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Original article

Torque Teno Virus quantification for monitoring of immunomodulation with biologic compounds in the treatment of rheumatoid arthritis

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Abstract

Objectives. RA patients who fail to respond to MTX can receive biologic dMARDs (bDMARDs). The Torque Teno Virus (TTV) is a potential novel candidate for monitoring of immunosuppression. We explore TTV in these patients and its association with clinical response to bDMARDs.

Methods. The BioBio Study is a multicentre randomized open-label trial, including RA patients with insufficient response to MTX. Patients were randomized to either TNFi (infliximab, INF), anti-IL-6 (tocilizumab, TCZ), CTLA4-Ig (abatacept, ABA) or anti-CD20 (rituximab, RTX) in addition to MTX. PCR was used to quantify TTV in the peripheral blood.

Results. TTV was measured in 95 patients (INF, $n = 23$; TCZ, $n = 22$; ABA, $n = 27$; RTX; $n = 23$). TTV increased by a median of 4.5×10^4 copies/ml [c/ml; interquartile range (IQR) 0–7.5×10^5] after 3 months. TTV levels at month 3 were associated with the Simplified Disease Activity Index (SDAI) ($P = 0.03$) and the Clinical Disease Activity Index (CDAI) response ($P = 0.026$) at month 6. A TTV cut-off level of 1.2×10^6 c/ml at month 3 had a positive likelihood ratio of 2.7 for prediction of an 85% reduction in SDAI at month 6.

Conclusion. Our data suggest that TTV levels increase upon TNF, CD20 and costimulation blockade and are associated with the clinical response to bDMARDs in RA patients.

Trial registration. ClinicalTrials.gov; https://clinicaltrials.gov; NCT01638715

Key words: rheumatoid arthritis, Torque Teno Virus, bDMARDs, outcomes, treatment response, methotrexate

Rheumatology key messages

- Torque Teno Virus (TTV) replication increases during treatment with rituximab, abatacept and TNFi.
- Higher TTV levels at 3 months coincide with better treatment response at 6 months.
- TTV seems to be an interesting candidate biomarker for monitoring of immunosuppression.

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Introduction

RA is an immune-mediated chronic inflammatory disease, mainly presenting with painful swelling and stiffness of synovial joints that, if untreated, leads to subsequent degradation of cartilage, destruction of bone and irreversible disability [1–4]. The anchor drug in treating RA is MTX, which should be started as early as possible, to prevent long-term damage by controlling inflammation and to restore quality of life [5–7]. Patients who fail to respond to MTX within the first 6 months are typically subjected to an addition of biologic DMARDs (bDMARDs) [8]. Currently, there are four main modes of action of bDMARDs available for treatment of RA: inhibitors of TNF alpha (TNFis), of the IL-6 receptor (anti-IL-6R), of T cell costimulation (CTLA4-Ig) and B cell–directed therapy (anti-CD20) [9]. All bDMARDs show comparable response rates at 6 months of treatment on the group levels in clinical trials of RA [9]. Differences in responses between individual patients are still unexplained, but may relate to variable levels of immunomodulation achieved by a given compound in a given patient. The lack of biomarkers predictive of a preferential treatment response is an unmet need [10], that currently leads to a rather arbitrary choice of one of the four modes of action after primary treatment failure with MTX [11, 12]. In the absence of predictive markers and in the setting of arbitrarily chosen compounds, the current challenge is to identify non-responders very quickly after they have been exposed to a certain compound, so that the time on insufficiently effective treatment is minimized.

Indeed, the early clinical response has so far been among the best predictors of achieving the treatment target of remission or low disease activity subsequently, but the predictive value is still not very high if this 3-month improvement does not exceed 80% [13, 14]. Currently, beyond the mere observation of a clinical response, or its absence, no biomarkers exist [15]. However, in other areas, such as solid organ transplantation, biomarkers for functional monitoring of the level of immunosuppression, such as peripheral blood copy number of the Torque Teno Virus (TTV), are promising candidates and are under clinical evaluation [16–18].

TTV is a non-pathogenic and highly prevalent virus that was first described in 1997 in posttransfusion hepatitis patients. It is a genetically highly heterogeneous circular, single-stranded DNA virus, mainly originating from the Anelloviridae family [19, 20]. The viral load has been shown to be dependent on age, gender, socio-economic status and immunosuppression of the host. It can be detected in up to 95% of healthy and up to 100% of immunosuppressed populations [21–23]. Aside from transplantation, TTV in the peripheral blood has also been shown to mirror the activity of the immune system of the host in HIV and malignancies. TTV load was associated with insufficient immunosuppression and thus the risk of allograft dysfunction, and with intense immunosuppression and consequently infection in patients after solid organ transplantation [16, 24–27]. In kidney and lung transplant patients, monitoring TTV to estimate the balance between the risk of infections and rejection is seen as a promising tool [17, 28–30].

With this background, quantification of the TTV load may be an attractive strategy for monitoring and/or predicting treatment response to bDMARDs in patients with RA. Based on the theory that TTV is associated with the immune function of its host, it might be indirectly associated with RA disease activity/the effectiveness of a bDMARD treatment. Here, using a randomized setting, we report on the responsiveness of TTV replication to four different modes of action of bDMARDs, which were thus initiated without any bias by indication, as well as the potential predictive value of TTV copy numbers with respect to later clinical responses in RA patients.

Methods

Study design and patients

We conducted a multicentre randomized open-label biomarker trial (BioBio Study; NCT01638715), including RA patients, who continued to have clinical disease activity index (CDAI) levels >14 after at least 6 months of MTX treatment with at least 20 mg weekly (alternatively LEF at 20 mg daily). Patients were randomized in a 1:1:1:1 ratio stratified by RF positivity (using a cut-point of 20 U/ml) to one of the four modes of action of bDMARDs (TNFi: infliximab, INF; anti-IL-6R: tocilizumab, TCZ; CTLA4-Ig: abatacept, ABA; anti-CD20: rituximab, RTX) in addition to ongoing MTX or LEF treatment. This allowed ruling out of bias by indication, a common problem in observational approaches to this topic.

Core set markers of disease activity and indicators of quality of life were collected at baseline, 3, 6, 9 and 12 months. Serum samples were collected at baseline, week 2 and month 3. This study was conducted adhering to good clinical practice guidelines and was approved by all ethics committees (ECs) of study sites (EC of the Medical University of Vienna, EC of the city of Vienna, EC of Oberösterreich, EC Ostschweiz, EC of Nasonova Research Institute of Rheumatology, EC of the Institute of Rheumatology Prague). All patients signed written informed consent before inclusion in the trial.

Real-world validation data

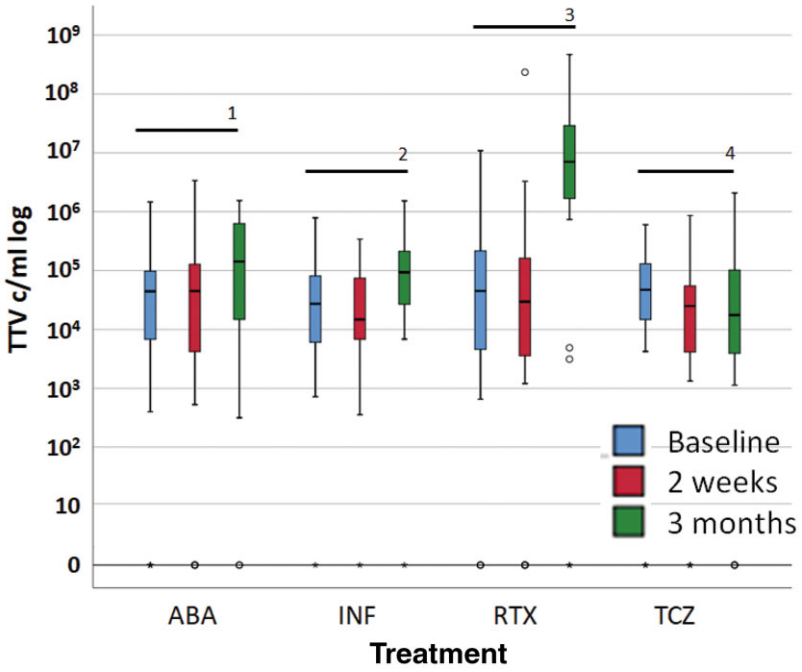
For validation of the results from the BioBio study, we identified RA patients from the longitudinal prospective RA database of the Division of Rheumatology of the Medical University of Vienna (EC-Nr: 2002/2014) [31, 32], who started a TNFi compound after insufficient MTX response with serum samples available in our biobank (EC-Nr: 559/2005) before TNFi-treatment initiation and between 50 and 120 days later for measurement for TTV. The validation was restricted to TNF inhibitors, since no other modes of action were commonly used as the first biologic compound in clinical practice.

Table 1 Descriptive characteristics at baseline and responses at 6 months

	Total	ABA	INF	RTX	TCZ
Baseline TTV; c/ml	4.2×10^4 (7.2×10^3 -1.1×10^5)	4.4×10^4 (6.4×10^3 -1.10×10^5)	2.7×10^4 (5.5×10^3 -9.4×10^4)	4.5×10^4 (2.4×10^3 -2.4×10^5)	4.7×10^4 (1.5×10^4–1.5×10^5)
TTV at 3 months; c/ml	1.1×10^5 (1.4×10^4 -1.4×10^6)	1.4×10^5 (1.3×10^4 -6.7×10^5)	8.4×10^4 (2.3×10^4 -1.7×10^5)	7.0×10^6 (1.4×10^6 -3.1×10^7)	1.8×10^4 (2.5×10^3 -1.1×10^5)
ΔTTV after 3 months; c/ml	4.5×10^4 (0–7.5×10^5)	1.1×10^5 (4.4×10^3–5.3×10^5)	5.4×10^4 (3.1×10^3 -8.8×10^4)	6.9×10^6 (1.4×10^6 -3.0×10^7)	-7.1×10^3 (-5.4×10^4 -8.9×10^3)
Female; %	76.8	81.5	73.9	73.9	77.3
HAQ	1 (0.8–1.5)	1 (0.6–1.4)	0.9 (0.8–1.5)	1 (0.8–1.5)	1.1 (0.8–1.6)
Pain (0–100 mm)	51 (32–70)	48 (38–70)	57 (40–70)	47 (24–58)	62 (30–73.3)
PGA (0–100 mm)	53.5 (40.5–68.5)	53 (42–70)	63 (48–69)	47 (25–59)	60.5 (37–68.8)
EGA (0–100 mm)	42 (31–60)	46 (31–61)	35 (27–57)	42 (33–51)	42 (30–64)
Fatigue (0–100 mm)	56 (36–69.5)	58 (38–75)	66 (38–75)	50 (33–65)	49.5 (34–68.3)
TJC28	8 (6–13.5)	10 (6–14)	8 (7–12)	10 (4–19)	8 (6–12.5)
SJC28	8 (5–11)	8 (6–10)	6 (4–10)	8 (5–10)	8 (5–12.3)
CRP; mg/dl	1.2 (0.3–2.4)	1.2 (0.2–2.3)	1.5 (0.4–2.8)	1.1 (0.4–2.2)	0.7 (0.2–3.7)
ESR	23.5 (13–40)	23 (12.8–40.3)	28 (20–43)	28 (11–58)	22 (8.5–35)
CCP; U/ml	49.6 (7–308.8)	44.8 (6.7–298.6)	49.6 (6.2–217)	59.3 (2–300)	146.5 (5.5–302.2)
RF; IU/ml	18.3 (10.3–136.1)	30.2 (12–392)	27.4 (12–143.2)	46 (12–230)	39.4 (11.7–167.3)
ACPA pos; %	72.6	74.1	78.3	73.9	63.6
RF pos; %	50.5	44.4	60.9	52.2	45.5
SDAI	28.8 (19.9–36.8)	30.2 (19.9–39.4)	24.4 (19.8–34)	31 (19.1–35.2)	28.1 (22.4–35.2)
CDAI	25.8 (18.8–35.4)	26.3 (19.7–38.6)	22.7 (18.1–33.5)	30.2 (17.2–35.8)	26.6 (21.4–34.2)
DAS28	5.4 (4.8–6)	5.6 (5.1–6.3)	5.3 (5–6)	5.3 (4.7–6.4)	5.3 (4.6–5.9)
Steroids use; %	34.7	44.4	21.7	34.8	36.4
Clinical response at 6 months					
ACR 20/50/70%	76.6/51.1/34	85.2/44.4/25.9	69.6/60.9/43.5	68.2/36.4/27.3	85.7/66.7/42.9
SDAI 50/70/85%	72.8/53.1/24.7	85/45/3.7	60/50/17.4	71.4/47.6/30.4	78.9/73.7/36.4
CDAI 50/70/85%	74.5/53.2/26.6	92.6/44.4/11.1	59.1/54.5/27.3	69.6/52.2/39.1	76.2/66.7/33.3
EULAR good/mod %	50.6/25.3	32.0/44.0	52.4/28.6	42.9/19.0	80.0/5.0
SDAI LDA/REM %	67.1	72.7	52.4	63.6	80.0
SDAI change	−17.6 (−25.03 to −11.31)	−17.6 (−27.8 to −11.4)	−16.7 (−20.7 to −5.8)	−17.0 (−24.2 to −9.4)	−21.3 (−27.9 to −11.3)
Relative SDAI change	−72.6 (−85.3 to −45.6)	−66.5 (−80.8 to −56.6)	−70.5 (−83.8 to −28.4)	−66.8 (−87.9 to −24.7)	−78.7 (−88.7 to −69.8)
CDAI change	−16.9 (−23.6 to −11.1)	−17.6 (−27.7 to −13.5)	−16.2 (−21.1 to −8.1)	−17.2 (−21.2 to −7.3)	−20.7 (−26.3 to −10.6)
Relative CDAI change	−72.8 (−85.7 to −51.6)	−67.3 (−79.3–58.1)	−75.4 (−90.5 to −34.8)	−70.2 (−88.3 to −36.3)	−73.5 (−88.0 to −55.9)
DAS28 change	−2.14 (−3.5 to −1.26)	−1.8 (−2.82 to −1.24)	−2.04 (−3.1 to −1.34)	−1.98 (−3.04 to −0.32)	−3.58 (−4.04 to −2.01)
DAS28 relative change	−40.9 (−59.1 to −24.7)	−33.6 (−48.0 to −25.7)	−42.9 (−54.9 to −21.0)	−38.8 (−57.4 to −4.8)	−66.5 (−75.2 to −42.6)

Descriptives of all patients at baseline, depicted separately by total and by treatment groups (ABA: abatacept; INF: infliximab; RTX: rituximab; TCZ: tocilizumab). Additionally, TTV (Torque Teno Virus) levels are also shown at 3 months and the absolute change between baseline and the level at 3 months. The second section outlines response outcomes after 6 months. PGA: Patient Global Assessment; EGA: Evaluator Global Assessment; TJC28: Tender Joint Count of 28 joints; SJC28: Swollen Joint Count of 28 joints; SDAI: Simplified Disease Activity Index; CDAI: Clinical Disease Activity Index; DAS28: DAS for 28 joints; EULAR good/mod: EULAR response either good or moderate; LDA/REM: Low disease activity/Remission; pos: positive.

FIG. 1 Torque Teno Virus levels by treatment and time points



TTV copy numbers (c/ml), illustrated in box-plots, shown at baseline (blue bars), 2 weeks (red bars) and 12 weeks (green bars) separately for ABA (abatacept), INF (infliximab), RTX (rituximab) and TCZ (tocilizumab). Comparisons (Mann–Whitney U): 1: $P = 0.071$, 2: $P = 0.018$, 3: $P < 0.001$, 4: $P = 0.263$. TTV: Torque Teno Virus; c/ml: cells per millilitre.

Measurement of Torque Teno Virus

Treating physicians were unaware of the TTV results. TTV copies within plasma samples of baseline, week 2 and month 3 were measured. TTV DNA was extracted from 200 µl of plasma using the NucliSENS easyMAG platform (bio-Merieux, France), as recommended by the manufacturer (see Supplementary Material, available at *Rheumatology* online).

Statistical analyses

We first investigated the changeability and responsiveness of TTV copy numbers (TTV c/ml; TTV levels) to different bDMARDs in RA. We used the Mann–Whitney *U* test, Kruskal–Wallis and Jonckheere–Terpstra to statistically assess whether TTV c/ml changed significantly during the first 3 months of treatment, and whether there was a difference between the four bDMARDs employed. We also investigated whether TTV c/ml or their changes were depending on sex, serology, glucocorticoids (GCs) use or disease activity at baseline and 3 months using univariate and multivariable regression analyses. Patients who had no detectable TTV c/ml at baseline were omitted from further analyses, as it must be assumed that they were not infected.

We then investigated the predictive value of baseline TTV levels for subsequent clinical responses, which were measured based on the SDAI and CDAI 50%, 70% and 85% responses evaluated at 6 months. Non-parametrical tests were used to assess differences in TTV c/ml at baseline, 2 weeks and 3 months between these response categories. Due to the distribution of TTV values, we performed univariate and forward-stepwise (adjusting for sex, age, ACPA status, baseline SDAI/CDAI, where appropriate) logistic regression analyses using 10 groups stratified by deciles of TTV as predictors for SDAI85%/CDAI85% response to obtain effect sizes. Forward-stepwise likelihood ratio-driven regression models were also used to assess whether TTV at 3 months further increased model accuracy for predicting SDAI70% or SDAI85% responses at 6 months in addition to CDAI50% response at 3 months and ACPA status. Spearman correlation tested the association between TTV at 3 months and absolute and relative SDAI, CDAI and DAS28 changes.

We also explored whether a predictive cut-off could be identified to predict SDAI/CDAI response levels or disease activity states at 6 months using diagnostic testing procedures (receiver operating characteristics, ROC, curve analyses). In an additional analysis, we grouped patients by the top and bottom quartiles of TTV levels at 3 months, and compared CDAI85%, SDAI85% and SDAI remission (REM) rates after 6 months, by χ^2 test.

In our real-world dataset of patients starting TNFi, generalized estimating equation analyses were used to assess TTV development over time due to heterogeneity in follow-up time points. All analyses have been done using SPSS v26 or STATA v15.

Table 2 Changes in level of Torque Teno Virus between baseline and 3 months (including levels for patients with baseline Torque Teno Virus level > 0, excluding TCZ

		TTV c/ml		ΔTTV c/ml
		Baseline	3 months	Baseline to 3 months
Sex	Female	4.2×10^4 (1.0×10^4–9.1×10^4)[a]	1.4×10^5 (3.4×10^4–1.9×10^6)[a]	9.4×10^4 (1.3×10^4–8.4×10^5)[a]
	Male	1.8×10^5 (2.6×10^4–5.2×10^5)[a]	1.4×10^6 (4.6×10^5–$1–6 \times 10^7$)[a]	7.5×10^5 (2.3×10^5–1.5×10^7)[a]
GCs use	Yes	5.2×10^4 (2.1×10^4–2.0×10^5)	5.1×10^5 (4.1×10^4–1.5×10^6)	3.0×10^5 (2.5×10^4–1.5×10^6)
	No	4.4×10^4 (1.3×10^4–9.7×10^4)	2.1×10^5 (5.3×10^4–3.9×10^6)	1.1×10^5 (2.0×10^4–3.9×10^6)
Age	≤40 years	4.5×10^4 (1.7×10^4–5.4×10^4)	9.5×10^5 (2.9×10^4–1.2×10^7)	4.7×10^4 (-1.3×10^4–4.6×10^6)
	41–60 years	4.5×10^4 (1.2×10^4–1.1×10^5)	1.9×10^5 (5.3×10^4–8.8×10^5)	6.0×10^4 (1.3×10^4–5.8×10^5)
	>60 years	1.8×10^5 (2.3×10^4–8.2×10^5)	6.5×10^6 (0.5×10^4–1.2×10^8)	3.6×10^5 (-4.2×10^4–1.3×10^7)
RF	Positive	5.1×10^4 (1.8×10^4–2.9×10^5)	4.6×10^5 (6.3×10^4–7.0×10^6)	2.3×10^5 (3.4×10^4–7.0×10^6)
	Negative	4.3×10^4 (1.1×10^4–9.2×10^4)	2.9×10^5 (4.3×10^4–2.2×10^6)	2.3×10^5 (2.4×10^4–2.2×10^6)
ACPA	Positive	5.0×10^4 (1.8×10^4–2.0×10^5)[a]	4.6×10^5 (5.5×10^4–6.5×10^6)	2.3×10^5 (3.5×10^4–6.3×10^6)
	Negative	2.5×10^4 (5.0×10^3–7.2×10^4)[a]	2.9×10^5 (2.3×10^4–1.9×10^6)	2.3×10^5 (1.8×10^4–1.8×10^6)

[a]Significant differences between comparison groups (regression analyses). ACPA: anti-citrullinated peptide antibodies; TTV: Torque Teno Virus; TCZ: tocilizumab; GCs: glucocorticoids.

Results

Clinical description and overall responses

Of 116 screened patients, 101 patients started on their randomized treatment (Supplementary Fig. S1, available at *Rheumatology* online). In 95 of the 101 patients receiving treatment, we were able to measure TTV adequately. Of these 95 patients, 23 were part of the INF, 22 of the TCZ, 27 of the ABA and 23 of the RTX randomization group; 77% were female, 73% were ACPA-positive and 51% RF-positive, and two-thirds showed high disease activity by CDAI (Table 1). There were no baseline differences in core set variables between the four treatment arms. Glucocorticoids were allowed at a stable dose throughout the trial and were utilized additionally in 35% of patients. Overall clinical responses to the various bDMARDs were within the expected range for this population at 6 months (see Table 1 and Supplementary Fig. S2A, available at *Rheumatology* online). Median changes in SDAI were similar across all treatment groups (ΔSDAI: 17.6, IQR: 11.3–25.0; ΔCDAI: 16.9, IQR: 11.1–23.6) (Supplementary Fig. S2B, available at *Rheumatology* online). Differences in response rates between treatments were not formally tested for, as this was neither the intent nor the design of the study.

TTV kinetics during treatment with different bDMARDs

Overall, the median TTV at baseline was 4.2×10^4 c/ml (IQR: 7.2×10^3–1.1×10^5) with no difference between the four treatment groups (Fig. 1 and Table 1; $P = 0.674$). No changes in TTV levels were detectable after 2 weeks of treatment (median ΔTTV: 0, IQR: -1.51×10^4–$1.0^7 \times 10^4$), and this time point was not further evaluated. After 3 months of treatment, an increase in TTV levels was observed compared with baseline (median ΔTTV c/ml: 4.5×10^4, IQR: 0–7.5×10^5; $P = 0.001$) with a particularly high increase in patients treated with INF (median ΔTTV: 5.4×10^4, IQR: 3.5×10^3–1.2×10^5; $P = 0.018$) and RTX (median ΔTTV: 6.9×10^6, IQR: 1.4×10^6–3.1×10^7; $P \leq 0.001$). Changes in TTV levels between baseline and 3 months in patients on ABA were in the same range but did not reach the predefined level of significance (median ΔTTV: 10.1×10^4, IQR: 4.4×10^3–5.3×10^5; $P = 0.071$). In patients treated with TCZ, TTV levels at 3 months did not change (median ΔTTV: -7.1×10^3, IQR: -5.4×10^4–8.9×10^3; $P = 0.263$). RTX showed a more profound increase in comparison with all others ($P < 0.001$), while in TCZ patients TTV levels were significantly lower compared with all other compounds ($P < 0.001$). The absence of TTV responsiveness to IL-6R inhibition led to the exclusion of the patient group treated with TCZ for the analysis of the predictive performance of TTV.

TTV in the context of clinical and patient characteristics

An overview of TTV levels stratified by clinical characteristics is provided in Table 2. In the total cohort there was higher TTV replication at baseline in male than in female patients (1.8×10^5, IQR: 2.6×10^4–$5.2.0 \times 10^5$ *vs* 4.2×10^4, IQR: 1.0×10^4–9.1×10^4; $P = 0.003$). There was no difference in TTV copy numbers regarding GCs use (GCs: 5.2×10^4 IQR: 2.1×10^4–2.0×10^5; no GC: 4.4×10^4, IQR: 1.3×10^4–9.7×10^4; $P = 0.214$), or disease activity level (high *vs* moderate disease activity: 4.6×10^4, IQR: 1.6×10^4–1.2×10^5 *vs* 5.6×10^4, IQR: 1.5×10^4–2.1×10^5; $P = 0.572$). However, patients that were positive for ACPA showed higher baseline TTV levels than those negative (ACPA positive: 5.0×10^4, IQR: 1.8×10^4–2.0×10^5 *vs* ACPA negative: 2.5×10^4, IQR: 5×10^3–7.2×10^4; $P = 0.027$). No differences in 3 months

Table 3 Copy numbers of TTV based on treatment response after 6 months

TTV in c/ml at	Non-response		50% response		70% response		85% response	
	CDAI	SDAI	CDAI	SDAI	CDAI	SDAI	CDAI	SDAI
Baseline	3.07×10^4 (3.6×10^3–1.07×10^5)	1.78×10^4 (3.3×10^3–6.7×10^4)	3.6×10^4 (1.6×10^4–8.1×10^4)	4.6×10^4 (2.5×10^4–2.0×10^5)	5.26×10^4 (2.1×10^4–1.8×10^5)	5.26×10^4 (2.1×10^4–1.8×10^5)	4.98×10^4 (1.5×10^4–6.6×10^5)	1.46×10^5 (1.4×10^4–6.7×10^5)
2 weeks	1.18×10^4 (4.1×10^3–1.1×10^5)	1.13×10^4 (3.2×10^3–5.9×10^4)	3.1×10^4 (4.3×10^3–1.1×10^5)	7.37×10^4 (2.7×10^3–1.4×10^5)	5.68×10^4 (2.5×10^4–1.6×10^5)	5.63×10^4 (1.6×10^4–1.7×10^5)	7.69×10^4 (1.4×10^4–4.79×10^5)	1.16×10^5 (2.4×10^4–6.2×10^5)
3 months	1.15×10^5 (1.0×10^4–2.5×10^6)	1.7×10^5 (1.5×10^4–4.2×10^6)	4.63×10^5 (4.8×10^4–7.3×10^5)	4.63×10^5 (1.8×10^4–7.0×10^5)	2.23×10^5 (5.3×10^4–1.4×10^6)	2.91×10^5 (5.4×10^4–3.6×10^6)	1.94×10^6 (1.2×10^5–1.3×10^7)	2.38×10^6 (5.8×10^5–1.6×10^7)

Median + IQR of TTV (=Torque Teno Virus) c/ml at baseline, 2 weeks and 3 months separately by CDAI (Clinical Disease Activity Index) and SDAI (Simplified Disease Activity Index) responses at 6 months.

TTV levels or their changes could be seen when subsetting patients by serologic status, GCs use or disease activity levels, but higher changes were seen in male compared with female patients at 3 months (7.5×10^5, IQR: 2.3×10^5–1.5×10^7 *vs* 9.4×10^4, IQR: 1.3×10^4–8.4×10^5; $P = 0.02$).

Nine patients showed no baseline TTV, and were thus excluded from time-dependent/predictive analyses (they are depicted in Supplementary Table S1, available at *Rheumatology* online).

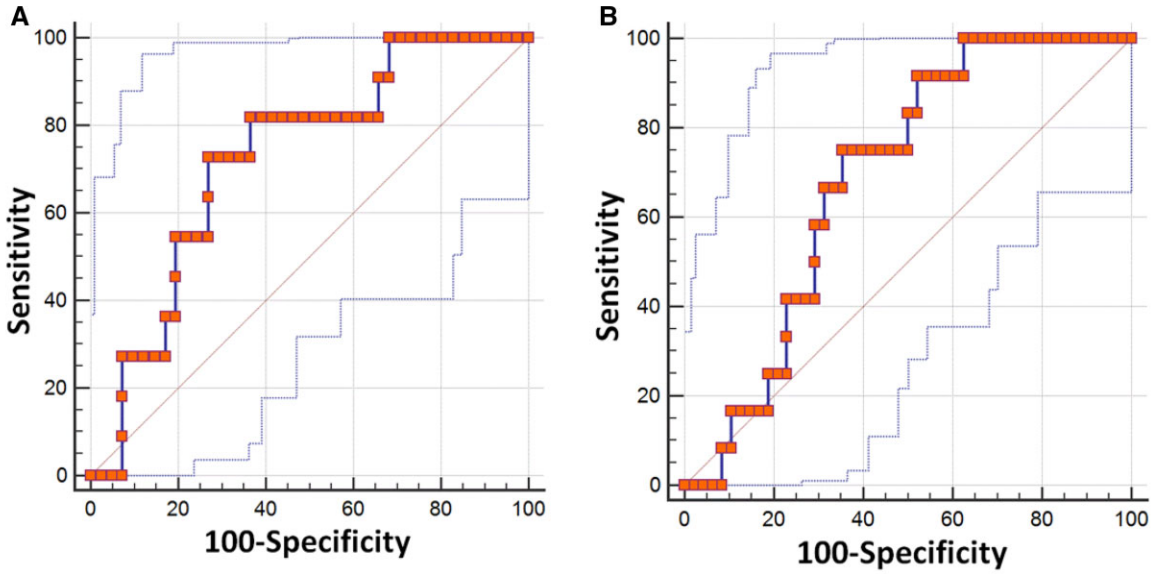
TTV replication and clinical response

TTV at baseline did not differ between patients showing different responses at 6 months using SDAI or CDAI definitions, but a trend towards higher baseline TTV levels in better SDAI response groups could be detected (Table 3). At 3 months, TTV levels were higher in SDAI85% responders *vs* non-responders at month 6 ($P = 0.023$; Supplementary Fig. S3A, available at *Rheumatology* online), and there was a trend between TTV increase and discrete SDAI response groups: SDAI non-responders, SDAI50-non70, SDAI70-non85, and SDAI85 responders ($P = 0.030$). Other covariates like SDAI, ACPA, age and sex were not significant predictors in a multivariable forward regression model. However, higher TTV at month 3 increased the odds of achieving an SDAI85% response at 6 months (OR: 1.5, 95% CI: 1.04, 2.1, $P = 0.028$). Similar results were found for CDAI response groups ($P = 0.026$; Supplementary Fig. S3B, available at *Rheumatology* online) and CDAI85% response *vs* those who did not (OR: 1.36, 95% CI: 1.04, 1.79; $P = 0.027$). Patients achieving SDAI remission at 6 months had higher 3 months TTV levels than those who did not ($P = 0.044$). Absolute changes in SDAI, CDAI and DAS28 after 6 months were not correlated with TTV load; however, a weak to moderate negative correlation was found for relative SDAI and CDAI changes (Supplementary Table S2, available at *Rheumatology* online). TTV at 3 months can add predictive value to reach SDAI70% or SDAI85% responses after 6 months of treatment, additionally, whether or not CDAI50% response at 3 months was achieved. Although a CDAI50% response after 3 months is informative, combining both considerably improved the R^2 for the SDAI85% model (R^2: 0.128 → 0.221) and the SDAI75% (R^2: 0.206 → 0.276)—Supplementary Table S3, available at *Rheumatology* online.

We next used the area under the ROC-curve (AUC) as the quality criteria for the usefulness of TTV levels as a test for SDAI85% and CDAI85% responses, as well as for reaching remission. ROC analysis showed an AUC of 0.725 (95% CI: 0.584, 0.840) for defining SDAI85% response at month 6 by TTV level at month 3 ($P = 0.004$, Fig. 2A). A TTV level of 1.2×10^6 c/ml at month 3 was associated with a positive likelihood ratio (+LR) of 2.7 (95% CI: 1.5, 5.0) for prediction of SDAI85% response at month 6 (−LR: 0.4, 95% CI: 0.1, 1.0). Alternative cut-offs based on ROC are provided in the table of Fig. 2. Comparable results were found for CDAI85% responses

FIG. 2 ROC for Torques Teno Virus levels at 3 months for SDAI85 response and SDAI remission

Outcome	TTV	Sensitivity	95% CI	Specificity	95% CI	+LR	95% CI	-LR	95% CI
SDAI85	$5.64x10^5$	81.8	48.2 – 97.7	63.4	46.9-77.9	2.2	1.4 – 3.7	0.3	0.1 – 1.0
	$1.20x10^6$	72.7	39.0 - 94.0	73.2	57.1 - 85.8	2.7	1.5 - 5.0	0.4	0.1 - 1.0
	$1.67x10^6$	54.6	23.4 – 83.3	80.5	65.1 – 91.2	2.8	1.2 – 6.4	0.6	0.3 – 1.1
SDAI-REM	$1.53x10^5$	83.3	51.6 – 97.9	46.5	31.2 – 62.3	1.6	1.1 – 2.3	0.4	0.1 – 1.3
	$5.64x10^5$	75.0	42.8 - 94.5	62.8	46.7 - 77.0	2.0	1.2 – 3.3	0.4	0.1 - 1.1
	$5.93x10^6$	25.0	5.5 – 57.2	81.4	66.6 – 91.6	1.3	0.4-4.3	0.9	0.6-1.3



(**A**) ROC curve of patients receiving abatacept (ABA), infliximab (INF) or rituximab (RTX), testing cut-offs for TTV c/ml at 3 months for SDAI85 response (Simplified Disease Activity Score) after 6 months of treatment. (**B**) ROC curve of patients receiving ABA, INF or RTX, testing cut-offs for TTV c/ml at 3 months for reaching SDAI remission after 6 months of treatment. The table provides an overview of a balanced TTV cut-off and cut-offs associated with either 80% sensitivity or 80% specificity, respectively. ROC: receiver operating characteristic; TTV: Torque Teno Virus; SDAI: Simplified Disease Activity Index; SDAI85: 85% reduction in Simplified Disease Activity Index; ABA: abatacept; INF: infliximab; RTX: rituximab, LR: likelihood ratio.

(AUC: 0.688; 95% CI: 0.555, 0.801; $P = 0.012$; TTV $>1.2 \times 10^6$ c/ml + LR: 2.5, 95% CI: 1.3, 4.7; −LR: 0.5, 95% CI: 0.3, 1.0). For SDAI remission, we could identify a TTV cut-off of 5.6×10^5 c/ml at 3 months identifying patients reaching remission (SDAI-REM) at 6 months (Fig. 2B, AUC: 0.676; 95% CI: 0.537, 0.796; $P = 0.018$), corresponding to a positive LR of 2.0 (95% CI: 1.2, 3.3).

To further explore the potential of TTV replication at 3 months for prediction of outcomes at 6 months, we analysed the top 25% and the lowest 25% of the 3 months TTV levels (TTV c/ml for lowest/highest quartiles were $<5.3 \times 10^4$ c/ml and $>2.6 \times 10^6$ c/ml, respectively). We saw higher rates of SDAI remission ($P = 0.037$), SDAI85% ($P = 0.016$) and CDAI85% ($P = 0.017$) responses in the top quartile of the 3-month TTV levels compared with the lowest quartile (Table 4); none of the patients in the latter group reached remission or SDAI85 response (Table 4). TTV load at 3 months could not differentiate between reaching SDAI low disease activity (LDA) and not reaching it ($P = 0.618$). In contrast to the absolute TTV levels at the 3 months' time point, the change in TTV copy numbers from baseline to 3 months did not correspond with responses at 6 months (data not shown).

Real-world data for patients starting TNFi

The real-life population of TNFi starters ($n = 23$) showed similar TTV c/ml compared with patients subjected to TNFi in the BioBio study (median: baseline 2.7×10^4 IQR: 8.5×10^3–1.1×10^5; ~3months: 3.8×10^4 IQR: 6.0×10^3–1.8×10^5). Similar to the BioBio cohort, TTV copy numbers increased with duration of treatment exposure ($P \leq 0.001$). Since the timing of the follow-up assessment varied between 50 and 120 days and response rates in the real-life cohort at 6 months were generally lower (35% reached an SDAI50% response at 6 months) than in the prospective trial ,we could not perform the predictive 3 months analyses in the routine patient population.

Discussion

This is the first study that has evaluated dynamics in TTV replication during RA treatment with four modes of

Table 4 Response rates at 6 months by top and lowest quartiles for TTV at 3 months

	SDAI REM		SDAI LDA/REM		SDAI85		CDAI85	
	No	Yes	No	Yes	No	Yes	No	Yes
Lowest 25% in TTV	100%	0%	44.4%	55.6%	100%	0%	92.90%	7.10%
Highest 25% in TTV	71.40%	28.60%	35.7%	64.3%	61.50%	38.50%	53.30%	46.70%

Cross-table: outlining rates of SDAI (Simplified Disease Activity Index) remission, SDAI 85% response, CDAI85% (Clinical Disease Activity Index) response and SDAI LDA/REM (low disease activity or remission) in the highest and the lowest 25% of patients according to TTV (Torque Teno Virus) copy numbers at 3 months.

action without bias by indication. We observed that TTV levels increase in patients receiving bDMARDs. This is particularly true for anti-CD20 blockade (RTX), which led to the highest increase in TTV copies. TTV levels increase within the first 12 weeks of treatment. At baseline, no differences in TTV c/ml between the four modes of action could be found. Our data reveal that TTV levels at 3 months might be considered a decision aid for responsiveness to bDMARDs, but are not sufficiently informative at treatment initiation. Replication of TTV seems to be differently stimulated depending on the mode of action, since RTX patients had significantly higher copy numbers than INF and ABA patients at 3 months. Interestingly, in TCZ patients TTV levels remained practically unchanged or even decreased. The increase in TTV levels during TNFi treatment was confirmed in our real-world cohort. T cell immunity is considered crucial in controlling TTV [33]; however, the strong effect of CD-20 blockade on TTV replication might be explained by a potential B cell involvement in controlling TTV. Prior studies have outlined that antigen-presenting cells and toll-like receptors play a role in TTV antigen recognition [34, 35], supported by an inverse correlation of TTV with antibody-mediated rejection in kidney-transplanted patients [36]. The missing effect of TTV in TCZ indicates the need for further study of the role of IL-6 (or its receptor components) in the pathomechanisms of TTV infection and host–cell interaction. Within a phase II study in patients with late antibody-mediated kidney transplant rejection, who were treated with clavakizumab (IL6-receptor antibody) or placebo, no differences in TTV replication could be found [37]. Data on $CD8^+$ T cells in patients with lymphoma and myeloma has shown an inverse correlation of T cell number and TTV load, but a better understanding of the immune compartments involved in TTV viral control and the interference of bDMARDs needs to be further explored [38–40].

Management of RA has arrived at the paradox point where multiple effective treatment regimens exist, but choice about their utilization is still enigmatic and highly subjective. The treat-to-target concept has provided a pragmatic approach to the prediction dilemma of RA, as it stipulates rapid switches if target levels of disease activity are not reached within 3–6 months [11, 41]. If no markers for preferential treatment choice exist, any new marker that has the ability to mirror the level of immunomodulation at least to some extent may warrant further investigations to understand its value for therapeutic steering in clinical practice. The TTV has been shown to be a potential candidate for this in other disease/therapeutic applications [16, 30, 36]. The increase in TTV during immunomodulatory therapy, as well as the link between TTV and clinical response, make TTV an interesting novel biomarker for further exploration for monitoring of immune-mediated diseases. The predictive value and clinical relevance of TTV seems suited to identifying patients at the greatest risk of non-response (i.e. those with very low copy numbers) or, although less pronounced, likely to have an outstandingly good response.

Based on our data, we also explored decision cut-points for this purpose, although their generalizability needs to be further studied. We found that those patients with $>1.2 \times 10^6$ c/ml of TTV at 3 months show a three times higher likelihood of achieving a SDAI85% response than those below. Still, not all patients above this threshold achieve a state of low disease activity or better. Generally, larger clinical responses were observed in those with higher TTV at 3 months. However, expectations about any novel predictive biomarker in RA need to be moderated by the fact that, currently, no reasonably accurate markers exist at all [42].

Although our study was four-armed, randomized and accounts for RF status, one limitation was the rather small patient number that prevented the observation of smaller effects or the implication of particularly high TTV c/ml, and whether this might be associated with adverse (in particular infectious) events. This also prevented us from stating a clear target range for TTV at 3 months after treatment initiation to achieve the best possible outcome. A pitfall related to this point is that we have not had access to similar trial data for biosamples that could have been used for TTV analyses to better replicate the cut-offs that we have found using ROC analyses. Even though the provided cut-offs are more inspirational than ready for application in clinical practice, optimal ranges of TTV c/ml in kidney transplant patients to avoid rejection were reported at between 1×10^6 and 1×10^8 c/ml [27, 43]. However, achieving

accuracy of TTV for prediction of response might be a challenging endeavour, since it has shown limitations when it comes to infections or transplant rejections in kidney-transplant patients [18].

A recently published observational cohort study investigated TTV in inflammatory arthritis patients treated with bDMARDs and conventional synthetic DMARDs (csDMARDs). No difference in TTV levels were seen between RTX-treated patients and healthy controls cross-sectionally [44]. However, this study looked mainly at cross-sectional levels observationally, while the present investigation was a longitudinal analysis in a clinical trial. A comparison with our findings was only possible concerning the increase in TTV during treatment initiation with TNFi, in which it is clearly confirmatory, likewise in the integrated real-life validation part of our study. Since 75% of patients received TNFi or secukinumab and only a few patients treated with TCZ, ABA or RTX were included, the analyses of TTV dynamics during treatment with other DMARDs were pooled in that study. It showed higher TTV after a mean of 4 months, similarly to our initial, and more descriptive, analysis. Associations with disease activity, or its prediction, were not analysed.

In summary, our data suggest that TTV levels at 3 months correspond to a clinical response to bDMARDs for RA, except for TCZ. This clearly bears potential in treatment monitoring of RA and bringing forward decisions about the need for treatment modifications. Future prospective trials and further modelling studies using biobanked samples may validate and better clarify the potential for TTV-tailored bDMARD dosing, while the lack of TTV increase with TCZ treatment needs better mechanistic understanding.

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Data availability statement

The data underlying this article will be shared on reasonable request to the corresponding author.

Supplementary data

Supplementary data are available at *Rheumatology* online.

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