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High Torque teno virus viremia predicts long-term mortality and reflects chronic low-grade inflammation (inflammaging) in geriatric inpatients

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ABSTRACT

Torque teno virus (TTV) is a ubiquitous virus whose viremia increases in conditions of immune dysfunction and aging, suggesting its potential role as a biomarker of immunosenescence. This study investigated the association between TTV viremia and all-cause mortality risk over seven years in a hospitalized older cohort, and its relationship with inflammatory markers including osteopontin (OPN) and growth differentiation factor 15 (GDF15). Data from 956 patients were analyzed, with high TTV load defined as ≥5 log DNA copies/mL. High TTV viremia was significantly associated with increased mortality risk at 1, 3, and 7 years independently of age, sex, comorbidities, and inflammatory markers. In stratified analyses, this association was significant at one year in both males and females, but persisted at three and seven years only in males. The strongest association was observed in participants aged 80–89 years, remaining significant across all follow-up periods. When patients were stratified by a composite immune score reflecting degrees of immunosenescence, high TTV viremia predicted increased mortality among those with intermediate or severe immune dysfunction, persisting up to seven years in the most immunosenescent subgroup. Patients with elevated TTV loads exhibited increased erythrocyte sedimentation rate (ESR), decreased serum albumin and hemoglobin, and significantly higher plasma levels of OPN and GDF15, whereas IL-10 tended to decrease. No significant differences were observed for neutrophil-to-lymphocyte ratio, IL-6, CD163, CCL22, or CXCL9 between high and low TTV viremia groups. These findings indicate that high TTV viremia independently predicts mortality risk and reflects a pro-inflammatory and immunosenescent state.

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1. Introduction

Torque teno virus (TTV), from *torques* and *tenuis*, Latin for 'necklace' and 'thin,' respectively, is a small, non-enveloped, single-stranded circular DNA virus belonging to the the *Anelloviridae* family. The discovery of TTV and other members of the *Anelloviridae* family led to the concept of "commensal viruses", defined as components of the human virome that are not clearly associated with disease. TTV has a small viral genome (3.8 kb), containing at least four partially overlapping open reading frames (ORFs) and a GC-rich region (Spandole et al., 2015). The untranslated region of genome (UTR) is highly conserved, whereas the coding regions exhibit extensive genetic variability (Bendinelli et al., 2001). Due to its high prevalence (70–90 %) in the global population TTV is generally considered to be a nonpathogenic virus, however, higher viral loads have been observed in various conditions involving altered immune function, including chronic viral infections, immunodeficiency, and aging (Abbate et al., 2023; Focosi et al., 2023; Giacconi et al., 2024; Gore et al., 2023). Like other components of the human virome, TTV replication is tightly regulated by the host immune system (Sabbaghian et al., 2024). Notably, current antiviral drugs do not affect TTV replication or viral load (Focosi et al., 2014; Peker et al., 2020), further supporting the notion that its dynamics mainly reflect the status of host immune control. For these reasons, measuring TTV viral load in the blood is emerging as a valuable biomarker for immune competence and dysregulation (Giacconi et al., 2023; Sabbaghian et al., 2024; Xie et al., 2021). Furthermore, it is being investigated as a promising predictor of graft rejection and infection risk after solid organ transplantation (Mrzljak and Vilibic-Cavlek, 2020; van Rijn et al., 2023; Zeng et al., 2023).

During aging, the immune system undergoes a gradual decline in the composition and function of immune organs and immune cells, along with dysregulated cytokine production, a process known as immunosenescence (Oh et al., 2019). Immunosenescent cells acquire a distinctive senescence-associated secretory phenotype (SASP) characterized by the secretion of various soluble factors, including cytokines, chemokines, growth factors, and proteases (Chandrasekaran et al., 2017; Pole et al., 2016; Saavedra et al., 2023). This altered "secretoma" contributes to chronic low-grade systemic inflammation, (*inflammaging*) (Franceschi et al., 2000; Fulop et al., 2017), tissue remodeling, and the age-related decline in immune function.

Several studies have shown that some viruses' DNA, including TTV, can directly or indirectly activate various immune cell types, and stimulate the production of pro-inflammatory cytokines, including interferon (IFN)γ, interleukin (IL) 6, and IL12 (Hayashi et al., 2006; Rocchi et al., 2009; Takeuchi and Akira, 2009).

To further investigate the link between TTV viremia and immune dysregulation, we focused on two immunomodulatory proteins with distinct but complementary roles in the inflammatory response: osteopontin (OPN) and growth differentiation factor 15 (GDF15). These markers were selected not only for their relevance in aging and chronic diseases, but also because they may reflect different facets of the immune response to chronic stress. OPN is a multifunctional phosphoglycoprotein involved in immune cell recruitment, activation, and cytokine production. It acts as a pro-inflammatory mediator in various pathological contexts, including atherosclerosis, viral infections, and cancer (Choi et al., 2014; Lok and Lyle, 2019; Moorman et al., 2020). In contrast, GDF15 is primarily considered a stress-induced cytokine, with anti-inflammatory properties, upregulated in response to mitochondrial dysfunction, cellular stress, and tissue injury (Basisty et al., 2020; Luan et al., 2019; Tsai et al., 2018; Wollert et al., 2017). Its elevation is interpreted as a protective, compensatory mechanism aimed at limiting excessive inflammation and promoting tissue tolerance (Luan et al., 2019). However, persistently elevated levels of GDF15 have been associated with poor outcomes in several chronic diseases, likely reflecting ongoing systemic stress and immune exhaustion (Lok and Lyle, 2019; Moorman et al., 2020).

Despite their clinical and biological significance, the relationship between TTV viremia and these two markers remains unexplored. Clarifying this link could reveal new insights into the immunoinflammatory mechanisms underlying TTV replication, immune senescence, and mortality risk in older adults.

This line of investigation is supported by emerging evidence linking higher TTV loads to immune dysfunction and poor clinical outcomes. For example, a recent study demonstrated an association between elevated TTV viremia and disease progression in patients with chronic obstructive pulmonary disease (Xie et al., 2021). In community-dwelling older adults, higher TTV viremia has been associated with an increased mortality risk, greater physical and cognitive frailty, elevated risk of ischemic heart disease (IHD), and an enhanced pro-inflammatory response, supporting the potential involvement of TTV in immunosenescence and age-related immune decline (Giacconi et al., 2023; Giacconi et al., 2018; Giacconi et al., 2024).

The purpose of this study was to investigate the association between TTV viremia and the risk for all-cause mortality in a hospitalized older cohort over a seven-year follow-up period, as well as to explore its relationship with inflammatory markers, including the potential immunomodulatory roles of osteopontin (OPN) and growth differentiation factor 15 (GDF15).

2. Materials and methods

2.1. Study population, recruitment, data, and blood collection

The Report-AGE project is a large-scale observational study involving older adults (mean age: 83.4 ± 7.0 years) hospitalized at the Italian National Institute of Health and Science on Aging (INRCA-IRCCS) (Trial Registration No. NCT01397682; (Bustacchini et al., 2015); Ethics Committee No CE INRCA 20031,04/02/2021).

In the present analysis, we used peripheral blood samples from 956 participants, collected within the first 24 h after hospital admission. Serum, plasma, and whole blood, were separated within 2 h, aliquoted and stored at −80 °C in the BioGer INRCA Biobank until analysis.

Only patients with two or more chronic conditions were included in the study. Additionally, to minimize statistical noise and reduce the risk of spurious associations, conditions with a prevalence below 2 % were not considered in the analysis.

Mortality was assessed at 1, 3, and 7 years. During the follow-up period, no participants received antiviral or immunomodulatory treatments that could have influenced TTV levels or study outcomes. Comorbidities were recorded and coded according to the International Classification of Diseases, Ninth Revision (ICD-9), including the following:

Hypertension: 401–405; Ischemic heart disease (IHD): 410–414; Congestive heart failure (CHF): 428; Cerebrovascular disease (CeVD): 430–438; Dementia: 290, 294, 331; Depression: 296; Chronic obstructive pulmonary disease (COPD): 491, 492, 494, 496; Parkinson's disease: 332; Chronic kidney disease (CKD): 585–587; Diabetes: 2500–2507; Cancer: 140–172, 174–195, 200–208. Multimorbidity was assessed using the Charlson Comorbidity Index (CCI). Frailty was evaluated using a Frailty Index (FI) based on the deficit accumulation model. A total of 30 health deficits were included (e.g., comorbidities, functional impairments, cognitive and sensory deficits, polypharmacy), with FI scores ranging from 0 (no deficits) to 1 (maximum deficits). FI was categorized as follows: non-frail (<0.10), prefrail (0.10–0.19), and frail (≥ 0.20)(Kim and Rockwood, 2024).

The study was approved by the local ethics committee, and all participants provided written informed consent.

2.2. TTV DNA detection and quantification

Viral DNA was extracted from whole blood samples using the QIAamp DNA Blood Mini Kit (Qiagen GmbH, Germany), following the

manufacturer's protocol. The presence and quantification of Torque teno virus (TTV) DNA were determined using a single-step, in-house TaqMan real-time PCR assay, as previously described (Maggi et al., 2005).

This "universal PCR" targets a highly conserved region within the untranslated region (UTR) of the TTV genome using specific forward and reverse primers, enabling quantification of the total TTV DNA load without discriminating between individual TTV species coexisting in the same sample.

Detailed procedures for viral DNA quantification, including assessments of assay specificity, sensitivity, intra- and inter-assay precision, and reproducibility, have been described elsewhere (Macera et al., 2022; Maggi et al., 2005). Briefly, the assay amplifies a 63-nucleotide fragment within the UTR and achieves a sensitivity of up to 10 viral genomes per mL of plasma or whole blood. The potential for inhibition was evaluated during the testing process. Inhibition was assessed by spectrophotometric analysis of extracted DNA (A260/280 ratio) and by spiking TTV-negative samples with plasmid DNA containing the target sequence (100 copies/μL, ~10× the detection limit) prior to extraction. Inhibition rates were calculated based on the failure to detect the target DNA-containing plasmid. The lower limit of detection was 1.0 log DNA copies/mL. TTV viremia equal to or exceeding 10,000 copies/mL (≥4 log copies/mL) was categorized as high-level viremia.

2.3. Clinical laboratory determinations

Peripheral venous blood samples were collected after an overnight fast and used for baseline biochemical analyses. Hemoglobin concentration, number and percentage of peripheral blood cells were determined by automated blood analyzers. All biochemical parameters, including albumin and venous erythrocyte sedimentation rate (ESR) were measured as part of routine laboratory analysis using standard laboratory methods. NLR (Neutrophil-to-Lymphocyte Ratio) was calculated for each patient dividing the absolute neutrophil count by the absolute lymphocyte count, as obtained from complete blood count. The SIRI index was calculated as the formula of neutrophil count * monocyte count/lymphocyte count. Estimated glomerular filtration rate (eGFR) was calculated using the BIS equation (Schaeffner et al., 2012).

2.4. Automated immunoassay for chemokine and cytokine serum assessment

Serum levels of IL6, IL10, GDF15, osteopontin, CCL22, CD163, CXCL9 were measured in triplicate using ELLA™ microfluidic immunoassays (ProteinSimple, Bio-Techne, USA) according with the manufacturer's specifications. Sensibility was: IL-6 (0.11 pg/mL), IL-10 (0.17 pg/mL) GDF15 (0.21 pg/mL), osteopontin (7.33 pg/mL), CCL22 (0.32 pg/mL), CD163 (3.23 pg/mL), and CXCL9 (8.8 pg/mL). Intra- assay coefficients of variation (CV) were all below 10 %. The calibration curve for each cartridge is generated by the manufacturer.

2.5. Statistical analysis

Descriptive statistics were used to summarize baseline characteristics, expressed as means ± standard deviations for continuous variables, and as frequencies and percentages for categorical variables. The Kolmogorov–Smirnov test was used to assess the normality of continuous variables. Comparisons between survivors and non-survivors were performed using the Student's *t*-test or Mann–Whitney *U* test for continuous variables, depending on data distribution, and the chi-square test for categorical variables. Kaplan–Meier survival curves and the log-rank test were used to evaluate differences in all-cause mortality across TTV viral load categories at 1, 3, and 7 years. Cox proportional hazards regression models were applied to assess the association between TTV DNA levels and mortality risk, adjusting for potential confounders including age, sex, medication count at discharge, frailty index, major diseases associated with mortality (Table 1), neutrophil count, albumin, eGFR, hemoglobin and ESR. A threshold of ≥5 log DNA copies/mL was used to define high TTV load based on previous literature (Russo et al., 2025), both for survival analysis and for evaluating changes in circulating inflammatory markers. Variables significantly associated with mortality in univariate analysis (Table 1) were included in multivariable models. Statistical significance was defined as $p < 0.05$. Analyses were performed using the SPSS/Win program (Version 29.0.1.0 (171); SPSS Inc., Chicago, IL).

3. Results

3.1. Characteristics of the study population

Among the 956 subjects studied, 172 individuals (18.0 %) died within one year of admission, 334 (34.9 %) within three years and 619 (64.7 %) within seven years of follow up (Table S1, Supplementary Material). Table 1 summarizes the characteristics of the study population, stratified by survival status. No significant differences were observed in sex distribution, lymphocytes count, neutrophil-to-

Table 1
Clinical characteristics of patients from the Report-Age study stratified by survival status.

	Survived N. 784	Deceased * N. 172	*P* value
Age (yrs) mean ± SD	82.5 ± 7.0	87.0 ± 5.8	<0.001
Males (%)	377 (48.0 %)	79 (45.9 %)	NS
Diabetes n (%)	172 (21.9 %)	37 (21.5 %)	NS
Hypertension n (%)	484 (61.7 %)	106 (61.6 %)	NS
AF (%)	143 (18.2 %)	47 (27.3 %)	0.007
IHD (%)	100 (12.8 %)	34 (19.8 %)	0.016
CKD (%)	163 (20.8 %)	61 (35.5 %)	<0.001
COPD (%)	99 (12.6 %)	42 (24.4 %)	<0.001
Cancer (%)	97 (12.4 %)	48 (27.9 %)	<0.001
Infections (%)	131 (16.7 %)	57 (33.1 %)	<0.001
Parkinson (%)	75 (9.6 %)	9 (5.2 %)	NS
Depression (%)	36 (4.6 %)	8 (4.7 %)	NS
Dementia (%)	201 (25.6 %)	35 (20.3 %)	NS
Stroke (%)	68 (8.7 %)	9 (5.2 %)	NS
Severe carotid artery stenosis (%)	41 (5.2 %)	6 (3.5 %)	NS
Osteoporosis (%)	54 (6.9 %)	11 (6.4 %)	NS
Gastrointestinal disorders (%)	123 (15.7 %)	19 (11.0 %)	NS
Hepatobiliary disorders (%)	78 (9.9 %)	11 (6.4 %)	NS
Hypothyroidism (%)	50 (6.4 %)	18 (10.5 %)	NS
Genito-urinary disorders (%)	286 (36.5 %)	70 (40.7 %)	NS
Number of pathologies mean ± SD	3.6 ± 1.8	4.7 ± 2.2	<0.001
Frailty Index, n(%)			
< 0.10	176 (22.4 %)	9 (5.2 %)	<0.001
0.10–0.19	304 (38.7 %)	39 (22.7 %)	
≥ 0.20	303 (38.5 %)	122 (70.9 %)	
RBC (x10^6/μlL)° mean ± SEM	4.11 ± 0.02	3.85 ± 0.04	<0.001
Hemoglobin (g/dl)° mean ± SEM	12.1 ± 0.1	11.3 ± 0.1	<0.001
NLR° mean ± SEM	5.69 ± 0.43	6.92 ± 0.61	NS
Neutrophils (x103/μL)° mean ± SEM	5.48 ± 0.12	6.78 ± 0.27	<0.001
Lymphocytes (x103/μL)° mean ± SEM	1.54 ± 0.03	1.43 ± 0.06	NS
Creatinine (mg/dL)° mean ± SEM	1.19 ± 0.03	1.47 ± 0.07	<0.001
eGFR°, mL/min/1.73 m^2 mean ± SEM	57.9 ± 0.8	49.8 ± 1.7	<0.001
ESR° (mm/h) mean ± SEM	34.5 ± 0.9	44.4 ± 2.0	<0.001
SIRI index° mean ± SEM	2.76 ± 0.17	3.75 ± 0.37	<0.01
TTV viral load° (log n. copies/mL) mean ± SEM	4.49 ± 0.03	4.81 ± 0.08	0.012
CMV° (U/mL) mean ± SEM	219.1 ± 10.5	253.2 ± 22.9	NS

AF atrial fibrillation; CHF: congestive heart failure; CKD: chronic kidney disease; COPD: Chronic obstructive pulmonary disease; eGFR estimated glomerular filtration rate; ESR Erythrocyte Sedimentation Rate; IHD: ischemic heart disease; NLR Neutrophil-to-lymphocyte ratio; RBC Red blood cells.
° ANCOVA analysis correcting for age and sex.
* one year after hospitalization.

lymphocyte ratio (NLR) and CMV IgG levels. The prevalence of hypertension, diabetes, dementia and stroke, Parkinson's disease, depression, severe carotid artery stenosis, osteoporosis, gastrointestinal disorders, hepatobiliary disorders, hypothyroidism, and genitourinary disorders was also comparable between the two groups. However, significant differences were observed in age, frailty index number of pathologies and in the prevalence of atrial fibrillation (AF), ischemic heart disease (IHD), chronic kidney disease (CKD), chronic obstructive pulmonary disease (COPD), infections and cancer. Additionally, individuals in the deceased group had significantly lower hemoglobin levels, red blood cell counts and estimated glomerular filtration rate (eGFR), ($p < 0.001$). Conversely, the deceased group showed significantly higher levels of neutrophil count, creatinine, erythrocyte sedimentation rate (ESR) ($p < 0.001$) and TTV viral load ($p < 0.05$) compared to survivors.

3.2. Survival analysis

The association between TTV viral load and all-cause mortality at one, three and seven years was assessed using Kaplan–Meier survival curves. TTV viremia was measured at baseline and a threshold of ≥5 log DNA copies/mL was used to define high TTV load, based on prior evidence (Russo et al., 2025). In a previous study conducted in community-dwelling older adults we identified a lower cut-off of 4 log viral DNA copies per μg of genomic DNA (extracted polymorphonuclear leukocytes), as predictive of increased all-cause mortality at three years (Giacconi et al., 2018). However, in hospitalized geriatric patients, TTV viremia levels are generally higher, likely reflecting more pronounced immune dysfunction and reduced immune surveillance (Giacconi et al., 2020; Giacconi et al., 2024). In addition, a recent study highlights the prognostic utility of TTV viremia when integrated with machine learning approaches (Querido et al., 2025). The authors report that TTV viremia may help assess infection risk in kidney transplant recipients and identified a TTV threshold of log 5 as a practical tool for clinical decision-making. Therefore, a threshold ≥5 log copies/mL (measure in whole blood) was considered more appropriate for this frail, acutely ill population, in line with findings from a recent study in a similar clinical setting (Russo et al., 2025). Using this threshold, a significant difference in survival was observed across all time points for individuals with TTV load ≥5 log DNA copies/mL (log-rank test: $p < 0.0001$; Fig. 1). In contrast, no survival differences were observed between subjects with intermediate TTV loads ranging from 4.12 to 4.99 log copies/mL and subjects with TTV loads <4.12 log copies/mL (Fig. S1 Supplementary material).

Cox proportional hazards models confirmed that, a TTV viral load ≥5 log copies/mL was independently associated with increased mortality

Table 2
Cox Regression for Mortality according to TTV viremia.

1-Year Mortality	Model 1	Model 2	Model 3
	HR (95 %CI)	HR (95 %CI)	HR (95 %CI)
Log TTV viremia ref. ≤4.99			
≥ 5.00	1.66 (1.15–2.38)	1.81 (1.25–2.62)	1.65 (1.19–2.31)
3-Year Mortality			
Log TTV viremia ref. ≤4.99			
≥ 5.00	1.42 (1.14–1.77)	1.51 (1.21–1.89)	1.34 (1.05–1.70)
7-Year Mortality			
Log TTV viremia ref. ≤4.99			
≥ 5.00	1.28 (1.09–1.51)	1.34 (1.13–1.58)	1.23 (1.03–1.48)

Model 1: age, sex, medication count at discharge and frailty adjusted.
Model 2: Model 1 and pathologies adjusted.
Model 3: Model 2 adjusted for neutrophil count, albumin, eGFR, hemoglobin and ESR.

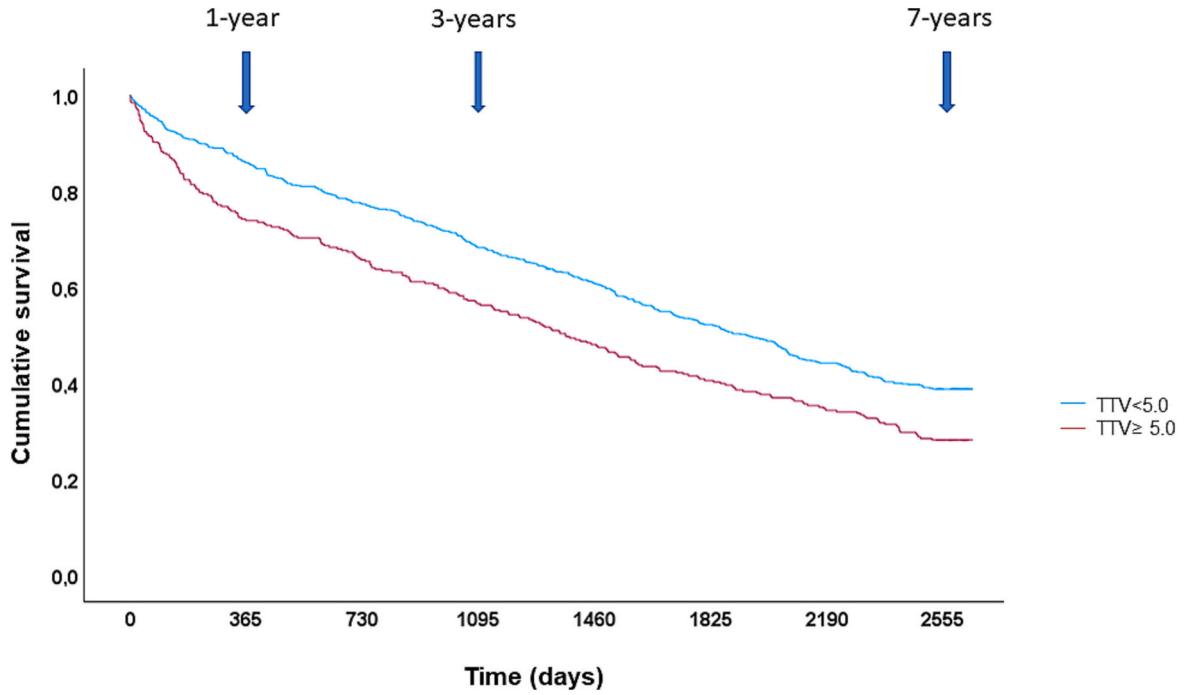

Fig. 1. Kaplan-Meier survival estimates according to TTV DNA loads in the Report-AGE cohort.
TTV viral load categories were defined as follows: TTV cat 1 = TTV log copies/mL < 5.0; TTV cat 2 = TTV log copies/mL ≥ 5.0. This figure represents Kaplan-Meier survival curve comparing all-cause-mortality between TTV log copies/mL < 5.0 (blue line) and TTV log copies/mL ≥ 5.0 (red line) at 1-year, 3-year, and 7-year follow-up.
Log-rank test of equality of survivor functions: $p < 0.001$ (for each time point). (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

risk at one year (HR: 1.81; 95 %CI: 1.25–2.62) after adjustment for age, sex, medication count at discharge, frailty, and comorbidities (Table 2, model 2). Significant differences were confirmed in an additional model constructed including further variables significantly associated with mortality in Table 1 (HR: 1.54; 95 %CI: 1.04–2.26; Table 2, model 3). Notably, in all models, the mortality risk associated with TTV loads ≥5 log copies decreased over time, although it remained significant at the 7-year follow-up (HR: 1.23; 95 %CI: 1.03–1.48; Table 2, model 3). This pattern may reflect a dilution of the effect over longer time periods, rather than a linear decrease in risk. In contrast, intermediate TTV DNA loads (4.12–4.99 log copies/mL) were not associated with differences in survival (Table S2, Supplementary Material). No significant association was observed between high TTV viremia and frailty status (Fig. S2). To validate whether the association between TTV viremia and mortality was independent of established inflammatory biomarkers, additional Cox regression analyses were performed including CRP and IL-6 as covariates. The results confirmed that high TTV viremia (≥5 log DNA copies/mL) remained significantly associated with mortality at 1-, 3-, and 7-year follow-up, even after adjustment for CRP and IL-6 levels (Table S3, Supplementary Material). Subgroup analyses (adjusted for age, sex, medication count at discharge, frailty, and comorbidity) were performed according to sex, age, and estimated immune status. Stratification by sex confirmed that the increased mortality risk associated with TTV ≥5 log copies/mL at one year was significant in both males and females (HR: 1.82, 95 % CI: 1.10–3.00; HR: 1.62, 95 % CI: 1.02–2.58, respectively), whereas at three and seven years significance persisted only in males (HR: 1.58, 95 % CI: 1.13–2.21; HR: 1.33, 95 % CI: 1.03–1.71, respectively) (Table S4, Supplementary Material). Age-stratified analyses showed that the association was significant in the 80–89-year age group at all follow-up time points (HR: 2.03, 95 % CI: 1.32–3.13; HR: 1.67, 95 % CI: 1.25–2.23; HR: 1.39, 95 % CI: 1.12–1.73, at one, three, and seven years, respectively), but not in the 60–79 or 90–103-year groups (Table S5, Supplementary Material). To estimate immune status in the absence of immune cell subpopulation data, z-scores were calculated for IL-6, IL-10, OPN, GDF-15, CRP, NLR, WBC, lymphocyte count, and inversed albumin. These standardized values were combined into a composite immune score, which was categorized into tertiles reflecting mild, intermediate, and severe degrees of immunosenescence. In analyses stratified by immune status, a TTV load ≥5 log copies/mL was significantly associated with increased mortality risk at one year among patients with intermediate or severe immunosenescence (HR:1.99, 95 % CI: 1.18–3.38; HR: 1.55, 95 % CI: 1.03–2.34), and at three and seven years among those with severe immunosenescence only (HR: 1.47, 95 % CI: 1.05–2.05); HR:1.37, 95 % CI: 1.05–1.80), (Table S6, Supplementary Material).

3.3. TTV and inflammatory markers

To investigate the presence of chronic inflammation potentially associated with TTV, key blood inflammatory markers were measured. A significant increase in erythrocyte sedimentation rate (ESR) was observed in patients with TTV viral loads ≥5 log copies/mL compared to those with TTV viremia <5 log copies/mL ($p < 0.05$), accompanied by a significant decrease in both serum albumin and hemoglobin levels ($p < 0.01$). Notably, conditions potentially causing acute reductions in hemoglobin and albumin, such as sepsis, pneumonia, or gastrointestinal bleeding, were present in less than 15 % of the patients, indicating that most reductions reflected chronic conditions rather than acute illness. No significant difference was found in the NLR according to TTV viremia (Fig. 2).

Additionally, a panel of key pro- and anti-inflammatory cytokines and chemokines and other inflammatory mediators was assessed in serum samples, as shown in Fig. 3. Among these, serum OPN and GDF15 levels were significantly elevated in patients with higher TTV viremia (Fig. 3 A and B, $p < 0.001$). To further investigate these associations, linear regression analysis was performed using TTV viremia as a continuous variable. As shown in Table S7 (Supplementary Material), significant associations were confirmed for albumin, hemoglobin, OPN, and GDF15 ($p < 0.05$), supporting a consistent linear relationship between TTV viral load and these inflammatory markers.

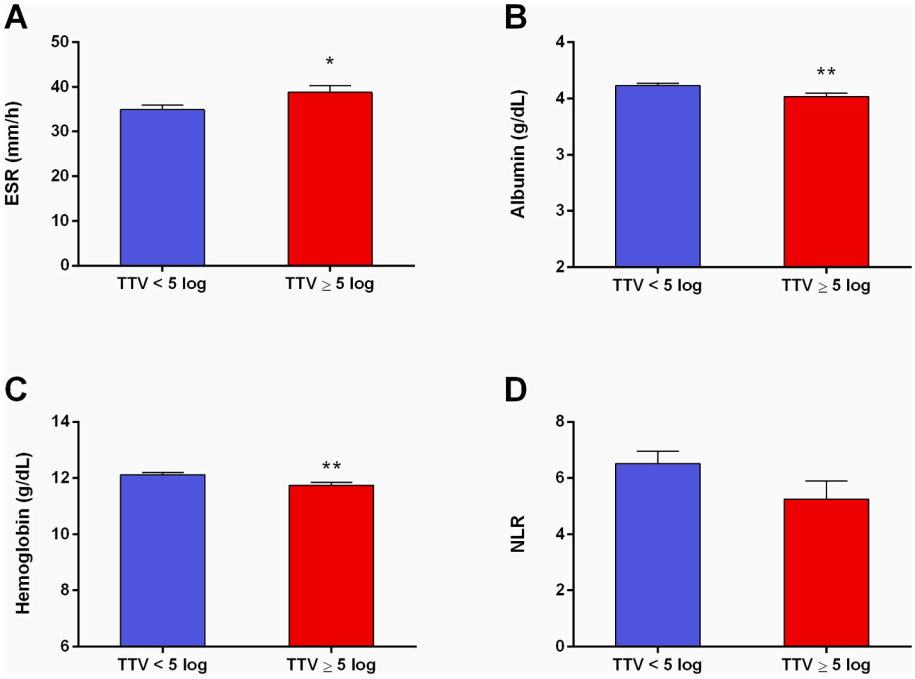

Fig. 2. Clinical inflammatory markers in the Report-AGE cohort according to TTV viral load.
Key blood inflammatory markers were measured in plasma sample of Report-AGE cohort. Increased TTV viral load was associated with elevated ESR (A) and reduced serum albumin and hemoglobin levels (B, C). Data are estimated marginal means and standard error.
* $p < 0.05$ and **$p < 0$ 0.01 ANCOVA analysis correcting for age, sex, and FI
ESR: erythrocyte sedimentation rate; NLR: neutrophils-lymphocyte ratio; FI: frailty index.

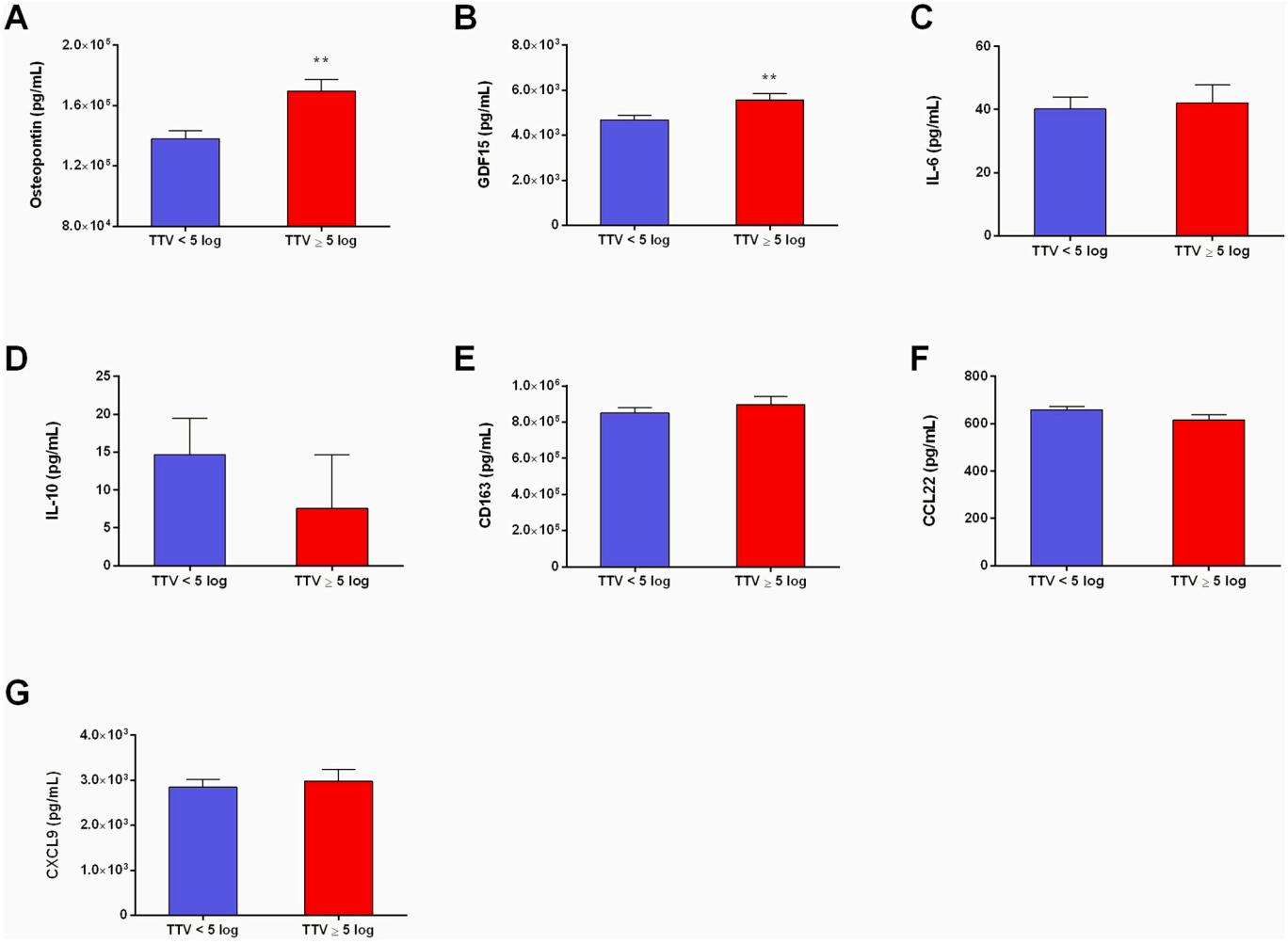

Fig. 3. Plasma cytokines and chemokines in the Report-AGE cohort according to TTV viral load
* $p < 0.05$ and **$p < 0$ 0.01 ANCOVA analysis correcting for age, sex, and FI
GDF15: Growth **D**ifferentiation **F**actor **15; IL:** Interleukin; **CCL22: C**hemokine (C—C motif) **L**igand **22; CXCL9:** Chemokine (**C-X-C** motif) **L**igand 9; CD163: **C**luster of **D**ifferentiation 163.

4. Discussion

TTV is generally considered to be a non-pathogenic virus due to its high global prevalence. In healthy populations, TTV loads are typically low, ranging from 2.3 to 2.8 log10 copies/mL, with no sex difference (Focosi et al., 2023). However, elevated levels of TTV viremia have been detected in various conditions involving immune system dysfunction, including chronic viral infections, immunodeficiency and aging (Giacconi et al., 2018; Gore et al., 2023). In addition, TTV viral replication tends to increase with age and is believed to contribute to immune system dysregulation, playing a role in immunosenescence. Multiple studies have demonstrated a correlation between higher TTV loads and unfavorable clinical outcomes or disease progression, particularly in infectious and inflammatory conditions (Giacconi et al., 2018; Giacconi et al., 2024; Gore et al., 2023; Russo et al., 2025). This suggests that TTV viral load could be used as a potential marker of immune competence and disease severity.

In this work we investigated the association between high TTV load and mortality risk in hospitalized patients from Report-AGE study. Our results showed a significant association between high TTV viral load (defined by a threshold of ≥5 log DNA copies/mL) and mortality risk across all time point investigated. However, the lack of association at lower viral loads highlights the importance of defining appropriate viral load thresholds for effective risk stratification. These findings suggest that a very high TTV viral load may serve as an independent biomarker of increased mortality risk among older patients.

To investigate whether high viral load (threshold of ≥5 log DNA copies/mL) was associated with a systemic pro-inflammatory state, key blood-based inflammatory markers were measured. Patients with TTV viremia ≥5 log exhibited significantly increased ESR and reduced serum albumin and hemoglobin levels, indicating an underlying inflammatory state. Interestingly, although NLR tended to increase among non-survivors (Table 1), we did not detect a significant increase in NLR in patients with elevated TTV viremia. In fact, a slightly lower NLR was observed in the high-TTV group, although this difference did not reach statistical significance. This apparent discrepancy may reflect the fact that NLR and TTV viremia capture distinct, though related, aspects of immune dysregulation. While NLR is a dynamic marker that often reflects acute or subacute systemic inflammation and has been widely associated with short-term mortality risk (Di Rosa et al., 2023), TTV viremia is thought to reflect chronic immune dysfunction and reduced immunosurveillance, particularly in the context of aging (Giacconi et al., 2018; Giacconi et al., 2020; Giacconi et al., 2024; Xie et al., 2021). Moreover, it is possible that the high-TTV group included patients with lower-grade or 'exhausted' inflammatory responses, which may not translate into elevated NLR. Finally, the high burden of multimorbidity and the clinical heterogeneity within our cohort may have attenuated the strength of associations in stratified analyses, not only for NLR, but

also for other inflammatory markers that did not show significant differences between TTV viremia groups. Further studies are needed to explore the complex interplay between acute inflammatory responses, immunosenescence, and TTV replication dynamics. Notably, high TTV viremia was also associated with elevated levels of osteopontin (OPN), a multifunctional matricellular protein involved in various biological processes, including bone remodeling, immune regulation, and tissue repair (Choi et al., 2014; Denhardt et al., 2001; Singh et al., 2018). Additionally, OPN has been implicated in the pathogenesis and progression of various diseases, such as atherosclerosis, glomerulonephritis, cancer, and chronic inflammatory conditions (Agnholt et al., 2007; Lok and Lyle, 2019; Lund et al., 2009). OPN exists both as a component of the extracellular matrix and as a soluble cytokine. As a potential immunomodulatory factor, OPN acts as a pro-inflammatory cytokine involved in regulating cell adhesion, migration and survival. During cell-mediated immune defenses against bacterial and viral pathogens, OPN plays a critical role in modulating T cell and macrophage activity (Moorman et al., 2020). It promotes the production of IL-12, thereby enhancing Th1 responses, while concurrently suppressing the expression of IL-10, which helps to regulate immune inflammation (Ashkar et al., 2000). Our results demonstrated a significant increase in OPN levels associated with elevated TTV loads and a slight decrease in IL-10 levels that may reflect the immunomodulatory effects of OPN. However, the reduction in IL-10 was not statistically significant and could be influenced by other factors, indicating the need for further investigation. Several viruses, including hepatitis B and C virus, influenza, dengue virus, and human immunodeficiency virus (HIV) have been associated with elevated OPN levels, which often correlate with increased disease severity (Brown et al., 2011; Chagan-Yasutan et al., 2014; Choi et al., 2014; Morimoto et al., 2011; Zhao et al., 2008). In this work, we report for the first time an association between elevated levels OPN and high TTV viremia. Previous studies have demonstrated that OPN can facilitate viral replication and exacerbate disease progression (Choi et al., 2014; Iqbal et al., 2018; Wang et al., 2022), suggesting that a similar mechanism might contribute to TTV replication.

Further investigations are warranted to elucidate the nature of these relationships. We also observed elevated levels of GDF15 in patients with high TTV viremia. GDF15 is a multifunctional anti-inflammatory cytokine belonging to the transforming growth factor-β (TGF-β) superfamily, widely expressed in several tissues, including the liver, intestine, kidneys, and placenta (Coll et al., 2020; Hsiao et al., 2000; Wang et al., 2021). It is primarily considered a stress-responsive molecule, whose expression is upregulated in response to cellular stress and mitochondrial dysfunction. Elevated circulating GDF15 levels have been reported in a variety of pathological conditions, including heart and kidney failure, chronic liver disease, cancer, chronic inflammatory disorders, and mitochondrial diseases (Lockhart et al., 2020; Poulsen et al., 2020; Tsai et al., 2018; Yatsuga et al., 2015). The biological effects of GDF15 are highly context-dependent and vary according to the disease setting and stage. For instance, in acute myocardial infarction models, GDF15 exhibits anti-inflammatory properties by inhibiting the recruitment of myeloid cells (Kempf et al., 2011), whereas in atherosclerosis models, it promotes disease progression by amplifying pro-inflammatory responses (Bonaterra et al., 2012; de Jager et al., 2011). Recent research also suggests a role for GDF15 in viral pathogenesis, where it may facilitate viral replication by suppressing antiviral cytokines such as interferon lambda 1 (IFN-λ1/IL-29), thus promoting a more permissive environment for virus propagation (Sometani et al., 2024; Wu et al., 2018). Conversely, GDF15 may also contribute to tissue tolerance and recovery after infection by modulating cellular metabolism (Luan et al., 2019). This functional duality highlights the complexity of its immunoregulatory role. GDF15 is also recognized as a component of the senescence-associated secretory phenotype (SASP), further implicating it in age-related immune dysregulation (Basisty et al., 2020; Di Micco et al., 2021; Guo et al., 2019). In addition, GDF15 has been widely studied as a prognostic biomarker in cardiovascular diseases (CVD), including acute coronary syndrome, atrial fibrillation, heart failure, and ischemic heart disease (Gurgoze et al., 2023; Li et al., 2020), and has been shown to independently predict cardiovascular mortality even in community-dwelling populations (Wollert et al., 2017). Interestingly, in a previous study, we reported that high TTV viremia was associated with an increased risk of IHD in older adults (Giacconi et al., 2024), a relationship that may be partially mediated by enhanced pro-inflammatory pathways involving GDF15. This potential interplay between TTV viremia and GDF15, possibly converging on shared inflammatory or metabolic pathways, deserves further mechanistic investigation. In conclusion, our findings demonstrate that high TTV viremia is independently associated with an increased risk of long-term mortality in hospitalized older adults, supporting its utility as a prognostic biomarker of immune system dysfunction. For the first time, we also report significant associations between elevated TTV load and increased levels of osteopontin (OPN) and growth differentiation factor 15 (GDF15), two immunomodulatory proteins involved in chronic inflammation and age-related disease processes. These results suggest that OPN and GDF15 may contribute to a pro-inflammatory milieu that facilitates TTV replication and exacerbates immunosenescence-driven pathophysiology. Elucidating these potential pathways could advance our understanding of the virome–host immune interface and guide future mechanistic studies.

Beyond its biological significance, these findings also have potential clinical implications regarding the use of TTV viremia as a biomarker of immune competence. TTV viremia is already being introduced into clinical practice as a potential biomarker to monitor the effectiveness of immunosuppression, particularly in solid organ transplantation. Several studies have shown that TTV load correlates inversely with immune competence and may reflect the balance between infection risk and graft rejection (Gorzer et al., 2023; Haupenthal et al., 2023; Querido et al., 2025). However, important challenges remain before its implementation in the geriatric setting, including the lack of standardized assay methodologies, the need for clinical validation in aging populations, the absence of universally defined cut-offs, and the current lack of evidence that TTV testing improves outcomes in routine geriatric care.

This study has some limitations. First, its cross-sectional design precludes the assessment of temporal or causal relationships between TTV viremia, inflammatory biomarkers, and mortality. Although we observed significant associations, we cannot determine the directionality of these links or whether elevated TTV viremia contributes to, or results from, chronic inflammation and immune dysfunction. **Second,** TTV viremia was assessed only at baseline, and no longitudinal measurements were available. Consequently, we could not evaluate temporal changes or trends in viral load, which may influence the dynamics of immune function and clinical outcomes. **Third,** the study did not include serum zinc and copper measurements, although their ratio was previously found to correlate with TTV viremia in an earlier cohort (Giacconi et al., 2020). The copper/zinc ratio has been identified as a predictor of all-cause mortality and cardiac risk in aging populations (Giacconi et al., 2024; Malavolta et al., 2010), and zinc deficiency has been linked to impaired antiviral type I interferon responses (Vallboehmer et al., 2025). Future studies incorporating micronutrient biomarkers are warranted to clarify whether altered zinc homeostasis may contribute to TTV replication, inflammaging, and mortality in hospitalized older adults. **Finally,** an important limitation is the absence of mechanistic data to elucidate the causal pathways linking TTV viremia with increased OPN and GDF15 levels. Since this is an observational study and no in vitro or in vivo experiments were performed, we cannot demonstrate whether elevated TTV replication directly induces the upregulation of these immunomodulatory proteins or whether their co-elevation reflects a shared response to chronic inflammatory and metabolic stress. Future mechanistic studies, including cellular and molecular investigations of TTV–host interactions, will be essential to clarify whether OPN and GDF15 actively contribute to sustaining TTV replication and immune dysregulation in aging. Altogether, our study

reinforces the clinical relevance of TTV as a biomarker of immunosenescence and opens new avenues for exploring its role in aging and inflammation-related conditions.

Altogether, our study reinforces the clinical relevance of TTV as a biomarker of immunosenescence and highlights its potential utility for monitoring immune competence in older adults, paving the way for future mechanistic and translational research aimed at unraveling the complex interplay between the human virome and the aging immune system.

CRediT authorship contribution statement

Laura Cianfruglia: Writing – original draft, Methodology. **Gretta Veronica Badillo Pazmay:** Methodology. **Carlo Fortunato:** Validation, Software, Methodology, Data curation. **Pietro Giorgio Spezia:** Methodology, Data curation. **Federica Novazzi:** Methodology. **Francesco Piacenza:** Data curation. **Marco Malavolta:** Writing – review & editing. **Francesca Marchegiani:** Writing – review & editing. **Rina Recchioni:** Writing – review & editing, Methodology. **Giulia Matacchione:** Methodology. **Chiara Giordani:** Methodology. **Maurizio Cardelli:** Writing – review & editing. **Tiziana Casoli:** Writing – review & editing. **Mirko Di Rosa:** Software. **Antonio Cherubini:** Writing – review & editing. **Giuseppe Pelliccioni:** Resources. **Riccardo Sarzani:** Writing – review & editing. **Francesco Spannella:** Writing – review & editing. **Fabrizia Lattanzio:** Funding acquisition, Formal analysis. **Anna Rita Bonfigli:** Resources. **Fabiola Olivieri:** Writing – review & editing. **Jacopo Sabbatinelli:** Writing – review & editing. **Fabrizio Maggi:** Writing – review & editing, Supervision. **Robertina Giacconi:** Writing – review & editing, Writing – original draft, Software, Investigation, Data curation.

Author contributions

Conceptualization, R.G., L.C., Methodology, L.C., C.F., P.G.S., F.N., G.V.B.P., G.M., C.G., R.R., F.Mar. Software, R.G., C.F., M.D.R.; BioBank. A.R.B., Recruitment of Subjects: A.C., G.P., R.S., F.S., Validation, C.F.; Investigation, R.G., L.C.; Resources, F.O., F.M.; Data Curation, C.F., R.G., P.G.S., F.P., L.C.; F.Mar., R.R.; R.Ga, J.S.; Writing – Original Draft Preparation, R.G., L.C., Writing – Review & Editing, R.G., F.O., F.M., M. M., M.C., A.C., J.S., T.C., F.Mar.; Supervision, F.M.; Formal Analysis: F. L.; Project Administration, F.L.; Funding Acquisition, F.L. All authors have read and agreed to the published version of the manuscript.

Ethical approval

All data collection and analyses adhered to the principles outlined in the Declaration of Helsinki. The Ethical Committee of the Italian National Research Center on Aging approved the study protocol (Ethics Committee No. CE INRCA 20031, 04/02/2021). This protocol does not include any studies involving animals performed by the authors.

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Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.exger.2025.112978.

Data availability

Data will be made available on request.

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