulting in lower TTV levels. Thus, diabetes seems to modify the relationship between adiposity and TTV through immunometabolic mechanisms that are disease specific. This may be tested prospectively in longitudinal and interventional studies with serial TTV quantification alongside immune phenotyping (e.g., cytokine responses and T-cell/NK-cell activation/exhaustion markers).Again, at the same time, results should also be interpreted considering findings from other chronic conditions, such as rheumatoid arthritis and hepatic diseases[36,37], where sometimes no differences in TTV load and significant individual variability were found. This suggests that TTV acts in a context-dependent way and may not serve as a consistent predictor across different diabetes phenotypes.

However, other mechanisms could mediate the association between glycemic control and TTVv. For example, there could be a direct effect of substances like glucose, methylglyoxal, advanced glycation products or palmitate, which are highly represented in the plasma of patients with decompensated diabetes, on TTV

replication. However, it would be difficult to test this hypothesis as currently there is no efficient culture system to support TTV replication[1]. Also, in these patients there could be an alteration in the hematopoietic stem cell (hSC) compartments, which is considered one of the main replication site for TTV[38]. In fact, T2D and its complication, both microvascular and macrovascular, are associated with the presence of clonal hematopoiesis of indeterminate significant (CHIP), an age-related condition characterized by the presence of somatic mutations in hSC[39].

In T2D patients, DPP-IVi treatment was an independent predictor of the presence of viremia, although with no difference in TTVv levels. This could be explained by the expression of this enzyme also on the surface of various immune cells, (particularly on T lymphocytes), where it is referred to as CD26[40]. The immunomodulatory effect of these drugs has been largely studied in model of T1D and insulitis[41]. However, to the best of our knowledge, we did not find any study exploring the role of CD26 in the control of TTV replication. Nevertheless, large-scale meta-analyses have consistently shown no increased risk of infections associated with DPP-IVi use[42], even in elderly or vulnerable populations[43]. Also, GLP1-RA were found to be associated with TTVv (Table 4). However, despite multivariable adjustment for age, sex, BMI, and HbA1c, a residual selection bias related to real-world clinical decision-making and guideline-driven prescribing patterns may persist and should be considered when interpreting these associations for each class of drug evaluated. Also, there could be residual confounding effect in the association by exposure duration, other effects of drugs such weight loss or clinical phenotype that cannot be ruled out in this study.

Our study is limited by the relatively small sample size, and by its cross-sectional design. For example, it would be of interest to evaluate the association between TTVv and long-term outcomes in patients with diabetes, in terms of microvascular and macrovascular complications, glycemic control and overall mortality. Also, since in the general population TTVv level is considered relatively stable over a period of up to 2 years[17], the association between variation in HbA1c over time and TTVv would need to be further explored. The relatively small T1D sample size ($n=61$) limits power for within-T1D associations and between-group comparisons involving T1D; however, exclusion of T1D in sensitivity analyses did not alter the main findings in T2D vs. ND, supporting robustness of the observed immunometabolic patterns. Another limitation is the lack of data about inflammatory markers and immunophenotyping of circulating immune cells, which would help to elucidate the association between TTVv and clinical characteristics of patients with diabetes.Because TTV was quantified on fasting samples obtained before any metabolic challenge, OGTT/MMTT is unlikely to directly affect TTV measures; observed between- cohort differences more plausibly reflect center-level heterogeneity. Center-adjusted and cohort-restricted sensitivity analyses confirmed that TTV detection/positivity alone is not discriminatory, whereas quantitative viral load carries the more informative signal in T2D. At the same time, the magnitude of the T2D-control viral-load contrast was center-dependent, supporting cautious interpretation of pooled estimates and underscoring the importance of fully standardized multicenter workflows in future prospective studies.

In our study, OGTT/MMTT-derived indices were not associated with TTVv; however, these indices do not capture day-to-day glycaemic variability and are therefore not suitable for assessing the impact of glycaemic variability on immunocompetence[44]. Future studies integrating TTVv with CGM-derived dynamic markers and CGM-based machine learning models, together with immunological and genetic information, may enable improved differentiation between diabetes phenotypes and more precise tracking of disease progression across the diabetes spectrum[45–47].

In conclusion, this study explored the association of TTVv with clinical characteristics of patients with diabetes, highlighting specific and complex immune-metabolic interactions. Diabetes and components of metabolic syndrome predict the presence of a detectable TTVv, whereas obesity in this context of metabolic derangement and glycemic decompensation is instead associated with lower TTVv. Taking together, these data suggest an intricate and U-shaped relationship between immune status, chronic inflammation and TTVv that needs to be further explored to evaluate the role of TTV as a prognostic marker in this clinical context.

Data availability

The data that support the findings of this study are not openly available due to reasons of sensitivity and are available from the corresponding author upon reasonable request.

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Author contributions
PGS performed TTV quantification. SC drafted the manuscript, performed the statistical analyses and participated in the interpretation of the data. CM performed TTV quantification. DF conceived the study design and participated in the interpretation of the data. MP collected the data. MB, GG, GC, LC, GDG contributed to the data collection. VSB handled part of the blood samples. FN performed TTV quantification. ER contributed to the data collection, performed the statistical analyses and participated in the interpretation of the data. PN, MJH contributed to the data collection. AT analyzed metabolic parameters. AD contributed to the data collection and participated in interpretation of the data. TM contributed to the data collection. FM conceived the study design and participated in the interpretation of the data. All authors performed critical revision of the text and accepted the final version of the text. GD conceived the study design, contributed to the data collection, performed the statistical analyses and participated in the interpretation of the data. GD had full access to the data and is the guarantor of the work.

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Declarations

Competing interests
The authors declare no competing interests.

Additional information
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